Immersion in Ultrasonic Cleaner at Various Frequencies Reduces Residual Monomer in Acrylic Temporary Restoration

การแช่ในเครื่องล้างอัลตราโซนิกส์ที่ความถี่ต่างๆ ลดมอนอเมอร์ตกค้างในวัสดุบูรณะฟันชั่วคราวอะคริลิก

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ABSTRACT

The study aimed to compare the amount of residual methyl methacrylate monomer in auto-polymerized acrylic resin after immersion in ultrasonic cleaner set at different frequencies, immersion in 50^{o}C water and non-treated resin. Twenty-four disc shaped specimens were randomly divided into four groups: Control (C), immersion in 50^{o}C water for 1 hour (PC), and immersion in 50^{o}C water in ultrasonic cleaners at 28 kHz (F1) or 40 kHz (F2) for 5 minutes. High-performance liquid chromatography was used to determine the amount of residual monomer. The data were analyzed with one-way ANOVA and Tukey's HSD. The results showed that the amount of residual monomer in group C was significantly higher than the other groups and there was no significant different in group PC, F1, and F2 at a 95% confidence level.

บทคัดย่อ

การศึกษานี้มีวัตถุประสงค์เพื่อเปรียบเทียบระดับของเมทิลเมทาคริเลตมอนอเมอร์ที่ตกค้างในอะคริลิกเรซินชนิดบ่มด้วยตัวเองที่ทำการลดมอนอเมอร์ด้วยวิธีการแช่ในเครื่องล้างอัลตราโซนิกส์ที่ความถี่ต่างๆ แช่ในอ่างน้ำที่อุณหภูมิ 50 องศาเซลเซียส และกลุ่มควบคุม โดยทำการเตรียมชิ้นงานรูปทรงแผ่นจานจำนวน 24 ชิ้น แบ่งเป็น 4 กลุ่มโดยสุ่ม ดังนี้ กลุ่มควบคุม (C) กลุ่มที่แช่ในน้ำอุณหภูมิ 50 องศาเซลเซียส เป็นเวลา 1 ชั่วโมง (PC) กลุ่มที่แช่ในเครื่องล้างอัลตราโซนิกส์ความถี่ 28 กิโลเฮิรตซ์ (F1) หรือ 40 กิโลเฮิรตซ์ (F2) เป็นเวลา 5 นาที หลังจากนั้นทำการตรวจหาปริมาณมอนอเมอร์ด้วยวิธีโครมาโทกราฟีของเหลวสมรรถนะสูง นำข้อมูลที่ได้มาคำนวณทางสถิติด้วยการวิเคราะห์ความแปรปรวนทางเดียว และ Tukey's HSD ผลการศึกษาแสดงให้เห็นว่าในกลุ่ม C มีปริมาณมอนอเมอร์สูงที่สุด และพบความแตกต่างอย่างไม่มีนัยสำคัญระหว่างกลุ่ม PC F1 และ F2 ที่ระดับความเชื่อมั่นร้อยละ 95

Key Words: Residual monomer, Ultrasonic cleaner, HPLC

คำสำคัญ: มอนอเมอร์ตกค้าง เครื่องล้างอัลตราโซนิกส์ โครมาโตกราฟีของเหลวสมรรถนะสูง

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Introduction

Temporary (provisional) restoration is an important procedure in fixed prosthodontics. After tooth preparation, a temporary restoration is used to provide immediate coverage to protect the pulp from irritation from thermal and chemical substances, keep the tooth in position, and maintain occlusal function. Furthermore, a temporary crown provides esthetics for the patient before the definitive crown is delivered (Shillingburg et al., 1997). The temporary or provisional crown is usually made from auto-polymerized poly-methyl methacrylate (PMMA) based acrylic resin, which is similar to acrylic denture base. However, one major problem of acrylic resin is “residual monomer”, i.e. unpolymerized monomer remaining in the acrylic resin after complete resin polymerization.

The amount of residual monomer is influenced by several factors, such as curing temperature, curing time, and powder to liquid ratio (Vallittu et al., 1998; Lamb., 1983). Several studies have reported cytotoxicity from the residual monomer that eluted from the acrylic resin and caused irritation of the oral mucosa and hypersensitivity (Weaver et al., 1980; Barclay et al., 1999; Strain et al., 1967). Other studies have shown that methyl methacrylate (MMA) monomer may produce formaldehyde by oxidization that is cytotoxic at a lower concentration than MMA (Tsuchiya et al., 1993; Tsuchiya et al., 1994). In addition, residual monomer affects water sorption into the acrylic resin and acts as a plasticizer. This affects the physical and mechanical properties of acrylic resin such as transverse strength, resistance to plastic deformation, and color stability (Dixon et al., 1991; Takakhashi et al., 1998; Arab et al., 2002).

Due to the many adverse effects of residual monomer, several methods have been proposed to minimize its presence in acrylic resin. These methods can be classified into two techniques. The first technique is post-polymerization treatment of the acrylic resin, such as using high watt microwave polymerization (Azzarri et al., 2003; Choukse et al., 2011), curing under higher temperature and pressure for a longer time (Lee et al., 2002), and secondary polymerization by boiling polymerized denture base in 100^{o}C water for 2 hours (Shim et al., 1999). The second technique is increasing the elution of the residual monomer from acrylic resin, such as by immersion in 55^{o}C water for 1 hour (Urban et al., 2007) or in 37^{o}C water for 24 hours (Vallittu et al., 1995). Although many methods have been recommended, the most frequently used method is immersion in 50^{o}C water for 1 hour, as recommended by Tsuchiya et al, (1994). However, these methods are not practical in clinical situations because they require extensive chair time.

Ultrasonic waves are a specific type of acoustic wave with frequencies above 20 kHz, which are above the hearing range of the average person. Ultrasonic waves have been used in diverse fields such as sonochemistry, underwater acoustics (SONAR), medical imaging (Ultrasound), and industrial cleaning (Ensminger et al., 2011). The operating frequency of an ultrasonic transducer has an effect on the amount of bubbles and implosion bubbles. Lower frequencies generate fewer bubbles but larger implosion bubbles that release more energy. In contrast, higher frequencies generate more bubbles but smaller implosive bubbles. A higher frequency may have less cleaning ability but results in greater fluid movement. In industrial applications, a single-frequency ultrasonic cleaner usually uses a 40 kHz ultrasonic transducer.

Ultrasonic cleaners have been proposed to increase the extraction rate of organic substances by the effect of cavitation, the formation and collapsing of microscopic vacuum bubbles. Demaggio et al. (1964) found that ultrasonic treatment was more effective in extracting alkaloid from *Datura stramonium* than conventional procedures. Wang (2006) recommended the use of ultrasonic treatment to enhance protein extraction from autoclaved soybean flakes. Ultrasonic treatment can also promote chemical reactions such as ultrasonic polymerization (Kuijpers, 2004). However, investigation into the effects of ultrasonic cleaners with different frequencies on the amount of residual monomer in acrylic resin has not yet been performed.

Objective of the study

The purpose of this study was to determine the effects of ultrasonic cleaners with different frequencies on the amount of residual methyl methacrylate monomer in auto-polymerized acrylic resin.

Methodology

Sample preparation

Twenty-four disc shaped auto-polymerized acrylic resin (Unifast Trad Ivory, GC, Japan) specimens were prepared and randomly divided into four experimental groups. The specimens were prepared by mixing powder and liquid according to the manufacturer's instruction (Powder to liquid ratio was 2.0 mg to 1 mL). When the acrylic resin was at the dough stage, it was packed into circular stainless steel investing molds (diameter 50 mm and depth 3.0 ± 0.1 mm). The molds were then placed in dental stone in dental flasks, which were pressed with hydraulic flask pressure at 3 bars. After processing, the specimens were kept in the dark for 24 ± 5 h prior to grinding.

P500 metallographic grinding paper (TOA, Thailand) was used to wet-grind both sides of the specimens to a thickness of 2.0 ± 0.1 mm and then polished with P1200 paper on the edge until smooth. The specimens were stored at -28^{o}C to prevent residual monomer evaporation. The specimens were divided in four groups (n=6) as follows: Group 1 was left untreated as the control group (C), Group 2 was immersed in 50^{o}C water for 1 hour as the positive control group (PC), Group 3 was immersed in 50^{o}C water with 28 kHz ultrasonic treatment for 5 minutes (F1), and Group 4 was immersed in 50^{o}C water with 40 kHz ultrasonic treatment for 5 minutes (F2). Subsequently, all specimens were stored in the dark for 24 ± 1 h prior to the monomer extraction procedure following ISO 20795-1 (2013).

Residual monomer extraction procedure

Each specimen disc was broken into small pieces, and pieces totaling approximately 650 mg were weighed to four decimal places using a digital scale (Sartorius BP110s, Sartorius, Germany), and were placed into a 10 mL volumetric flask (Duran, Germany). In each group, three specimen discs were divided into three sample solutions for the pass/fail determination test for residual monomer and three were used in one sample solution each. Tetrahydrofuran diluting solution (Merck Co., Germany) was then added to a 10 mL final volume. Each flask was stirred using a clean 3 mm polytetrafluoroethylene coated magnetic stirring bar (Cowie Technology, UK) on a magnetic stirrer (PMC 509C, Barnstead, USA) for 72 ± 2 hours at room temperature. 2 mL of the resultant slurry was then transferred to another 10 mL volumetric flask with a micropipette. Methanol diluting solution (RCI Labscan, Thailand) was added to a 10 mL final volume and the solution was shaken to cause resin precipitation. 5 mL of the solution from each flask was transferred to glass centrifugation tubes. Each solution was then centrifuged at 3000 rpm for 15 minutes at 25°C (Avanti J-E, Beckman Coulter, USA). 1 µL of the supernatant of each sample analyzed by a HPLC system (Shimadzu 20A Prominence HPLC, Shimadzu Corporation, Japan) using a reverse-phase LC-18 (5 µm particle diameter, 4.6 cm internal diameter x 150 mm length) analytical column maintained at 40°C with a 66% methanol and 34% water isocratic elution. The flow rate was 1.5 mL/min and the UV wavelength was detected at 205 nm.

Residual monomer determination

The amount of MMA was determined from a standard calibration curve ($R^2 > 0.99$) (Fig. 2) that was prepared by plotting the peaks of known amounts of MMA (Approximately 6 mg, 60 mg, 150 mg, 300 mg, and 400 mg).

The standard curve was used to determine the concentration in micrograms of MMA, c_{MMA}, per milliliter of analyzed sample solution. The total quantity of MMA in the sample solution, m_{MMA}, in micrograms, was calculated according to the following equation:

$$m_{MMA} = \left[c_{MMA}\ x \left(\frac{10}{2}\right)^{a)} x\ 10^{b)} \right]$$

The residual monomer (%wt) was calculated using the following equation:

$$Residual\ monomer\ (\%wt) = \frac{m_{MMA}}{c_{MMA}}\ x\ 100$$

The three specimens that were divided into three solutions (nine solutions in total) were tested for pass/fail determination of residual monomer. The three solutions per sample were averaged to generate the representative value of each specimen. Therefore, six values were obtained for each experimental group (Fig. 1). The data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD test at a 95% confidence level.

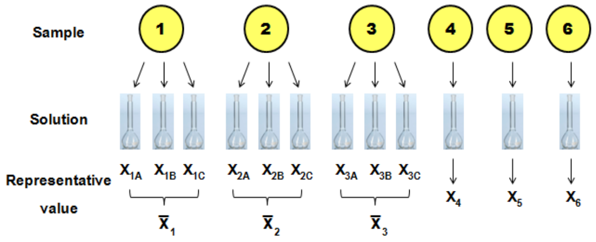

Figure 1 Solution preparation in each group.

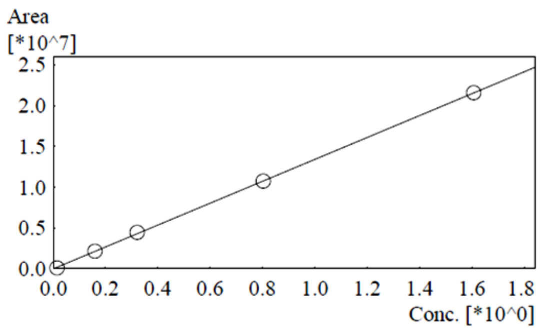

Figure 2 MMA Standard calibration graph

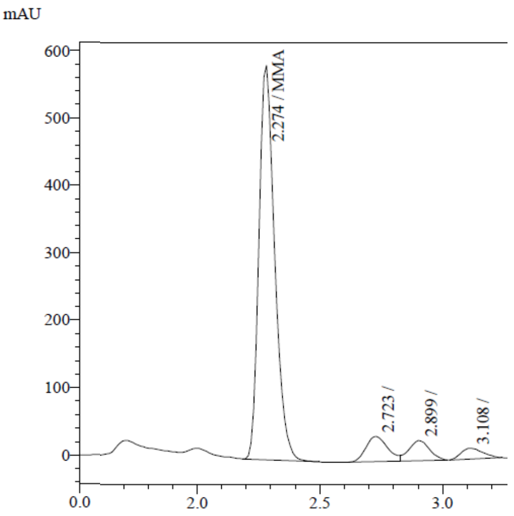

Figure 3 Example of an MMA HPLC chromatogram

Results

The standard calibration curve was calculated, which had the following equation ($R^2 > 0.995$):

$$f(x) = 1.38539e + 007*x - 372563$$

The %wt of residual monomer in each acrylic resin sample was calculated from the peak area of MMA observed on the chromatograms using the standard curve equation. Table 1 shows the mean amount and standard deviation of residual MMA monomer in each group. The control group had a significantly higher amount of residual monomer than the other groups ($p < 0.05$) but there were no significant differences between the amount of residual monomer in groups PC, F1, and F2 ($p > 0.05$).

The three specimens that were divided into nine solutions were tested for pass/fail determination of residual monomer per ISO 20795-1, which states that the upper limit of residual monomer is 4.5 %wt. The results indicated that all groups passed this requirement.

Table 1 The mean and standard deviation of residual monomer

	Residual monomer (% wt)	
Group	Mean	SD
C	3.271^{A}	0.090
PC	2.032^{B}	0.143
F1	2.013^{B}	0.077
F2	2.118^{B}	0.041

* The means with identical letters were not significantly different ($P>0.05$)

Discussion and Conclusions

In the present study, we investigated the effects of ultrasonic treatment on the amount of residual monomer in acrylic resin. Residual monomer has been demonstrated to cause hypersensitivity of the oral mucosa, altered acrylic resin color stability, and decreased acrylic resin mechanical strength (Jagger, 1978; Craig, 2002). We found that the amount of residual monomer in all the groups was lower than the upper limit of residual MMA for auto-polymerized acrylic resin as recommended by ISO 20795-1.

There are two methods for residual monomer determination that are suggested by ISO, Gas chromatography (GC) and High-performance liquid chromatography (HPLC). In the present study, the HPLC method was chosen because of its advantages over GC analysis. Philips et al. (1996) compared the use of GC-MS and HPLC for urinalysis to determine the amount of cocaine present. Their results showed that HPLC was more sensitive and precise than GC-MS in quantitative analysis and HPLC required fewer sample pretreatments. In contrast, Wahlen (2002) showed that GC-ICP-MS was superior in terms of sensitivity than HPLC-ICP-MS. However, HPLC analysis is less expensive and can analyze large sample batches more rapidly.

Previous investigations into the amount of residual monomer focused on the elution of monomer from acrylic resin into the environment, saliva or water, and how much monomer remained in the acrylic resin (Baker et al., 1988; Vallittu et al., 1995;Shim et al.,1999; Lee et al., 2002;). However, in the present study, we analyzed the amount of remaining residual monomer in acrylic resin following the method of residual monomer determination in ISO. We determined that the control group (C) had the highest mean amount of residual monomer. The positive control group (PC), immersion in 50^{o}C water for 1 hour, had a significantly lower mean amount of residual monomer than the control group. These results are in agreement with the recommendation of Tsuchiya (1994), who suggested the immersion of acrylic resin dentures in 50^{o}C water before insertion, especially for auto-polymerized acrylic resins, to minimize the risk of adverse reactions in patients who wear acrylic dentures. In our study, the specimens that were immersed in 50^{o}C water with 28 kHz (F1 group) or 40 kHz (F2 group) ultrasonic treatment for 5 minutes had significantly lower mean amount of residual monomer than the control group, but were not significantly different than the positive control group. This suggests that was no difference between these methods on minimizing residual monomer. Therefore, immersion of acrylic resin in 50^{o}C water with 28 or 40 kHz ultrasonic treatment for 5 min is preferred because this results in less chair time. Previous studies have shown that when residual monomer was minimized, the mechanical properties of acrylic resin were improved (Dogan et al., 1995; Patil et al., 2009). Future studies should investigate the mechanical properties of acrylic resin when minimizing residual monomer by ultrasonic treatment.

Patil et al. (2009) and Azzarri et al. (2003) recommended using microwave treatment at 650 watts and 800 watts for 5 minutes, respectively as post-polymerization treatment of acrylic resin. These treatments use the same chair time as ultrasonic methods but may have an effect on the dimensional stability of acrylic resin. The study of Chia et al. (1995) demonstrated that during polymerization using 500 watts microwave, the temperature of the acrylic resin rose to approximately 100^{o}C. Ghassan (2008) demonstrated a negative correlation between temperature and dimensional stability. Wagner (2013) found that post-polymerization treatment of acrylic resin with microwave curing at either 420 or 700 watts for 3 minutes caused acrylic resin deformation. The dimensional stability of acrylic resin after ultrasonic treatment has not yet been investigated. However, we suggest that dimensional stability would not be affected because the main purpose of ultrasonic treatment is to enhance the elution of residual monomer from acrylic resin, rather than the post-polymerization of residual monomer.

Ultrasonic treatment may affect the amount of residual monomer in two ways. First, ultrasonic treatment increased the flow rate of water in the tank, which may affect the elution of the residual monomer into the environment. Second, the implosive bubbles release energy to the surface of the specimens and may cause polymerization of the remaining monomer. However, in the present study we did not determine how ultrasonic treatment affects the residual monomer. Future studies should focus on the elution of residual monomer into the environment and the comparison of the degree of conversion of MMA before and after ultrasonic treatment. The surface of the acrylic resin should also be investigated, because the high energy of the implosive bubbles may damage the surface of specimens and cause increased roughness of the polished surface, which is undesirable.

In conclusion, the amount of residual MMA monomer in acrylic resin can be significantly minimized by immersion in 50°C water with 28 or 40 kHz ultrasonic treatment for 5 minutes, and this method is preferred to immersion in 50°C water for 1 hour because less chair time is required.

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