EFFECT OF LIGHT SOURCE DISTANCE ON MONOMER RELEASE FROM LIGHT-CURED DENTAL RESIN RESTORATIVE MATERIALS

by

Alan H. Chen
Lieutenant Commander, Dental Corps
United States Navy

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Research Committee:

[redacted]

Ling Ye, DDS, PhD
CDR, DC, USN
Research Committee Chair

[redacted]

Andrew Avillo, DMD, MS
CAPT, DC, USN
Program Director, Comprehensive Dentistry

[redacted]

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[redacted]

David Evers, PhD
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Alan H. Chen
Comprehensive Dentistry Graduate Program
Naval Postgraduate Dental School
June 2019

NAVAL POSTGRADUATE DENTAL SCHOOL
Alan H. Chen

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ABSTRACT

EFFECT OF LIGHT SOURCE DISTANCE ON MONOMER RELEASE FROM LIGHT-CURED DENTAL RESIN RESTORATIVE MATERIALS

ALAN H. CHEN, DDS, M.S.,
COMPREHENSIVE DENTISTRY, 2019

Directed by: LING YE, COMMITTEE CHAIR, DENTAL RESEARCH DEPT
Naval Postgraduate Dental School

Introduction: Light-cured dental resin-composites plays a significant role in today's dentistry. While it does not contain Mercury like amalgam, it is, as the name states, a composite of monomers which can have adverse biological effects. The release of monomers is related to the polymerization condition, in this case, composite to light distance.

Purpose: To determine the difference in monomer release from light-cured dental resin-composites under ideal (0 mm) and clinically relevant (4 mm) light cure distances.

Materials and methods: Five light-cured dental resin-composites that are commonly found in military dental clinics were selected, Filtek Supreme Ultra, Paradigm ^{TM}, Esthet X HD, Herculite XRV, and SonicFil 3. Four samples of 4 mm radius and 2 mm thickness were fabricated for each composite at curing distances of 0 mm and 4 mm. Samples were submerged in 1.25 ml of artificial saliva and Liquid Chromatography-Mass Spectrometry (LC-MS) was used to determine the concentration of monomer released at day 1 and day 7.

Results: In general, monomer release was not different at 0 mm and 4 mm curing distance. At day 7, significant differences in monomer concentration released were found between the two curing distances in Filtek Supreme Ultra for HEMA and UDMA; Paradigm™ for HEMA; and Sonicfil 3 for UDMA. HEMA showed detection only at day 7 data for several materials.

Conclusions: These results confirmed that monomers are released from composite resins after polymerization at ideal and clinical conditions. Based on the day 1 result, it is suggestive that even at the clinically relevant distance (4 mm), the light-curing unit is able to deliver proficient energy to facilitate sufficient polymerization of the composite material. Future research on monomer and composite degradation and its effect on monomer concentration detection over time is required to provide further conclusions regarding the concentration of monomer released.

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LIST OF ABBREVIATIONS

1.	BisGMA	Bisphenol A-glycidyl methacrylate
2.	HEMA	Hydroxylethyl methacrylate
3.	HPLC	High Pressure Liquid Chromatography
4.	LC-MS	Liquid Chromatography – Mass Spectrometry
5.	PDA	Photodiode Array
6.	TEGDMA	Triethylene glycol dimethacrylate
7.	UDMA	Urethane dimethacrylate

CHAPTER 1: REVIEW OF LITERATURE

Dental materials are among the most extensively used artificial materials in humans (Van Landuyt et al., 2011) and resin-composites play a significant part within the materials used. Based upon a 2005-2006 American Dental Association survey, the number of posterior teeth restorations with resin-composite have surpassed the number of amalgam restorations and at least 146 million resin-based composite restorations and sealants are placed annually in the United States (American Dental Association, 2007).

Dental resin-composites are versatile esthetic materials that promote the conservation of natural tooth structures. They are generally composed of inorganic or organic filler particles embedded in an organic resin-based matrix. While it is considered a stable restorative material, it remains susceptible to degradation and to leaching of monomers (Ferracane, 1994). In various storage media, more than 30 chemical substances were released from resin-composite (Michelsen et al., 2003). Since composite dental materials are widely used, it is important to be aware of their potential in exposing patient with monomers that can have toxic effects.

Light-curing is a common requirement for the majority of dental resin-composites that are employed in dental practice. Blue light at wavelength 468 nm is used to stimulate the photo-initiator camphorquinone, the most commonly used initiator, triggering the polymerization reaction. The amount of energy delivered to the resin-composite is affected by several factors, such as the intensity of the light source, distance between the light source and the material, and the duration of time the material is exposed to the light. Only when the required amount of energy is received by the light-cure resin-composite can it adequately polymerize for the restoration to exhibit the desired physical properties. However, even when appropriate polymerization conditions are applied, only 40-70% of resin materials are cured (Sideridou & Karabela, 2009), leaving almost one-half as monomers that can potentially leech out.

The conversion/polymerization of the monomer not only affects the physical properties of the restorative material (such as wear resistance, hardness, and the tendency to discolor (Price et al., 2010)), the release of monomers could also lead to health issues. They could enter the oral cavity or pulp and cause local reactions (Van Landuyt et al., 2011). Monomers with potential toxicity include, but are not limited to, bisphenol A (BPA), bisphenol A-glycidyl methacrylate (BisGMA), urethane dimethacrylate (UDMA), and triethylene glycol dimethacrylate (TEGDMA).

The toxicity of BPA is well-studied. BPA is usually present as a contaminant from the manufacture of plastics. Infants, young children and pregnant/lactating women are most sensitive to BPA (Shelby, 2008). BPA can affect metabolism, cause obesity (Anses 2013), affect parahormonal activity, and imitate estrogen group hormones to possibly contribute to female infertility (Olea et al., 1996; Chao et al., 2012). The "safe" intake amount of BPA is estimated at 0.04 to 0.05 mg/kg/day (European Food Safety Authority, 2015; Sideridou & Karabela, 2009). The relevance of this amount to exposure from dental materials is unclear, but it is likely that the average oral consumption of BPA poses no risk to human health.

The toxicities of the remaining monomers have been poorly studied. The representative compound for resin monomer toxicity studies, BisGMA, is a derivative of BPA and is widely used in dental resin-composites (Peutzfeldt, 1997). UDMA is often used as a replacement for BisGMA for desired handling property. TEGDMA is used as a diluent that also improves handling properties, and is suspected to promote the growth of cariogenic bacteria (Hansel et al., 1998). BisGMA, UDMA and TEGDMA could disturb cell differentiation, the immune response, and wound healing. HEMA is utilized for dentin adhesion, and while it is deemed to be far less risky, may still disturb physiological pulp homeostasis and repair. Clinical concerns in regard to the monomers released from resin-composite materials include contact reactions in the oral mucosa, stomatitis, lip swelling, and perioral dermatitis (Nathanson et al., 1979; Lind, 1988; Blomgren et al., 1996; Martin et al., 2003; Kanerva & Alanko, 1998). Monomers may also cause gastrointestinal issues (Polydorou et al., 2009) (**Fig. 1**).

A number of research groups have studied the release of monomers from dental composites. The release of BPA is frequently too little to quantify. BisGMA, TEGDMA and UDMA are frequently present. However, the amounts released vary from study to study. This can be partially explained by the different conditions used by each group. Extraction of materials in organic solvents such as alcohols (Alshali et al., 2015; Van Landuyt et al., 2011) is often employed because, predictably, it is more capable of taking up hydrophobic monomers that are poorly soluble in water. Three aqueous preparations are often used: water, artificial saliva and human saliva. Surprisingly, human saliva gives poor yields of monomers, possibly due to digestive enzyme activity (Rothmund et al., 2015).

While formulating this study we looked for a model system that was (i) as close to physiologically relevant as possible, (ii) easily compared to other studies and (iii) relevant to the Defense Health Agencies dental programs. Based upon the literature reviewed, we chose extractions in commercially available artificial saliva at human body temperature for 1 day and 1 week. We pursued standardized conditions described by Van Landuyt et al., 2011. Finally, 5 composites often found in the Navy Dental Clinic were selected.

Samples were tested under ideal light curing conditions (0 mm) to verify existing data on monomer leaching. Samples were also tested under a 4 mm curing light to resin distance, which simulates the light to material distance of a Class I restoration (Price, et al., 2010.) (**Fig. 2**), to provide clinically relevant data for analysis. Light curing conditions are based upon literature precedent (Price, et al., 2010.)

CHAPTER II: MATERIALS AND METHODS

Sample Preparation

Five dental light cured resin-composite materials were tested: Filtek Supreme Ultra (3M), SonicFill (Kerr Dental), Paradigm (3M), Esthet-X HD (Dentsply Sirona) and Herculite XRV (Kerr Dental). Since the shade of the material can affect the light penetration and polymerization, the commonly used shade A2 was selected. Each material was prepared as a cylinder of 2 mm height and 8 mm diameter. To best standardize surface areas, each sample was processed from the same silicone mold. Glass slabs of 1 mm thickness provided a flat surface for smoothing the material and acted as standardized height adjustments between curing conditions.

The rationale for the surface area of samples is to simulate a large single composite restoration that approximates one-half of the average total crown surface area of a first molar ($315/2 = 157.5$ mm^2) as reported by Van Landuyt et al., 2011. The 2 mm depth of samples is based upon the common recommended maximum curing depth of dental resin composites, according to the manufacturer, and the corresponding radius was calculated to be 4.11 mm. For the ease of measurement, the radius was set at 4 mm, leading to a total surface area of the sample of 150.8 mm^2, with a volume of 100.53 mm^3 **(Fig. 3)**.

Curing light used was the ESPE Elipar S10 (3M) with 10 mm light guide which would provide full coverage of the sample with 8 mm diameter. The unit has the designed output of 1200 mW/cm^2. The LED Radiometer (Kerr) was used to verify the consistent output between each resin material every 8 cures.

The curing light was mounted on the test tube rack perpendicular to the material at the predetermined height to ensure the even exposure and the curing distance was adjusted by moving the sample mold.

Samples were cured for 20 seconds (the common recommended curing time according to the manufacturer) with the light source at distances of 0 mm (perpendicular and directly on top of the sample) and 4 mm as reported in Price, et al., 2010 (**Fig. 3**). Based on Price, et al. 2010, up to 24 J/cm^2 of energy per unit area should adequately polymerize a dental resin sample with 2 mm thickness. With the 20 seconds exposure time and the expected output of this LED curing light, the energy delivered at the tip of light guide is calculated to be 24 J/cm^2 which ensures the adequate polymerization of the monomers at 0 mm light to material distance.

Exposed cylindrical samples were transferred to glass test tubes and 1250 μL of artificial saliva (Pickering Laboratories) was added. Glass vials were used to avoid contamination from plastics. Samples were covered with foil and incubated at 37°C. 250 μL aliquots was then removed immediately from each solution as control for contamination and again at day 1 and day 7 for data analysis. Artificial saliva without resin served as a negative control and artificial saliva spiked with monomers (TEGDMA, HEMA, BisGMA and UDMA; Sigma) served as a positive control. Caffeine was used as an internal standard.

Liquid Chromatography-Mass Spectrometry Leeched monomers were detected and quantified using a Shimadzu LCMS-2020 liquid chromatograph mass spectrometer equipped with a 3.9x150 mm C18 column (Waters, cat. no. WAT046980) and an analytical guard column (Phenomenex, cat. no. KJO-4282).This system contained a Shimadzu SIL-20A auto sampler, two LC-20AD pumps, a DGU-20A5 degasser, a CTO-20A column oven, and an SPD-M20A Photodiode Array detector (PDA). The mass spectrometer was a single quadrupole equipped with electrospray ionization and corona discharge needle sources for a dual ionization source interface.

Samples were diluted 1:2 in acetonitrile with caffeine as internal standard. These mixtures were centrifuged, then 25μLof the supernatants were injected for LC-MS analyses. Separation was achieved using a 0.4 mL/min flow rate with the column oven set at 37°C.The mobile phase consisted of (A) 0.1% formic acid in water, and (B) 0.1% formic acid in acetonitrile as mobile phase B with the program of 95% A at 0-2 minutes, 95% to 5% A at 2-5 minutes, 5% A at 5-18 minutes, and 5% to 95% A at 18-20 minutes.

The following ion source parameters were used: interface voltage switching between 4.5 kV for positive mode and -3.5 kV for negative mode, nebulizing gas at 1.5 L/min, drying gas at 15.0 L/min, desolvation line temperature at 250°C, heat block temperature at 350°C, and detector voltage at 1.15 kV. These parameters were set after tuning and calibrating according to a tuning solution (Shimadzu, cat. no. 225-14985-01).

Monomers were identified according to retention times in relation to those of chemical standards (**Table 1**), and quantified according to peak area ratios relative to the caffeine internal standard, using m/z values of: 195.0 (caffeine), 227.1 (BPA), 287.1 (TEGDMA), 471.2 (UDMA), and 513.0 (BisGMA) (**Table 1**). All monomers were monitored in positive ion mode, except for bisphenol A, which was monitored in negative ion mode (**Table 1**). HEMA was quantified using the PDA detector at a wavelength of 210 nm, relative to the caffeine internal standard that was quantified at a wavelength of 274 nm (**Table 1**). Standard curves were developed using at least five, and at least duplicate, points. Samples were set up in quadruplicate and their mean and standard deviation of replicates reported in $\mu mol/mm^2$ and absolute values of release expression; the standardized reporting conditions suggested in Van Landuyt, *et al.*, 2011.

Statistics

Collected data was processed according to the Nonparametric Mann-Whitney U test to determine the significance in the concentration difference between the two tested distances.

CHAPTER III: RESULTS

HEMA

Leaching of HEMA was only detected from Filtek Supreme Ultra after 1 day of incubation (**Fig. 4**). A noticeable increase observed between day 1 and day 7 for Filtek Supreme Ultra. 3 out of 5 materials (Paradigm ™, Esthet-X HD, Herculite XRV) only show detectable HEMA concentrations on day 7. While no HEMA was detected from

SonicFil after 1 or 7 days. No differences in concentration between 0 and 4 mm curing distance were detected at day 1. Discrepancies were found with HEMA for Filtek Supreme Ultra and Paradigm ™ for day 7 alone, where significantly lower concentration were detected at 4 mm curing distance compared to the 0 mm curing distance. Both materials are from 3M and were modified from the original Filtek™ Z250 Universal.

UDMA

No differences were detected between 0 mm and 4 mm at day 1. On day 7 at 4 mm curing distance, Filtek Supreme Ultra showed significantly lower concentration compared to the 0 mm curing distance. Again on day 7 at 4 mm curing distance, Sonicfill 3 showed significantly higher concentration compared to the 0 mm curing distance (**Fig. 5**). This is interesting since Sonicfill 3 is advertised to be capable of 5 mm depth of cure compared to the usual 2 mm increment suggested for the other materials. If it performed as advertised, Sonicfill 3 should have had the best chance to reach adequate polymerization at 4 mm curing distance. Very low concentration were detected for Esthet-X HD and Herculite XRV. Between day 1 and day 7, some data suggested a decrease of concentration on day 7. Since solution samples were extracted from the same test tubes, and the only difference was time, this requires further investigation.

TEGDMA

No significant difference were detected for all materials tested between 0 mm and 4 mm curing distance. Very low concentrations were detected for Filtek Supreme Ultra, Paradigm ™ and Sonicfill 3. Noticeable increase were observed between day 1 and day 7 data for Esthet-X HD (**Fig. 6**).

BisGMA

Only Herculite XRV had detectable monomer concentration, no significant difference were found between 0 and 4 mm (**Fig. 7**).

CHAPTER V: DISCUSSION

In general, monomer concentrations were detected for all light-cure resin composites tested at different curing distance across 7 days. There were no significant differences in monomer concentrations detected at 0 and 4 mm curing distance on day 1. Monomer concentration differences were detected between day 1 and day 7 for HEMA and TEGDMA, suggesting continued leaching of these monomers beyond 1 day of incubation. While these results may overall suggest a need for longer term experiments than the 1 day previously indicated, further studies would need to determine the stability of these monomers in solution for longer time periods as well. This could explain surprising small but significant decreases in monomer concentrations from day 7 compared to day 1 in a few cases.

A lack of significant differences in the concentration data observed indicates that the curing light to material distances tested here had no effect on curing efficacy. During the course of the resin composite curings, the radiometer indicated fluctuations in curing light output of less than 100 mW/cm^2 (one twelfth of the designed output). Therefore, the data was confirmed to not have been affected by curing light output fluctuations.

Future studies should include larger light-to-material distance, larger sample size, stability tests for the monomers in artificial saliva, other considerations to prevent or account for evaporation of the solution, and data collection for day 2 and day 3. These would provide clarification on the findings of this study. An experimental setup more representative of physiological conditions would consist of a semi-continuous flow setup, rather than the batch setup performed in our study. This would also determine whether continued leaching is dependent on a concentration gradient in the saliva.

CHAPTER VI: CONCLUSION

No significant difference in the monomer release were found between the ideal (0 mm) and clinically relevant (4 mm) light to material distance, especially with regards to the day 1 data. Further studies are needed to clarify any differences seen with the day 7 data.

CHAPTER VII: ACKNOWLEDGEMENTS

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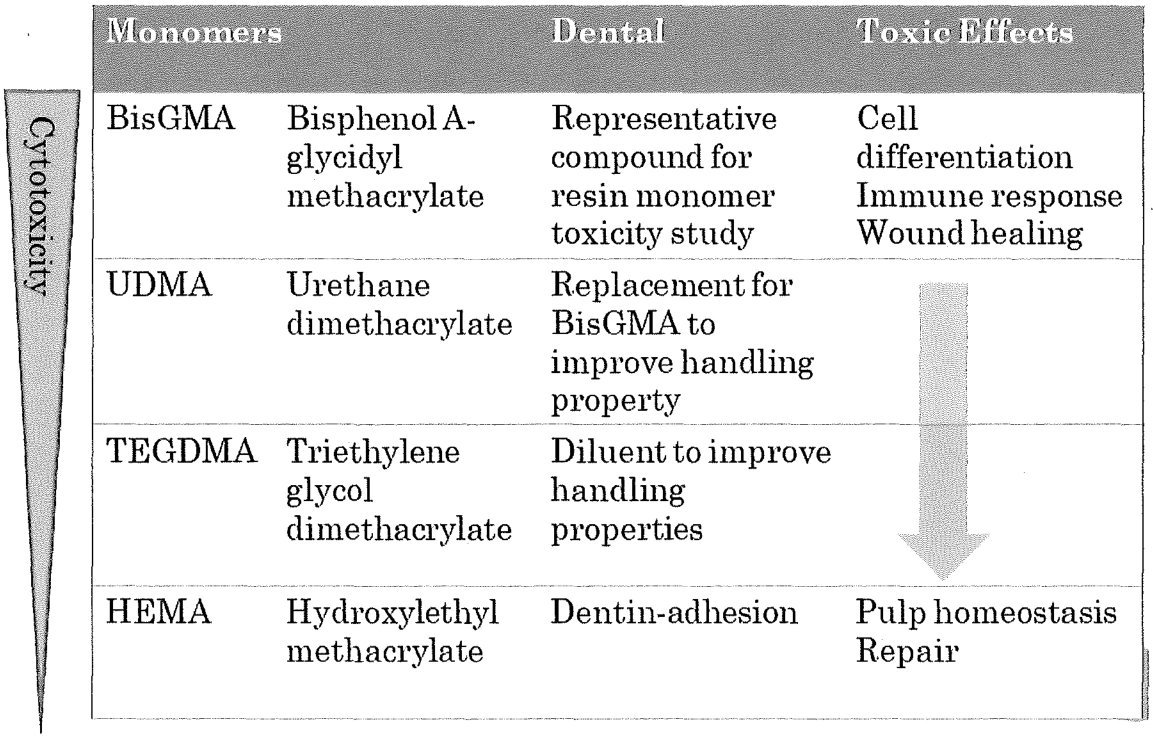
Table

Limits of quantification						
Chemical	Mode of detection	Retention time (mins)	Wavelength used for quantification (nm)	m/z used for quantification	Limit of detection for Day 1 (µmol/mm2)	Limit of detection for Day 7 (µmol/mm2)
BPA	MS (negative mode)	8.1	-	227	0.000073	0.000097
HEMA	PDA	7.2	210	-	0.000255	0.000340
UDMA	MS (positive mode)	8.9	-	471	0.000014	0.000019
TEGDMA	MS (positive mode)	8.7	-	287	0.000002	0.000003
Bis-GMA	MS (positive mode)	9.0	-	513	0.000097	0.000129
Internal standard (caffeine)	MS (positive mode) or PDA	7.0	274	195	---	---

Table 1. Limits of quantification for monomers test

Figures



Monomers		Dental	Toxic Effects
BisGMA	Bisphenol A-glycidyl methacrylate	Representative compound for resin monomer toxicity study	Cell differentiation Immune response Wound healing
UDMA	Urethane dimethacrylate	Replacement for BisGMA to improve handling property	
TEGDMA	Triethylene glycol dimethacrylate	Diluent to improve handling properties	
HEMA	Hydroxylethyl methacrylate	Dentin-adhesion	Pulp homeostasis Repair

Fig. 1. Cytotoxic effects and dental properties of the monomers studied.

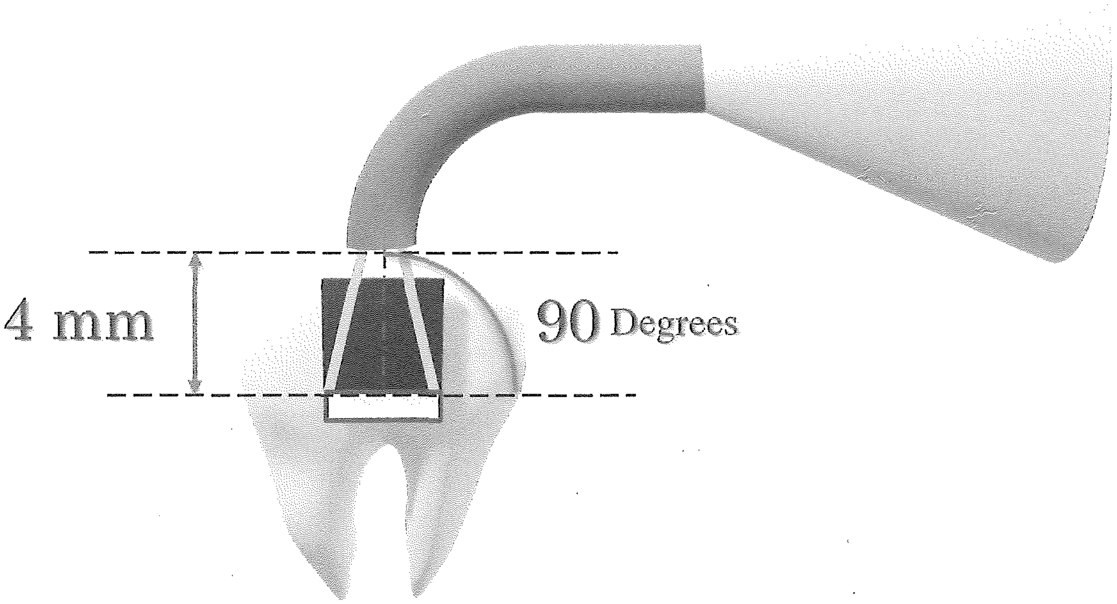


Fig. 2. Curing condition estimated for a Class I preparation in cross section.

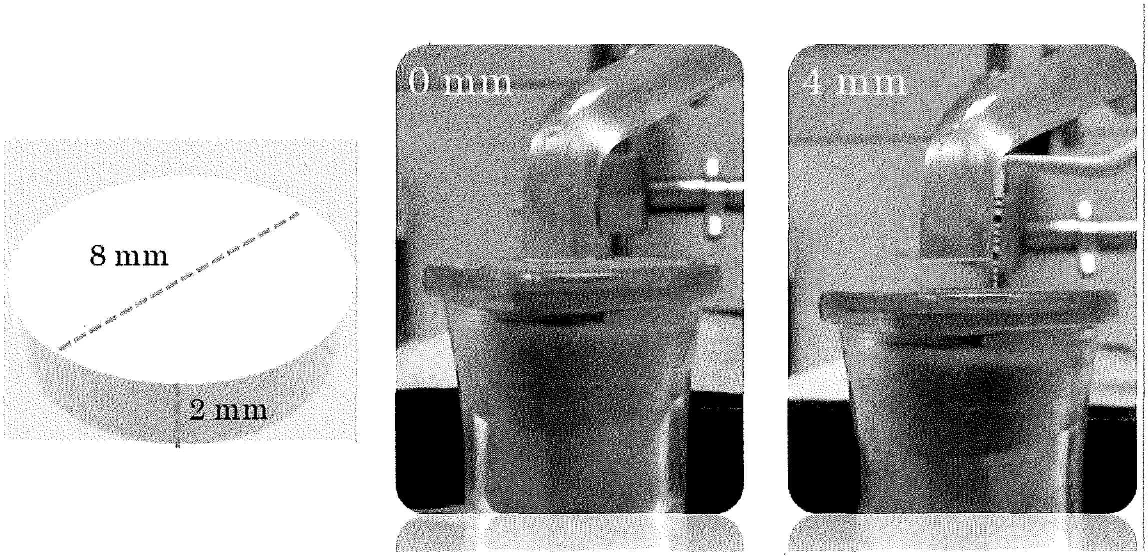


Fig. 3. Demonstration of sample preparation.

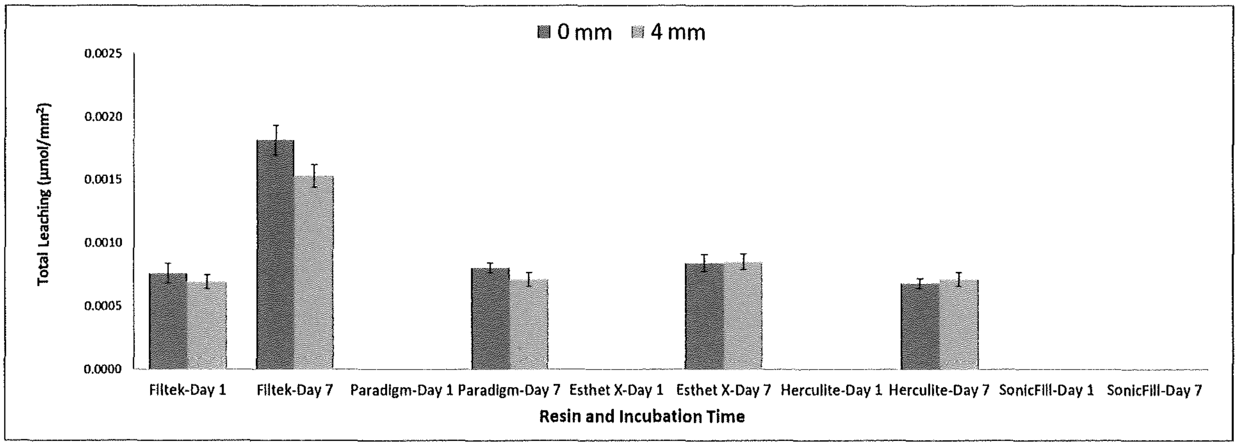


Fig. 4. Leaching amounts of HEMA from the dental resin composites tested.

Leaching is quantified as total amount leached into solution in relation to surface area of the resin composite material. Data is organized according to resin material, curing distance (0 and 4 mm), and incubation time. All values represent means ± standard deviations from four replicates.

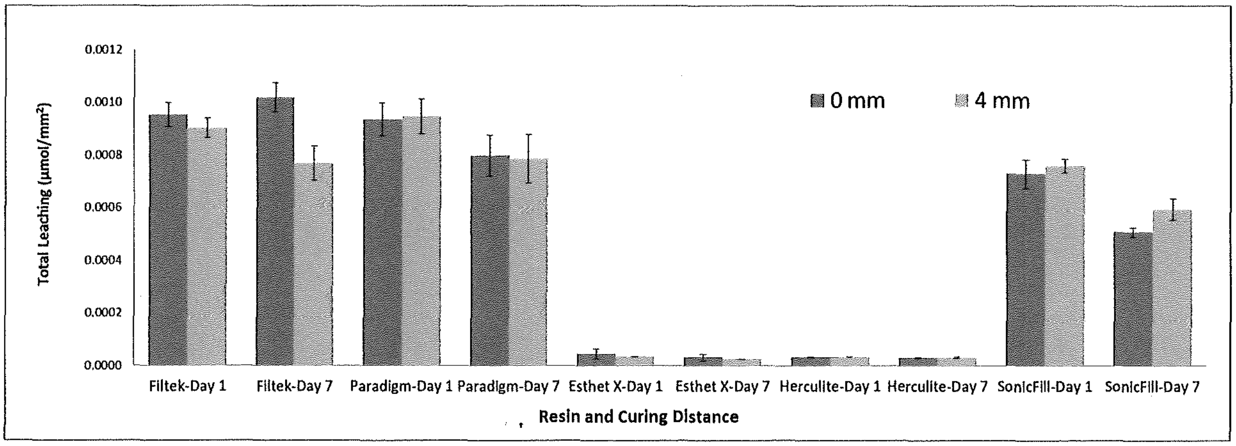


Fig. 5. Leaching amounts of UDMA from the dental resin composites tested.

Leaching is quantified as total amount leached into solution in relation to surface area of the resin composite material. Data is organized according to resin material, curing distance (0 and 4 mm), and incubation time. All values represent means ± standard deviations from four replicates.

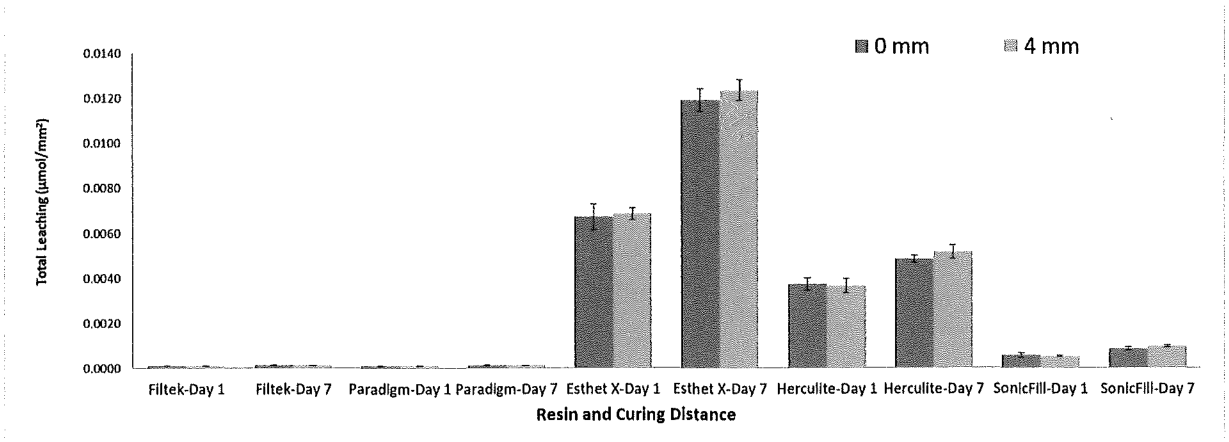


Fig. 6. Leaching amounts of TEGDMA from the dental resin composites tested. Leaching is quantified as total amount leached into solution in relation to surface area of the resin composite material. Data is organized according to resin material, curing distance (0 and 4 mm), and incubation time. All values represent means ± standard deviations from four replicates.

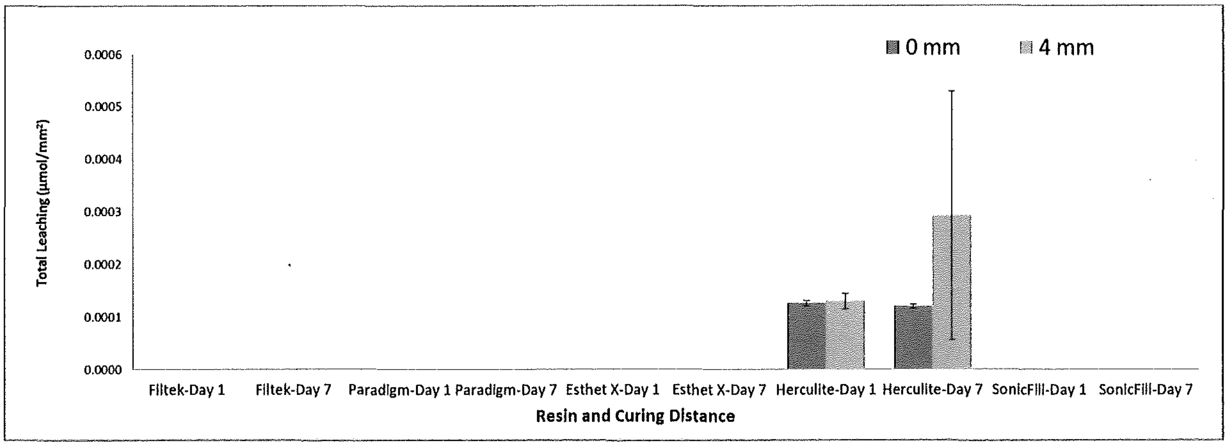


Fig. 7. Leaching amounts of BisGMA from the dental resin composites tested. Leaching is quantified as total amount leached into solution in relation to surface area of the resin composite material. Data is organized according to resin material, curing distance (0 and 4 mm), and incubation time. All values represent means ± standard deviations from four replicates.

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