Heliyon 10 (2024) e24369

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Effects of three disinfection solutions on residual monomers released from resin nanoceramic CAD/CAM blocks

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A R T I C L E I N F O

Keywords:
Residual monomer
HPLC
Resin nanoceramic CAD/CAM block

A B S T R A C T

The aim of this study was to evaluate the effects of three disinfection solutions on the amount of monomers released from resin nanoceramic CAD/CAM blocks using high performance liquid chromatography (HPLC). Forty resin nanoceramic CAD/CAM (Cerasmart, GC, Japan) samples (12x14 × 2 mm) were divided into four groups; each group was disinfected using one of four solutions (Group 1: no disinfectant; Group 2: 70 % ethanol; Group 3: 2 % glutaraldehyde; and Group 4: 1 % sodium hypochlorite) for 5 min. Analysis of residual monomers (UDMA and Bis-EMA) amounts was performed using an HPLC instrument (Dionex Ultimate 3000, Thermo Fisher Scientific). After 30 days, the amounts of monomers found were as follows: 14.54 ppm for Group 1; 9.28 ppm for Group 2; 10.60 ppm for Group 3; and 2.76 ppm for Group 4 (the smallest monomer amount) ($p < 0.001$). Disinfection of indirect restorations prior to cementation can reduce the amount of residual monomers remaining from resin nanoceramic CAD/CAM blocks.

1. Introduction

Computer-aided design and computer-aided manufacturing technology (CAD/CAM) is commonly used in restorative dentistry. CAD/CAM technology provides dental professionals the option to produce aesthetically pleasing restorations with excellent physical properties in a single appointment [1,2].

There are different CAD/CAM blocks with different formulation. Resin composites and glass-matrix ceramics are frequently used in CAD/CAM restorations due to their enhanced physical and optical properties [3,4]. Resin composite CAD/CAM blocks are less costly and easier to repair than ceramic-based blocks; therefore, they have become increasingly popular in dentistry [5]. However, resin composite CAD/CAM blocks have some disadvantages.

Resin composite CAD/CAM blocks can release monomers (such as urethane dimethacrylate (UDMA), bisphenol A-glycidyl dimethacrylate (Bis-GMA), triethylene glycol dimethacrylate (TEGDMA) and bisphenol A ethoxylate dimethacrylate (Bis-EMA)) into the oral environment [6–8]. Harmful residual monomers can be absorbed by the oral environment and easily diffused by body fluids such as blood and saliva. Different studies have shown that the release of residual monomers can have toxic effects on the human body [7,9,10]. This toxicity depends on the chemical composition and amount of residual monomers.

The American Dental Association and Centers for Disease Control and Prevention guidelines recommend that dentures and indirect dental restorations should be disinfected before they are sent to the laboratory and delivered to patients [11,12]. Glutaraldehyde and sodium hypochlorite are among the most preferred solutions for dental prostheses disinfection [13–16]. Infection control is an

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https://doi.org/10.1016/j.heliyon.2024.e24369
Received 7 August 2023; Received in revised form 5 January 2024; Accepted 8 January 2024
Available online 19 January 2024

important aspect of contemporary clinical dentistry, as saliva can be contaminated with oral commensals and opportunistic pathogens, and can harbor certain pathogens (including SARS-CoV-2) during infection and in a carrier state. Given the nature of clinical dentistry, exposure to blood and saliva aerosols is inevitable. Direct contact with fluid-contaminated environmental surfaces, tools, and equipment is also a predictable source of pathogen contamination [17].

Although it is known that the residual unreacted monomers acts as a plasticizer and reduces the mechanical properties of the resin composite materials [18], which disinfection protocol provides optimal mechanical properties for CAD-CAM materials is unclear [19].

The aim of this study was to evaluate the effect of three disinfection solutions on the amount of monomers released from resin nanoceramic CAD/CAM blocks using high performance liquid chromatography (HPLC). The null hypothesis, which states that different disinfection solutions have no effects on the amount of monomer released from resin nanoceramic CAD/CAM blocks, was investigated.

2. Materials and methods

Information regarding nanoceramic CAD/CAM blocks and disinfection solutions used in this study are listed in Table 1.

Resin nanoceramic CAD/CAM blocks were vertically sectioned along the longitudinal axis using a 0.3 mm diamond-coated, low-speed precision cutting blade (Microtome, Mecatome T180, Presi SA, Angonnes, France) under copious water cooling. The final samples' thickness (2.00 ± 0.01 mm) and dimensions (12 × 14 mm) were measured using a digital micrometer (Carbon Fiber Composites Digital Caliper, Zhejiang Precision Instrument Co., Zhejiang, China). Forty samples were prepared using resin nanoceramic CAD/CAM blocks, and cleaned in an ultrasonic bath (Digital Ultrasonic Cleaner, Chalk, China) for 5 min using distilled water. Samples were then divided into four groups and immersed in disinfection solutions for 5 min, as follows:

Group 1 (G1): no disinfection (n:10)
Group 2 (G2): 70 % ethanol, 5 min (n:10)
Group 3 (G3): 2 % glutaraldehyde, 5 min (n:10)
Group 4 (G4): 1 % sodium hypochlorite, 5 min (n:10)

Next, each specimen was immediately immersed in 5 ml of 75 % ethanol/water solution in a glass vial, and stored in a general-purpose incubator (Memmert UN110, Schwabach, Germany) at 37 °C for 30 days. After 30 days, extraction solutions were filtered and transferred to 2 ml amber-colored glass vials (Allpro, BMS Kimya, Turkey). Solutions were analyzed using an HPLC instrument (Dionex Ultimate 3000, Thermo Fisher Scientific) equipped with a reverse-phase 250 mm × 4.6 mm Thermo ODS Hypersil C18 column and 5 μm particle size. (Inc, Sunnyvale, CA, USA). An isocratic method consisting of acetonitrile and water (80:20) and a flow rate of 1 ml/min was used, and UV detection was set to 205 nm. The column temperature was 37 °C, with a run time of 30 min for each sample. Residual monomers in solutions were identified by comparing retention times with those of reference standards under the same HPLC conditions. UDMA and Bis-EMA standard solutions (Sigma Aldrich, St. Louis, MO, USA) had retention times of 6.40 and 25.79 min, respectively (Fig. 1).

Statistical analysis was performed using Statistical Package Software (version 26.0, SPSS Inc., Chicago, IL, USA). Data normality was checked using the Shapiro–Wilk test, and continuous variables were expressed as mean values (minimum–maximum). One-way analysis of variance (ANOVA) was used to determine whether there was a significant difference between disinfection solutions in terms of the amount of monomers released from resin nanoceramic CAD/CAM blocks. $P < 0.05$ was considered statistically significant. The Games–Howell test was used for *Post hoc* analysis because of Levene's test showed significant differences in the variance of the groups.

3. Results and discussion

The effects of different disinfectants on monomers released from resin nanoceramic CAD/CAM blocks were statistically significant ($P < 0.001$). The mean (+standard deviation) amounts of UDMA and Bis-EMA (ppm) released from resin CAD/CAM blocks at day 30 are shown in Table 2.

UDMA monomer release was higher than Bis-EMA monomer release in all groups. Group 1 (control group) released the highest amounts of Bis-EMA, UDMA, and total monomers; Group 4 (disinfected using NaOCl) released the fewest monomers.

Group 4 (disinfected using NaOCl) released fewer monomers than groups 1, 2, and 3 did, which was statistically significant ($p < 0.05$). Group 2's (disinfected using ethanol) decrease in monomer elution was not statistically significant compared to Group 3's (disinfected using glutaraldehyde) ($p > 0.05$) (Table 2).

The dental literature contains few studies that used resin nanoceramic CAD/CAM blocks for monomer elution [6,20], although there are many studies regarding monomer elution from conventional composite materials [18,21,22]. To the best of our knowledge, this is the first study of the effects of disinfection solutions on the amounts of monomers released from resin nanoceramic CAD/CAM

Table 1
Resin nanoceramic CAD/CAM block and disinfection solutions used in this study.

Material	Classification	Composition	Manufacturer
Cerasmart	Resin Nanoceramic CAD/CAM block	UDMA, Bis-EMA	GC, Tokyo, Japan
70 % ethanol solution	Disinfectant	CH_3CH_2OH	Derhand Plus, Deren İlaç, Turkey
2 % glutaraldehyde	Disinfectant	OHC(CH2)3CHO	ACTOSED® Forte Ready, ActoPharma, Turkey
1 % sodium hypochlorite	Disinfectant	5.25 % NaOCl	Wisard, Rehber Kimya, Turkey

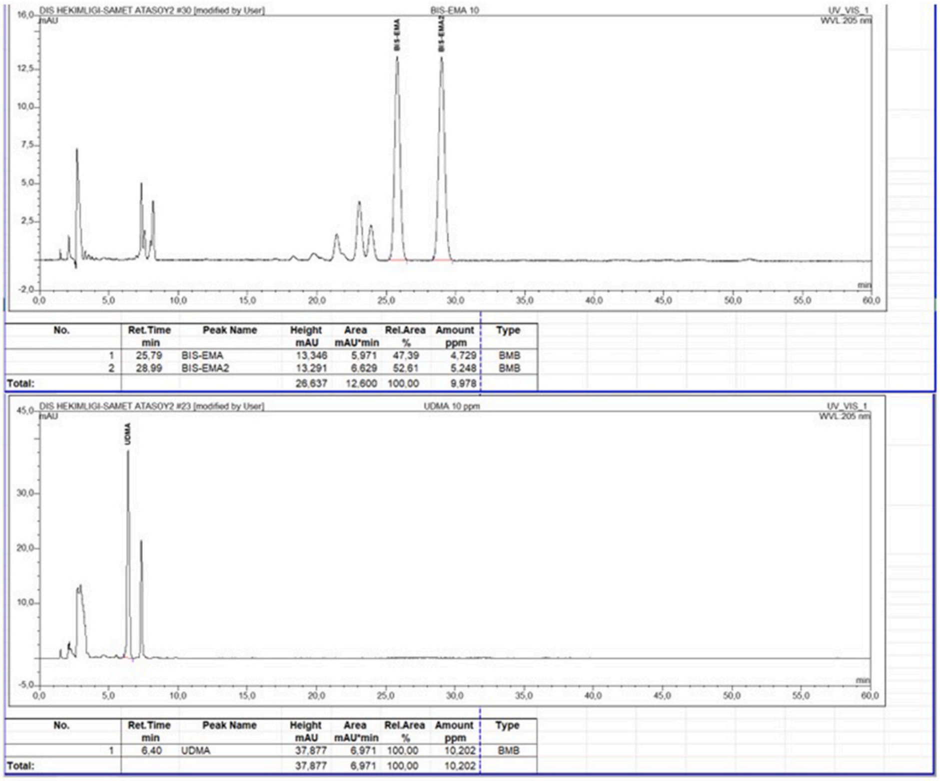

Fig. 1. Monomers' (UDMA and Bis-EMA) peaks and retention times.

blocks.

This study found significant differences in monomer amounts released from resin nanoceramic CAD/CAM blocks immersed in three disinfection solutions and a control solution. Thus, the null hypothesis was rejected. Monomer elution in Group 1 (control, without disinfectant) was higher than in the other groups. Group 4 (1 % NaOCl) showed the lowest monomer elution among all groups.

CAD/CAM materials release less residual monomers than conventional resin composites. There were differences in the amounts of monomers released from resin nanoceramic CAD/CAM blocks in previous studies that investigated monomer elution. Alamush et al. showed that 7.3 ppm of UDMA eluted from a resin nanoceramic CAD/CAM block after 1 month [23]. Mourosis et al. reported that 0.029 ng/mL of UDMA and 0.006 ng/mL of Bis-EMA eluted from resin nanoceramic CAD/CAM blocks after 30 days [24]; Barutçugil et al. reported that 6.20 μg/ml of UDMA and 6.98 μg/ml of Bis-EMA eluted after 3 months [6]. The amounts of monomers eluted in this study were different from those eluted in previous studies. In this study, Group 1, which was not immersed in a disinfection solution, eluted 8.00 ppm of UDMA and 6.54 ppm of Bis-EMA. The degree and rate of elution of monomers from resin composites depends on several factors, including the monomers' conversion degree (DC), the extraction solvent's composition and solubility properties, and the samples' dimensions and chemical properties [25]. Differences between eluted monomers amounts in previous studies may have depended on the dimensions of the CAD/CAM block samples used. This study used 12x10 × 2 mm CAD/CAM block samples.

Ethanol, sodium hypochlorite, isopropyl alcohol, phenol, glutaraldehyde, chlorhexidine, and peracetic acid effectively reduce the risk of cross infection by eliminating pathogens. Although some studies show that they do not affect resins' physical properties [16], it is known that most chemical disinfection solutions have negative effects on resins' microhardness and surface roughness. The decrease in microhardness and the increase in surface roughness depend on the disinfection solution's chemical composition and immersion duration [26].

The reduction in resins' microhardness was partially attributed to the amount of residual monomer released from the resin [27]. Residual monomer may adversely affect resins' physical properties through a plasticizing effect [28]. These results are supported by previous studies, which reported that using both chlorhexidine and 1 % sodium hypochlorite slightly reduced acrylic resins' surface hardness [29]. Polymer exposure to a solution results in hydrolytic degradation arising from the chemical interaction between the solution and the organic matrix in the free spaces between the chains in the polymer system [30,31]. Additionally, the active agents could result in accelerated chemical degradation [15].

In the present study, HPLC was used to detect amounts of monomers released from resin nanoceramic CAD/CAM blocks. The complexity of the oral environment, the saliva buffering system, and saliva flow could potentially influence dental restoration materials' chemical properties. These factors were not included in this in vitro study [20]. The amounts of residual monomers obtained from Group 1 (no disinfection) were higher than those obtained from groups 2, 3, and 4. This reduction in the amounts of monomers

Table 2
Mean amounts (ppm) released from resin nanoceramic CAD/CAM blocks and *Post hoc* analysis.

					One Way ANOVA	The Games–Howell test for Post hoc analysis					
	Group1	Group 2	Group 3	Group 4	$P < 0.001$	Group1-2	Group1-3	Group1-4	Group2-3	Group2-4	Group3-4
UDMA	$8{,}00 \pm 3{,}67^{A}$	$5{,}29 \pm 2{,}53^{B}$	$6{,}19 \pm 1{,}17^{B}$	$1{,}90 \pm 1{,}40^{C}$		0,001	0,001	0,001	0.100	0.001	0,001
Bis-EMA	$6{,}54 \pm 2{,}26^{A}$	$3{,}99 \pm 2{,}14^{B}$	$4{,}41 \pm 0{,}23^{B}$	$0{,}80 \pm 0{,}78^{C}$		0,001	0,001	0,001	0,664	0,001	0,001
TOTAL	$14{,}54 \pm 5{,}85^{A}$	$9{,}28 \pm 4{,}66^{B}$	$10{,}60 \pm 1{,}29^{B}$	$2{,}76 \pm 2{,}01^{C}$		0,001	0,001	0,001	0.302	0.001	0,001

Group 1: control; Group 2: 70 % ethanol, 5 min; Group 3: 2 % glutaraldehyde, 5 min; Group 4: 1 % NaOCl, 5 min; and *: at $p < 0.001$.
For each monomer within a row, groups with different uppercase letter are significantly different (Games-Howell test, $p < 0.05$).

released from groups 2, 3, and 4 may be due to the amount of monomer released into the disinfectant solutions during the disinfection process before HPLC residual monomer analysis.

4. Conclusion

Within the limits of this study, it was found that.

- The disinfection of indirect restorations prior to cementation could reduce the amount of residual monomer remaining from resin nanoceramic CAD/CAM blocks.
- After 30 days, disinfection using 1 % NaOCl reduced the monomers remaining from resin nanoceramic CAD/CAM blocks to a greater extent than the other disinfection solutions studied.

Data availability

Data will be made available on request.

CRediT authorship contribution statement

Serdar Akarsu: Writing – review & editing, Supervision, Methodology, Data curation, Conceptualization. **Samet Atasoy:** Methodology, Investigation, Funding acquisition, Data curation. **Merve Arıkan:** Writing – review & editing. **Bengisu Koca:** Writing – review & editing. **Sena Nur Yiğin:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by the Scientific Research Projects Department of Ordu University (A-2135).

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