EFFECT OF A LOCALLY MANUFACTURED TOOTHBRUSHING SIMULATOR ON THE MONOMER ELUTION OF CAD/CAM RESIN COMPOSITE BLOCK - A PILOT STUDY

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Abstract

Objective: This pilot study aimed to calibrate the high-performance liquid chromatography (HPLC) protocol for detection and quantification of monomer that may be eluted from a computer-aided design and computer-aided manufacturing (CAD/CAM) resin composite block (RCB) when subjected to an abrasion test with a toothbrushing simulator. **Materials and methods:** CAD/CAM RCB (Shofu Block HC) were sectioned and randomly allocated into two groups made up of Control group (CT) group (n = 4), and Toothbrush Abrasion (TA) group (n = 4). CT group was subjected to no surface treatment and TA group was subjected to a toothbrushing wear test following immersion of both groups in artificial saliva for 7 days. High Performance Liquid Chromatography (HPLC) was used to separate and identify urethane dimethacrylate (UDMA) and triethylene glycol dimethacrylate (TEGDMA) that were eluted from the slurry produced following the toothbrushing abrasion test of the TA group and from storage solution of artificial saliva of the CT group. **Results:** After the toothbrush wear test, 6.192 to 16.937 ng/µL of UDMA eluted from the TA group and no UDMA was detected in the CT group. TEGDMA was not detected from both the CT and TA groups. **Conclusion and clinical significance:** The HPLC protocol has been calibrated and toothbrushing wear resulted in detectable UDMA released from Shofu HC Block at a level that is not harmful to the patient.

***Keywords*:** Toothbrushing Abrasion, Monomer Elution, UDMA, TEGDMA, Resin Composite Block

Introduction

In dentistry, there has been a substantial surge in the utilization of computer-aided design and manufacturing (CAD/CAM), particularly for the fabrication of indirect dental restorations over the last decades. This is due to the comparableness of the CAD/CAM prostheses with those produced using standard manufacturing processes in terms of high reproducibility of the prostheses, time effectiveness, and lower overall production costs. Furthermore, the advancement in digital technology and the development of new tools have made it possible for current CAD/CAM machines to process a wide range of restorative materials, including porcelain, glass-ceramics, glass infiltrated ceramics, polycrystalline ceramics, resin ceramic, resin composite, and acrylic resin, amongst others (1).

According to Ruse and Sadoun (2), machinable aesthetic dental materials employed in the fabrication of CAD/CAM restorations can be classified into two main categories: glass–ceramics/ceramics or resin-based composites. While both ceramics and glass ceramics are non-metallic materials bonded by ionic and covalent bonds, ceramics are crystalline materials while glass ceramics consist of reinforcing ceramic fillers in an amorphous glass matrix. Meanwhile, resin composite materials consist of a polymeric matrix reinforced by either ceramic, glass ceramic, glass or composite fillers. Glass ceramics and ceramics exhibit robust strength and rigidity attributed to their elevated elastic modulus, coupled with commendable mechanical and aesthetic characteristics (2). Nevertheless, their inherent brittleness renders them more prone to chipping, particularly in the milling process. While the mechanical attributes of resin composites

intended for CAD/CAM application have been thoroughly characterized and significantly improved for the production of indirect restorations such as inlays, onlays, veneers, and crowns, advancements have been achieved through the incorporation of innovative compositional elements. These elements include resin monomers like UDMA, characterized by a higher concentration of double bonds compared to Bis-GMA, as well as initiation systems such as BPO. Additionally, polymerization modes involving high temperature and/or high-pressure polymerization have been employed, all designed to improve the degree of conversion of CAD/CAM composite blocks. Furthermore, an increase in filler loading has been pursued to enhance mechanical strength and wear properties (3-5).

Toothbrushing constitutes a widely recognized preventive strategy against dental and periodontal disease (6). Nevertheless, it can inadvertently result in undesirable outcomes, such as the induction of physical abrasion on both natural dentition and dental restorations. This, in turn, may contribute to increased surface roughness, susceptibility to staining, and the potential for gingival irritation (7). In vitro studies of toothbrushing abrasion on dental material is usually performed using customized tooth brushing simulators (8-14) and commercial automated tooth brushing simulators to simulate manual toothbrushing action (15-19).

Commercial automated toothbrush simulator is well able to replicate the motion sequences (forwards, backwards and zigzag motion, as well as circular) and the contact pressure applied during teeth cleaning. However, its main disadvantage is the relatively high cost compared to a locally customised machine. Customised tooth brushing simulators in published studies include those using commercial electrical toothbrush in a customised toothbrush-holding device (13, 14) and devices with manual toothbrush in holders that replicate horizontal linear motion at a load that ranges from 350 to about 500 gf (8-10). In spite of the extensive body of literature detailing the construction of personalized toothbrushing simulators, there remains a notable absence of locally developed toothbrushing simulators to date.

When subjected to oral fluids, including saliva and various chemical compounds within the oral environment, RCBs can undergo corrosive wear. The situation is worsened by the regular changes in oral temperature. This process can soften the outer layer of RCBs, making the material more prone to additional wear. In the case involving direct composite, potentially harmful components like filler particles, free radicals, photo initiator molecules, and monomers such as low molecular weight like HEMA or TEGDMA, high molecular weight like BisGMA or UDMA may be released, posing biological hazards to the patient (20). Several factors that affect monomer elution from a resin composite include molecule structure, solvent type, degree of monomer conversion, microstructure, and filler composition (21). Studies have investigated the monomer elution of CAD/CAM composite resins blocks in different storage media, including distilled water, 75% ethanol, water and artificial saliva for short-term and long-term storage time (21, 22). However, it remains unclear whether toothbrush wear will result in the leaching of monomers from RCBs, along with its recognized implications for cytotoxicity and potential health hazards.

Evidently, in terms of adverse effects and health hazards of monomer elution from resin-based dental materials, unreacted and leachable monomers have the potential to permeate into the oral cavity and may interact with soft tissues, resulting in three primary outcomes which are local effects, systemic effect, and allergic reaction (23). In local effects, monomers possess the capacity to incite irritation within oral tissues, manifesting symptoms such as burning sensations, erythema, and ulcerations. Although the practical implications of systemic effects of monomer elution are still being investigated, previous studies show that monomer elution can lead to cytotoxic effects and endocrine disruption (24). Allergic reactions to monomers such as dermatitis or respiratory complications can occur in certain individual where TEGDMA impedes the growth of oral epithelial cells, triggers mitochondrial impairment, and exhibits cytotoxicity to pulmonary cells at a rate 2–5 times higher than that of HEMA (25).

Other than these three main effects, adverse reactions of monomer elution can also be seen in progression of secondary caries. Unbound monomers act as substrates for cariogenic bacteria, particularly when compounded with polymerization shrinkage which allows bacteria to penetrate gaps and potentially resulting in secondary caries (26). Concern regarding the effect on dental pulp specifically the toxicity effect of free monomer on dental pulp cells (DPCs) has been raised due to the extensive utilization of resin-based dental materials. Even in the absence of direct contact with the pulp, these monomers engage with dental composites, permeating through dentin and perturbing DPC physiology. Elevated levels of unpolymerized monomers, notably TEGDMA and HEMA, have the potential to impede DPC differentiation and crucial mineralization mechanisms (27). Potential genotoxicity effect can be observed in TEGDMA, UDMA, and Bis-GMA monomers where it displays minor DNA migration enhancement and induces the deletion of significant DNA sequences in mammalian cells (28). Despite all the adverse reactions and health hazards of monomer elution claimed, the current body of evidence regarding the biocompatibility of RCB remains insufficient, posing a challenge for clinicians and consumers alike in making well-informed decisions.

This pilot study aimed to evaluate the probability of monomer elution from RCB when subjected to abrasion test using a locally manufactured toothbrushing simulator and to calibrate the toothbrushing and high-performance liquid chromatography (HPLC) analysis in view of further testing of other RCBs. The null hypothesis is that no detectable monomer (below the limit of detection) will be released from the HC Block when subjected to toothbrushing using the local toothbrushing simulator machine.

Materials And Methods

The composition of the RCB used in this pilot study is given in Table 1.

Table 1: CAD/CAM materials used in this study

Type	Brand	Compositions		Manufacturer
		Monomer	Filler	
CAD/CAM Hybrid Composite	HC Block	UDMA +TEGDMA	61% silica powder, microfumed silica, and zirconium silicate	Shofu Japan

A block of RCB (Shofu HC, SHOFU INC., Kyoto, Japan) size 14 x 12 x 18 mm shade A3 was sectioned into three samples to the dimension of rectangular cross-section 14 x 12 x 5 mm using a diamond saw (IsoMet 4000 Precision Cutter, Buehler, Il., USA). A total of eight samples were prepared and subsequently allocated randomly into two distinct groups, Control group (CT) group, n=4 and Toothbrush Abrasion (TA) group, n=4. All samples were polished with alumina polisher impregnated with 73% by weight with aluminium oxide particles (Al2O3) (Dura-Polish, SHOFU INC., Kyoto, Japan). The polished slabs were subjected to ultrasonic cleaning in distilled water for a period of five minutes, aiming to eliminate any impurities. Following this, both CT and TA samples were immersed in artificial saliva for seven days and only TA samples were subjected to toothbrushing simulation after the immersion.

Tooth brushing simulator machine

The locally customized toothbrushing machine used in this pilot study simulates manual toothbrushing action in a horizontal manner. The machine was constructed in collaboration with the Faculty of Mechanical Engineering, Universiti Tun Hussein Onn (Figure 1). It operates with two independent brushing station and 2 corresponding chambers where the specimens are held for the toothbrushing intervention and collection of the toothpaste slurry. The presence of stroke counter allows for the number of strokes to be recorded and a Force Centre Resistance (FCR) enables the detection of force, while the force applied by the toothbrush head is adjustable by adjusting the tightness of the toothbrush holder.

Toothbrushing protocol

Prior to the test, all TA samples underwent a seven-day conditioning in artificial saliva (29) at (37 ± 1°C) as per ISO technical specifications ISO/TR 14569-1:2007 (30). Subsequently, the specimens underwent rinsing with tap water and subjected to deionized cleaning using an ultrasonic bath containing 1% Sodium Lauryl Sulfate (SDS,

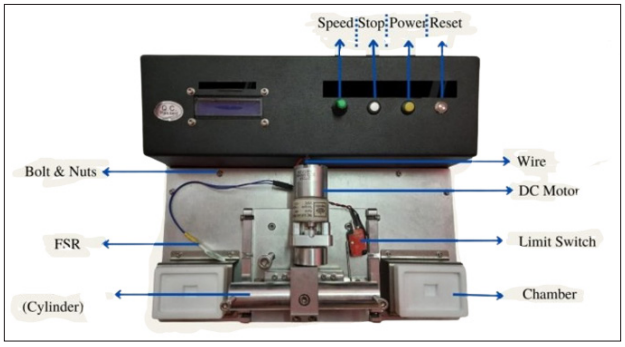

Figure 1: Customized toothbrushing simulator machine

Chemiz, Malaysia) as a detergent. For the toothbrushing wear test, an abrasive slurry was formulated by combining fluoride toothpaste (Colgate Total, Colgate-Palmolive Co., Malaysia) with artificial saliva in a weight ratio of 1:2. The samples underwent brushing using soft nylon-bristled toothbrush heads (Colgate Twister, Colgate-Palmolive Co., Vietnam) with a load of 2.5 N at a rate of 170 cycles per minute, reaching up to 10,000 cycles. It is noteworthy that 10,000 cycles approximate the equivalent of 12 months of typical tooth brushing (31). The toothbrush heads were replaced after every 5,000 cycles which corresponds approximately to a change of the toothbrush head of every six months (32, 33).

Validation of HPLC analysis

Prior to quantification of UDMA (Sigma-Aldrich, Mo, USA) and TEGDMA (Sigma-Aldrich, Mo, USA) using HPLC analysis, it was first validated for the following parameters: linearity, specificity, limit of quantification (LOQ), and limit of detection (LOD) (34). The HPLC analysis was conducted using a HPLC machine (Agilent Infinity Quaternary LC, Agilent Technologies Inc., Ca., USA) equipped with a UV detector and a quaternary pump. Mobile phase was prepared using acetonitrile (Merck, Darmstadt, Germany) and non-ionized water (70:30% v/v 1L). To achieve this, 700 ml of acetonitrile and 300 ml of non-ionized water were measured separately, mixed, and degassed in an ultrasonic vibrator to eliminate any air that could potentially impact the readings.

Specificity

The capacity to differentiate the analytes when coexisting with anticipated components is denoted as specificity. The specificity was assessed by introducing methanol as a blank sample into the HPLC system and to assess the presence of interference between the blank sample and the UDMA and TEGDMA peaks.

Linearity of the calibration curve

The linearity of the calibration curves was established through the analysis of mixed standard solutions containing UDMA and TEGDMA. Standard solutions were prepared using the stock monomer of interest (UDMA, TEGDMA) that were dissolved in methanol at three different

concentrations (80-120% expected content of the analyte) ranging from 3 to 12 ng/µL for UDMA and 0.3 to 1.5 ng/µL for TEGDMA and assessed by HPLC to assess the linearity of the response to allow for quantification of each monomer.

Limit of detection (LOD) and limit of quantification (LOQ)

The lowest analyte concentration in a sample that can be detected by an analysis is known as the LOD, whereas the lowest analyte concentration that can be quantitatively quantified with sufficient accuracy and precision is known as the LOQ. LOD can be expressed as: DL=3.3σ/S while LOQ was expressed as: QL=10σ/S. Where S represents the slope of the calibration curve, and δ was determined based on the residual standard deviation of the calibration curve.

Monomer elution measurement protocol with high-performance liquid chromatography (HPLC)

Following calibration, the quantification of monomer from the extracted solution which included artificial saliva for incubation of CT and toothpaste slurry from TA was carried out. The solution extracted was subjected to filtration using a 0.2 µm polytetrafluoroethylene (PTFE) Agilent Captiva premium syringe filter (Agilent Technologies, California, USA). This precautionary measure was implemented to prevent potential column obstruction or damage. The solutions were injected into 1 ml screw cap vials and entered to the HPLC system. HPLC samples of 10µL were injected into PerfectSil Target ODS-3 (250 mm × 4.6 mm, 5 µm) analytical column (MZ AnalysenTechnik, Mainz, Germany). Chromatographic separation was accomplished utilizing the mobile phase at a flow rate of 5.0 ml/min. The column temperature was maintained at 23°C, and each sample underwent a 23-minute run time. The UV detector settings were configured at 205 and 210nm. Notably, the retention time for UDMA was recorded at 3.39 minutes, while TEGDMA exhibited a retention time of 4.05 minutes.

Statistical analysis

All data were entered into Microsoft Excel version 16 and the assessment of the linearity of the calibration curves for UDMA and TEGDMA was conducted through the application of simple linear regression analysis.

Results

Validation criteria of the HPLC analysis: specificity

The specificity was affirmed through the absence of endogenous interference at retention times proximate to the peaks of interest, as determined by examining chromatograms of the blank sample and a blank sample spiked with standard monomer. In the spiked sample, the retention times for UDMA and TEGDMA were recorded at 3.39 minutes and 4.05 minutes, respectively (Figure 2).

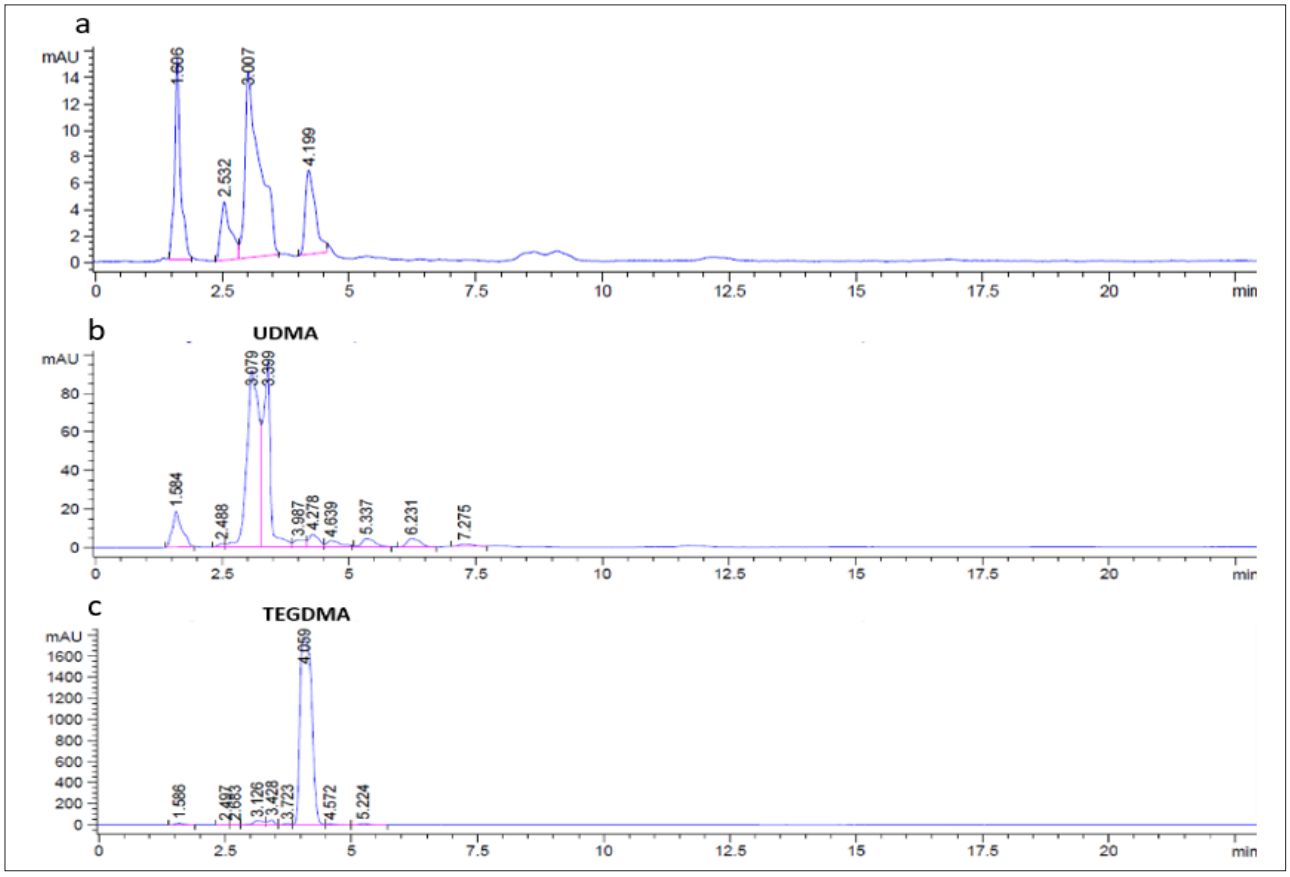

Figure 2: HPLC chromatogram for (a) blank sample: methanol and the standard monomer's peaks (b) UDMA (c) TEGDMA and their retention times, with UDMA at 3.39min, and TEGDMA at 4.05min respectively.

Linearity

Simple linear regression was applied to derive the calibration curves for the monomers UDMA and TEGDMA over the concentration range tested. (Figure 3 and Figure 4). A linear correlation between the peak area and analyte concentrations was established, as affirmed by the correlation coefficient (R^2) values, all of which exceeded 99% (UDMA =0.990 and TEGDMA=0.933). The calibration curve's slope and intercept were computed, yielding the subsequent linear equation: y = 168.46x – 70.28 for UDMA and y = 20424.29x – 504.44 for TEGDMA. Therefore, it can be concluded that the calibration curves for both UDMA and TEGDMA exhibit linearity within the tested concentration range.

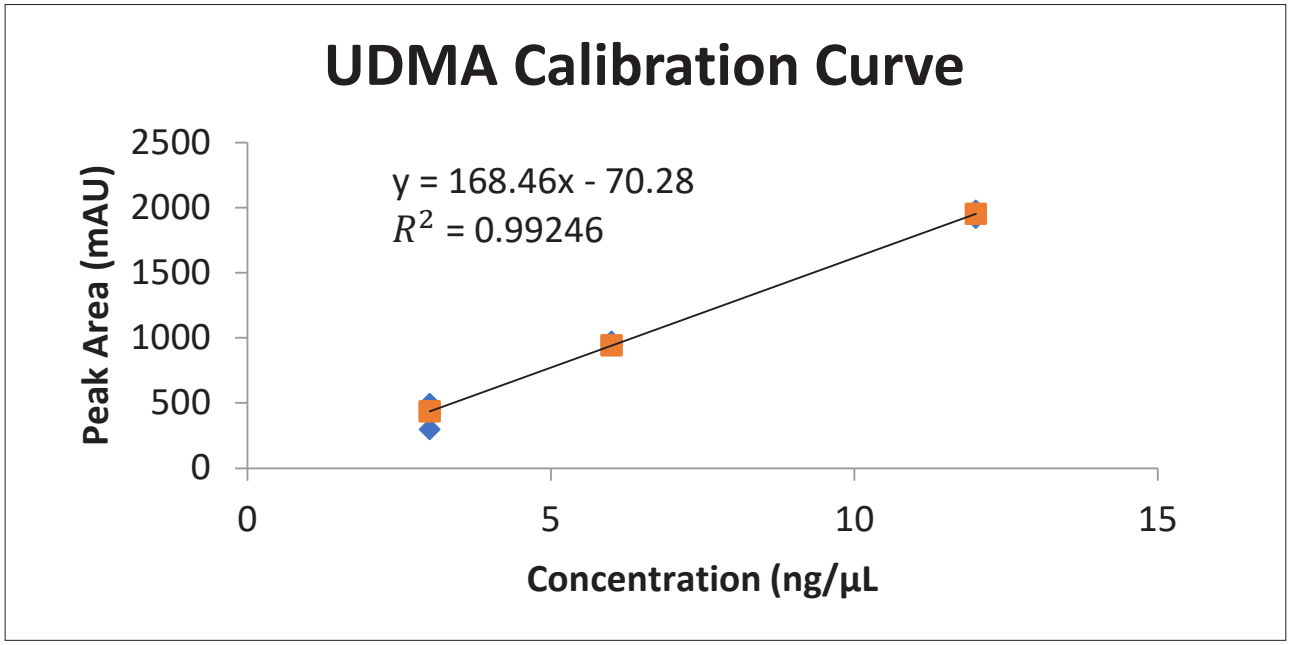

Figure 3: Calibration curve for UDMA whereby data were fitted by linear regression model

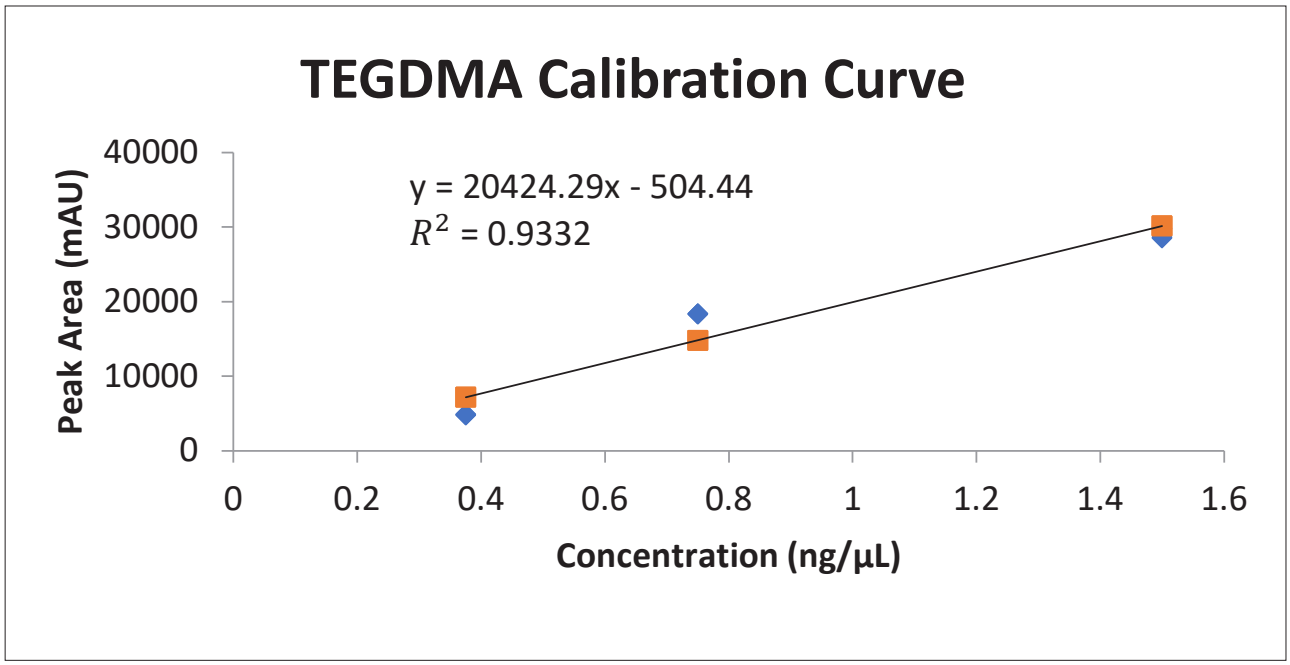

Figure 4: Calibration curve for TEGDMA whereby data were fitted by linear regression model

Limit of detection and limit of quantification

The Limit of Detection (LOD) for UDMA and TEGDMA, as determined from the residual standard deviation of the regression line, were found to be 0.369 ng/mL and 0.709 ng/mL, respectively. Additionally, the Limit of Quantification (LOQ) for UDMA and TEGDMA, derived from the residual standard deviation of a regression line, were determined as 1.68837 µg/mL and 2.147668 µg/mL, respectively.

Analysis of the HPLC chromatograms revealed that Shofu HC block in the Toothbrush Abrasion (TA) group eluted UDMA (Figure 5) in a concentration that ranged between 6.192 ng/µL to 16.937 ng/µL. UDMA was not identified in the CT group, while TEGDMA was absent in both the TA and CT groups. The mean ± standard deviation of each monomer released from the Shofu HC Block that were subjected to the different interventions is shown in Table 2.

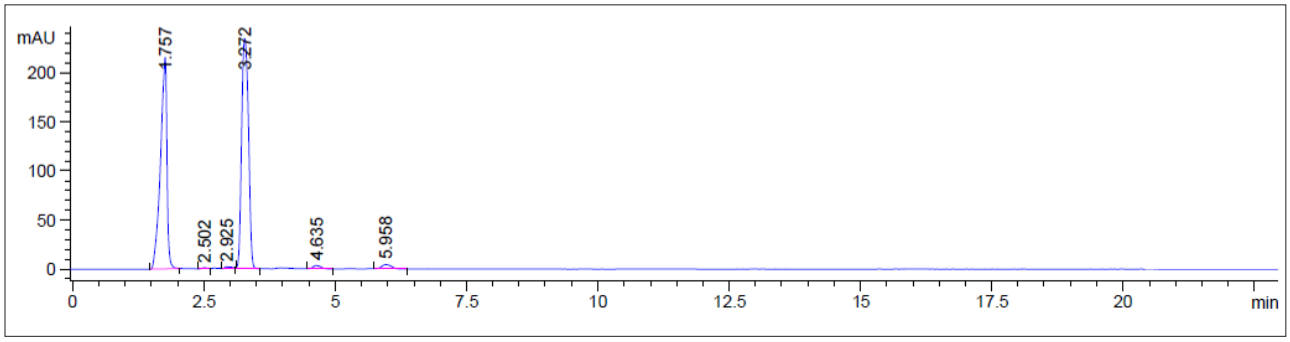

Figure 5: HPLC chromatograph for eluted UDMA detected at 3.27min for TA sample

Table 2: Mean ±standard deviation (ng/µL) of eluted monomers

Group	Sample	Mean concentration (ng/µL) ± SD	
		UDMA	TEGDMA
CT	1	ND	ND
	2	ND	ND
	3	ND	ND
	4	ND	ND
TA	1	8.798 ± 2.153	ND
	2	16.141 ±9.917	ND
	3	15.409 ±6.743	ND
	4	24.996± 1.291	ND

ND: not detected

Discussion

This in vitro pilot study was conducted to investigate the type and quantity of monomer elution from HC Blocks when subjected to an abrasion test using a locally manufactured toothbrushing simulator. Toothbrushing simulators typically consist of essential components, including a toothbrush, slurry, programmed controls for adjusting force, time, temperature, and the number of cycles, as well as a drawer system designed to accommodate both samples and the slurry. An observational study by Wiegand et al. (35) reported that the average toothbrushing force exerted by an individual during manual toothbrushing using a manual toothbrush is approximately 1.6 ± 0.3 N. However, the published technical specification for load for a tooth brushing wear test by International Organization for Standardization (ISO) (30) is between 0.5 N and 2.5 N. Therefore, in the present study, a range between 1.5 N to 2.5 N of force was applied on all samples by adjustment of the toothbrush head that was monitored by the Force Centre Resistance (FCR) of the toothbrushing simulator. According to a review of the existing literature, the predominant toothbrushing method among the majority of individuals in the studied population is the "horizontal scrub method" (36). Hence, in the present study, we decided to simulate this 'horizontal scrub toothbrushing action'. The objective in configuring the toothbrushing simulator setup was to replicate, as closely as possible, an individual's typical toothbrushing style by applying a consistent force value and a horizontal toothbrushing action.

The aim of validating an analytical procedure is to verify its suitability for the intended purpose. In this study, the validation of the HPLC analysis was carried out in accordance with the guidelines set forth by the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) (37). The validation tests determined specificity, and working range limit which included the linear response and lower range limit verification. Within the limits of the tests, specificity and linear response were demonstrated for both TEGDMA and UDMA, indicating the suitability of the HPLC analysis for monomer quantification.

Considering the result of monomer elution from this study, the null hypothesis can be rejected due to the detectable monomer eluted which was mainly UDMA from the TA group when compared to the CT group. No study has evaluated the effect of toothbrushing on monomer release from composite resin or any CAD/CAM block. However, various extraction media had been investigated for the release of residual monomers from resin composite used for direct restorations (38-42). In these studies, there were mainly two types of extraction media used: I) aqueous mixtures or water, which included cell culture media, artificial saliva, human saliva, water-based buffer solutions, or II) various organic extraction media, which included ethanol, methanol, acetone, acetonitrile, tetrahydrofuran, and chloroform. The extraction medium used has an impact on both the concentration of the eluted monomer and the duration of elution (32). The choice of extraction medium depends on the specific research objectives. Following ISO specifications, distilled water is deemed appropriate for resin-based filling materials as it emulates the moist intraoral environment, encompassing both saliva and water. However, as per the finding of Moharamzadeh et al. (34), the release of TEGDMA into various water-based extraction media, including distilled water and those designed to simulate an intraoral environment such as saline solution, artificial saliva, and Dulbecco's Modified Eagle Medium (DMEM) without serum, exhibited no statistically significant differences. Therefore, the usage of artificial saliva as a storage medium in this study instead of distilled water as recommended by ISO is justified. In this study, both monomers UDMA and TEGDMA were not detected in CT group that were stored in artificial saliva for 1 week. This might be due to the very low concentration level of these monomers following the short storage time that cannot be detected by HPLC. According to Mourouzis et al. (22), a distinction exists in the leaching pattern of monomers from CAD/CAM materials as compared to conventional resin composites. Notably, the leaching of monomers in CAD/CAM materials diminishes over time. The difference is attributed to the fact that the CAD/CAM materials have already been pre-polymerized into ready-to-mill blocks and thus have better chemical properties. The same study proved that the highest quantity of monomer was released into the 100% ethanol, whereas the least amount was released into distilled water, and the difference was highly significant. Therefore, the type of extraction medium too may have contributed to the reason why both monomers were not detected in the CT group following 1 week of storage.

In the present study, TEGDMA was not detected from the samples when subjected to an abrasion test using the locally manufactured toothbrushing simulator. This result is a unique observation that contrasted with other studies which have found that TEGDMA is the major monomer eluted from resin composite (21, 41, 43, 44). According to Tanaka et al. (45) small molecular weight monomers could be extracted in a significantly greater amount than large molecular weight monomers. Smaller molecules, such as TEGDMA, have higher mobility and will be eluted faster than larger molecules, such as BisGMA and UDMA. These differences might be due to the different intervention and storage solutions compared to the present study, where the samples were subjected to wear test using the toothbrushing simulator and were stored in artificial saliva. In addition to this, UDMA serves as the primary monomer utilized in composite CAD/CAM blocks (21). However, further investigation is needed to confirm the reason behind this result.

The monomers leached from resin-based materials have the potential to trigger a wide range of unwanted side effects. Consequently, their hazardous effects and mechanisms need to be clarified for safety of usage. Study by Geurtsen et al. (46) in 1998, investigated the cytotoxic effects, indicated by the effective dose for 50% cell death (ED50 concentrations), of 35 monomers or additives found in commercially available dental composite resins were assessed using monolayers of permanent 3T3 cells and three primary human fibroblast types derived from oral tissues, specifically gingiva, pulp, and periodontal ligament, as the test system (39). The result of this study showed that ED50 values of UDMA ranged 0.06–0.47 mM and TEGDMA was 0.12–0.26 mM. With these values, they also reported that the base monomer UDMA is highly toxic in which persistent toxicity by the monomer could slow down cellular metabolism and increase susceptibility to harm from other system. However, Chang et al. (40) investigated the effects of UDMA on the growth, cell cycle progression, reactive oxygen species (ROS) production and glutathione (GSH) alteration in CHO-K1 cells revealed that UDMA exhibits a toxicity level that is marginally lower than that of BisGMA, yet surpasses the toxicity of both TEGDMA and HEMA. Notably, the cytotoxic effects and growth inhibition induced by UDMA are observed to be associated with cell cycle arrest, necrosis, and apoptosis. In the present study, it was found that the monomer levels found in Shofu HC Block when it is converted from ng/µL to mM ranged between 3.60 x 10 -5 mM and is below the ED50 cytotoxicity levels when compared to the result by Geurtsen et al. (46).

Conclusion

It can be concluded from this study that the toothbrushing wear resulted in release of detectable amount of UDMA monomer. However, the amount is well below the published cytotoxic level for UDMA.

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Competing interests

The authors declare that they have no competing interests.

Ethical Clearance

This research does not include any human and animal subject or product and has been exempted from ethics review by the Research Committee of Faculty of Dentistry, Universiti Teknologi MARA (UiTM).

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