Original Article

Level of residual monomer released from orthodontic acrylic materials

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ABSTRACT

Objective: To quantify, with high-pressure liquid chromatography (HPLC), the amount of residual monomer leached from different orthodontic acrylic materials prepared with two different manipulation methods.

Materials and Methods: Eighty cylindrical specimens (5 × 25 mm) were divided into eight groups (n = 10). The specimens were prepared with four acrylic materials—Orthocryl Neon Blue (Dentaurum), Orthocryl EQ (Dentaurum), Orthoplast (Vertex), and O-80 (Imicryl)—and with two different manipulation methods: doughing and spray-on. HPLC measurements were made at intervals of 2 hours, 6 hours, 1 day, 1 week, and 3 months. One-way analysis of variance (ANOVA) and Tukey's honestly significant difference multiple-comparison test were used to assess the amount of monomer eluted from the various groups. To assess the differences within each group over the various periods, repeated-measures ANOVA and paired *t*-tests were used.

Results: Statistically significant differences were found within the groups in the amount of residual monomer in the specimens at different time intervals ($P < .001$). HPLC showed statistically significant differences among the groups ($P < .05$) in the amount of eluted monomer. Evaluation of the manipulation techniques showed that the monomer release rate was higher in the specimens prepared with the doughing method. When the four acrylic materials were compared, the specimens made from Orthoplast (Vertex) showed the highest rate of monomer release with both manipulation techniques.

Conclusion: The spray-on method can be recommended to clinicians for the preparation of orthodontic appliances. (*Angle Orthod.* 2014;84:862–867.)

KEY WORDS: Monomer release; Acrylic material; Manipulation methods

INTRODUCTION

Since the 1930s, polymeric parts of dentures and removable orthodontic appliances have usually been fabricated with acrylic resins or polymethyl methacrylates (PMMAs). Furthermore, acrylic resins are used for maxillary orthopedic appliances for newborn cleft lip and palate patients and splints for orthognathic surgery patients. Acrylic resins can be polymerized by different procedures: autopolymerization, photopolymerization, microwave polymerization, or thermopolymerization. Autopolymerized resins are used commonly in orthodontic clinics because of their low cost and practical applications.[1,2]

Various methods can be used to fabricate removable orthodontic appliances. For manipulation, the spray-on (salt and pepper) method and the doughing method are generally used. The spray-on (salt and pepper) method, in which the polymer is saturated by its monomer, is popular in orthodontic clinics, and the doughing method, in which liquid and powder are mixed together, is widely utilized in prosthodontics.[3]

Changes in the humidity levels in the oral cavity may affect the materials used in orthodontic treatment. This situation results in the release of elements and chemically modified reaction products. Leaching of these components into the mucous membranes or into the whole body can produce a local or systemic

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Accepted: November 2013. Submitted: June 2013.
Published Online: March 6, 2014

 DOI: 10.2319/060713-435.1

Table 1. Chemical Composition and Manipulation of Materials

Brand	Components	Chemical Composition	Manufacturer	Setup Proportion for Spray-on	Setup Proportion for Doughing	Pressure Temprature	Time
Orthoplast	Liquid	Methyl methacrylate, cross linker, accelerator	Vertex	Liquid: 1 scale	Liquid: 1 scale	2.2–2.5 bar 45°C–55°C	Dough: 9 min Polymerization: 20 min
	Powder	Methyl methacrylate		Powder: 3 scale	Powder: 2.6 scale		
Orthocryl EQ	Liquid	Methyl methacrylate, ethylene glycol dimethacrylate, N,N-dihydroxyethylene-p-poluidine	Dentaurum	Liquid: 1 scale	Liquid: 1 scale	2.2–2.5 bar 45°C–55°C	Dough: 3–5 min Polymerization 20 min
	Powder	Polymethyl methacrylate		Powder: 3.1 scale	Powder: 2.5 scale		
O-80	Liquid	Methyl methacrylate	Imicryl	Liquid: 0.9 scale	Liquid: 1 scale	2.2–2.5 bar 45°C–55°C	Dough: 9 min Polymerization: 20 min
	Powder	Methacrylate copolymerizates		Powder: 3 scale	Powder: 2.5 scale		
Orthocryl Neon Blue	Liquid	Methyl methacrylate, ethylene glycol dimethacrylate, N,N- dihydroxyethylene-p-poluidine	Dentaurum	Liquid: 0.9 scale	Liquid: 1 scale	2.2–2.5 bar 45°C–55°C	Dough: 3–5 min Polymerization 20 min
	Powder	Polymethyl methacrylate with color concentrate		Powder: 3 scale	Powder: 2.5 scale		

inflammatory reaction.[4] The residual monomer has the potential to create irritation, mucosal inflammation, and an allergic reaction. Clinical symptoms include erythema, erosion, and a burning sensation in the oral area.[5]

Although a large amount of residual monomer has been observed in heat-cured PMMA denture-base resins, self-curing acrylic resins, such as those often employed in removable orthodontic appliances, have greater amounts of free residual monomer.[6] The residual monomer released from acrylic denture-base materials has been examined in several studies, but no comprehensive studies have been done to compare the amount of released residual monomer with the two different preparation methods (spray-on and doughing).

The aim of this study was to test the null hypothesis that no differences exist in the level of residual monomer released from orthodontic acrylic resins prepared by spray-on or doughing manipulation methods using high-pressure liquid chromatography (HPLC).

MATERIALS AND METHODS

Orthoplast (Vertex Dental, Zeist, The Netherlands), Orthocryl EQ (Dentaurum, Ispringen, Germany), Orthocryl Neon Blue (Dentaurum), and O-80 (Imicryl, Konya, Turkey) were selected for testing. All these acrylic materials have been developed for the fabrication of removable orthodontic appliances. Table 1 shows the chemical composition and preparation procedures of the materials used in this study.

The suggestions of the manufacturers (powder-to-liquid ratios, polymerization time, and polymerization temperatures) were taken into account in the preparation of the test specimens. The specimens were prepared according to EN ISO 10993-12:2012 under aseptic conditions using sterile polytetrafluoroethylene standard discs measuring 25 mm in diameter and 5 mm in thickness.

Specimens were prepared with the four different acrylic materials and the two different manipulation methods. Thus, eight groups were created according to the different combinations of acrylics and preparation methods. Each group included 10 samples, and a total of 80 samples were used.

HPLC (Agilent 1100 System, Woldbronn, Germany) was used to identify the residual methyl methacrylate (MMA) content. The analysis used an Agilent UV detector at 230 nm, an Ace 5 C18 column, a binary pump, and manual injector systems. For the mobile phase, a 50:50 mixture of acetonitrile (HPLC grade) and distilled water was used, and the flow rate was 1 mL/min. The calibration curve was constructed with an MMA concentration of 100 to 3000 ppm in acetonitrile.

All of the specimens were stored in a glass flask, which was sealed with a leak-proof cap and tightly wrapped parafilm to avoid leakage. As an extraction solvent, 20 mL of methanol were added. The specimens were maintained at 37°C during the experiment. To determine the release of the MMA over time, 20 μL of the extract solution were injected into the HPLC at regular intervals, and these solutions were evaluated to determine the rate of residual monomer release. The time intervals were 2 hours (T1), 6 hours (T2), 1 day (T3), 1 week (T4), and 3 months (T5).

Table 2. Comparison of the Amounts (%) of Residual Monomer[a]

	T1 (2 h)		T2 (6 h)		T3 (24 h)		T4 (1 wk)		T5 (3 mo)		ANOVA Paired *T*-Test
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	All Comparisons
Orthocryl Neon Blue spray-on	0.63[d]	0.04	0.81[c,d]	0.01	1.08[b]	0.02	1.50[b,c]	0.05	1.82[b,c]	0.08	***
O-80 spray-on	0.55[b]	0.02	0.79[b]	0.03	1.05[b]	0.06	1.20[b]	0.07	1.79[b,c]	0.09	***
Orthoplast spray-on	0.53[b]	0.03	0.80[c,d]	0.02	1.23[c]	0.07	1.55[d,e]	0.06	2.05[d,e]	0.15	***
Orthocryl EQ spray-on	0.55[b]	0.05	0.78[c]	0.05	1.06[b]	0.05	1.23[b,c]	0.04	1.80[b]	0.13	***
Orthocryl Neon Blue doughing	0.79[e]	0.03	0.90[d]	0.01	1.05[b]	0.05	1.26[b,c]	0.08	1.85[b,c]	0.14	***
O-80 doughing	0.61[c,d]	0.01	0.86[c,d]	0.02	1.07[b]	0.04	1.54[d]	0.07	2.35[e]	0.15	***
Orthoplast doughing	0.62[d]	0.02	1.03[e]	0.05	1.46[c]	0.08	1.78[e]	0.03	2.68[f]	0.21	***
Orthocryl EQ doughing	0.57[b,c]	0.04	0.80[c,d]	0.05	1.06[b]	0.05	1.51[c]	0.11	2.03[c,d]	0.12	***

[a] The residual monomer released from various orthodontic acrylic materials at five different time intervals (mean ± standard deviation [SD]).
[b,c,d,e,f] Means with the same lowercase letter are not significantly different at $\alpha = .05$ according to ANOVA and Tukey HSD.
*** $P = P < 0.001$.

Statistical Analysis

The SPSS 14.0 software program (SPSS Inc, Chicago, IL, USA) was used for statistical analysis. One-way analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) multiple comparisons were used to assess the amount of eluted monomer from the groups. To determine the differences within each group over the various periods, repeated-measures ANOVA and paired *t*-tests were used. The data are presented as means and standard deviations. A value of $P < .05$ was accepted as statistically significant.

RESULTS

The results revealed statistically significant differences within the groups in the amount of (%) residual monomer in the specimens at different time intervals ($P < .001$) (Table 2). According to ANOVA, the maximum rate of release occurred in the Orthoplast group with both the spray-on (T3, T4, and T5) and the doughing (T2, T3, T4, and T5) methods (Figure 1).

According to HPLC, the amount of leached residual monomer ranged from 0.1% to 0.7%. The lowest rate (0.1%) of residual monomer occurred with the O-80 and the spray-on method at T1 and T2, and the highest rate (0.7%) occurred in the Orthoplast group prepared with the doughing method at T1.

Among the groups, the Orthocryl Neon Blue group showed the highest rate of eluting monomer with both manipulation methods at T1. Statistically significant differences were found between the two manipulation methods, except in the Orthocryl EQ group at T1. The residual monomer rate was higher in the specimens using the doughing method (Table 2; Figure 2).

Among all the groups, the highest rate of eluted monomer occurred in the Orthoplast group prepared with the doughing method at T2. The O-80 and Orthoplast groups showed statistically significant increases in the specimens prepared with the doughing method at T2 (Table 2; Figure 2).

The Orthoplast group showed a statistically significantly higher rate of monomer elution with both methods at T3 and T4. There was no difference in the rate of eluting monomer between the two manipulation methods at the same time points (Figures 1 and 2).

There were statistically significant differences between the doughing and spray-on methods in the amount of monomer eluted. The residual monomer rate was higher in the specimens using the doughing method, especially in the Orthoplast, Orthocryl EQ, and O-80 groups after the 3-month period. There was no significant difference between the two manipulation methods in the Neon Blue applied group (Figures 1 and 2).

Figure 3 shows that the amount of monomers eluted from the specimens began to decline after the first day. The amount of monomer elution in the first 24 hours was higher than during the subsequent 1-week and 3-month periods.

DISCUSSION

Although many studies have examined the release of monomers from prosthetic acrylics, few studies in the orthodontic literature have focused on the release of monomers from orthodontic acrylics.[7–10] This study investigated the effects of manipulation methods on the amount of residual monomer released from four different dental acrylic resins.

Although PMMAs are commonly used for the fabrication of acrylic materials, there is no consensus on the most appropriate polymerization process for converting a monomer to a polymer. Sufficient polymerization is a critical factor to maximize the physical properties and biocompatibility of acrylic resin–based materials.[11] The biological effects of MMA must be considered in orthodontic removable appliances because MMA remains in contact with the oral mucosa for

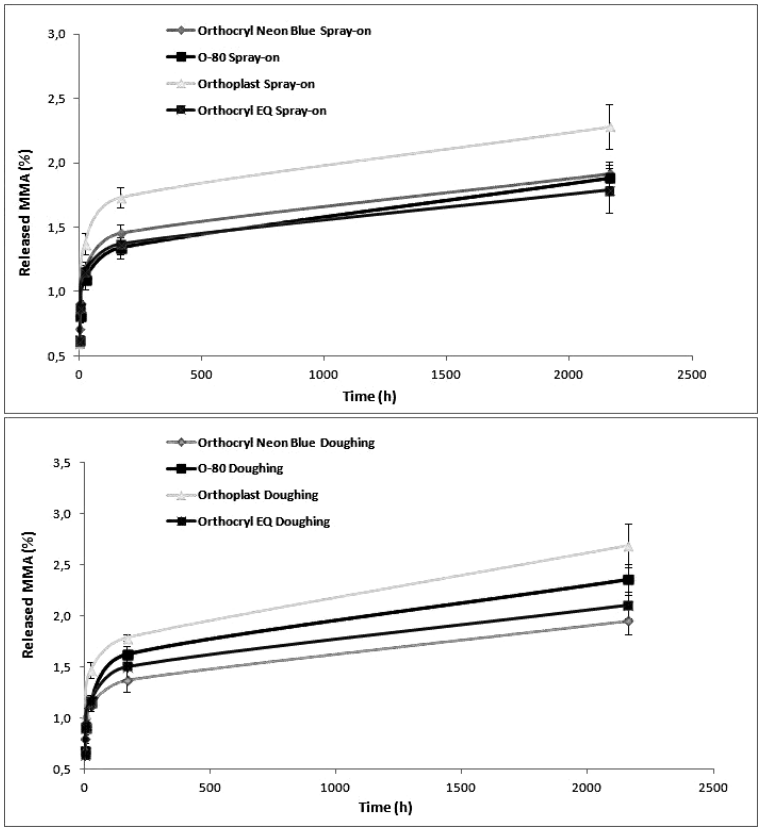

Figure 1. The amount of released monomer from four different orthodontic acrylic materials.

a long period. MMA is a known allergen and can cause local adverse reactions, such as necrosis, a burning sensation, erythematic lesions, fissures, pain, edema, and even some systemic reactions.[3] Gonçalves et al.[12,13] indicated that acrylic resins exert cytotoxic effects on cells, especially epithelial cells. Consequently, it is desirable to reduce the residual monomer content in dental acrylic resins to as low a level as possible before they are placed in the mouth.[14] Moreover, health care workers should be taken into account in any consideration of the biocompatibility of materials. In many instances, the risk of adverse effects of biomaterials is much higher for the personnel in the dental clinic than for the patient because of the former's constant exposure during the handling of the materials in the laboratory and clinic. Hence, researchers must determine the risk of these materials in orthodontic practice.[15]

Several methods have been described to evaluate the levels of residual MMA monomer. In the present study, the amount of residual MMA leaching from acrylic resins was determined with HPLC.[7,8,16] Some researchers have used HPLC for a similar purpose, but other chromatographic methods, such as gas chromatography, infrared spectrometry,[16] chemical detection,[7] and HPLC,[8,17] can also be used to determine the amount of residual MMA in acrylic resins. Recently, HPLC has become popular in analytical chemistry because it is a nondestructive method, enables simultaneous analysis of various substances, and provides correct estimates of the degree of residual monomer in acrylic resins.[9]

The use of various extraction solvents, including tetrahydrofuran, acetonitrile, methanol, and ethanol, has been reported in the literature.[18,19] In the present study, methanol was considered the most appropriate for use with the HPLC analysis because it provides separate peaks for all the extracted compounds, thereby enabling measurement of the residual monomers released into the solution.[20] The temperature, time, polymerization technique, method of specimen preparation, and thickness of the acrylic resin may influence the release of residual monomers.[10] Stafford and Brooks[21] showed that the amount of leached residual monomer is generally around 1.5% to 4.5% in self-curing acrylic resins but that it is only around 0.3% with heat curing. Yilmaz et al.[22] used the international patterns of ISO 1567 as a reference for the release of residual monomer. This reference limits the level of residual monomer to 2.2% for heat-curing and 4.5% for self-curing acrylic resins. The polymer:monomer ratio can also influence residual monomer levels.[23] Kedjarune et al.[20] and Lamb et al.[23] examined the influence of

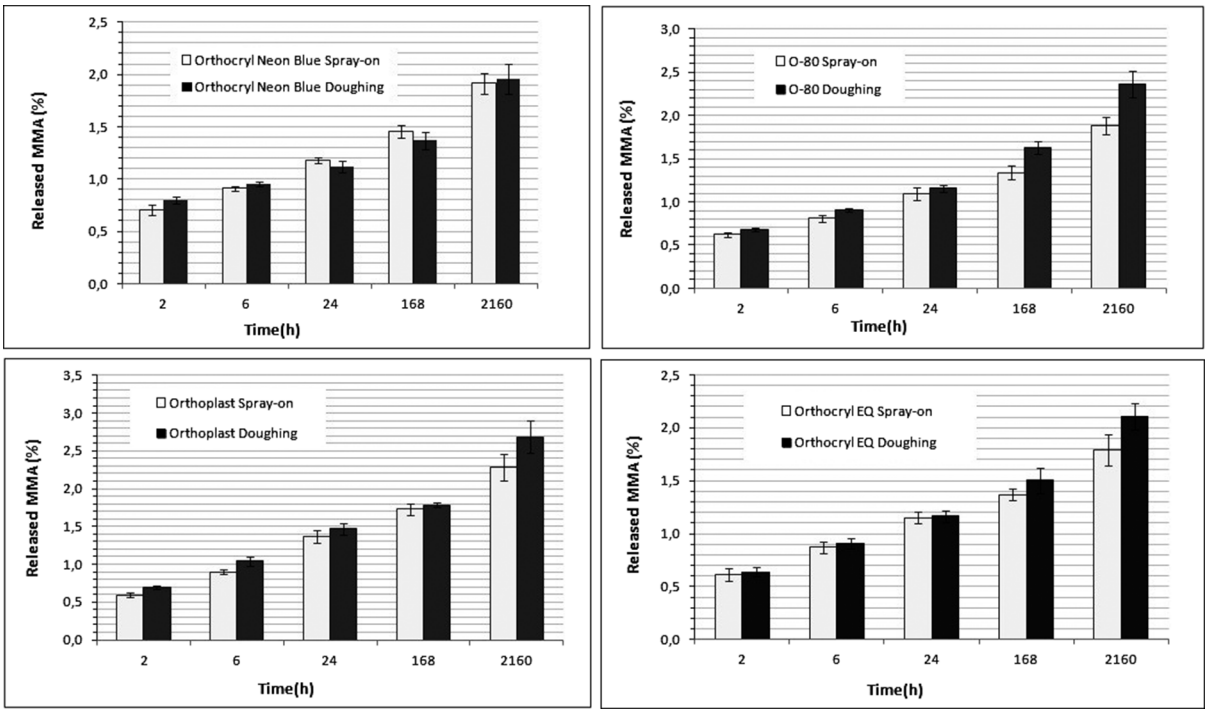

Figure 2. Comparison of the amount of released monomer from acrylic prepared by the doughing method or the spray-on method.

the polymer:monomer ratio on residual monomer levels. They found that a mixture containing a high proportion of monomer results in a greater amount of residual monomer and therefore has more potential for cytotoxicity. According to the current study, the residual monomer rate was higher in the specimens created using the doughing method, especially in the Orthoplast, Orthocryl EQ, and O-80 applied groups after 3 months. The polymer:monomer ratio used in the preparation of the specimens was 2.5:1 with the doughing method and 3:1 with the spray-on method.

The monomer elution rate in the first 24 hours was higher than at the 1-week and 3-month time points. This may be explained by alterations over time in the structure of the toxic substances that were released or by combination of the substances with other materials in the environment, leading to a change in their cytotoxic characteristics.[24] Previous studies have recommended procedures for reducing the residual monomer content of polymerized acrylic resins.[5,8,25] Several researchers have suggested that acrylic resin–based appliances should be placed in a water bath after polymerization for at least 24 hours before application in the patient.[8,25]

CONCLUSIONS

- Within the limitations of this in vitro study, residual MMA content was higher in specimens which were prepared with doughing method.
- In this study, Orthoplast acrylic resin showed the highest residual monomer amount compared to the other acrylic resins.
- The eluting monomer rate in the first 24 hours was higher than at the 1-week and 3-month time points.

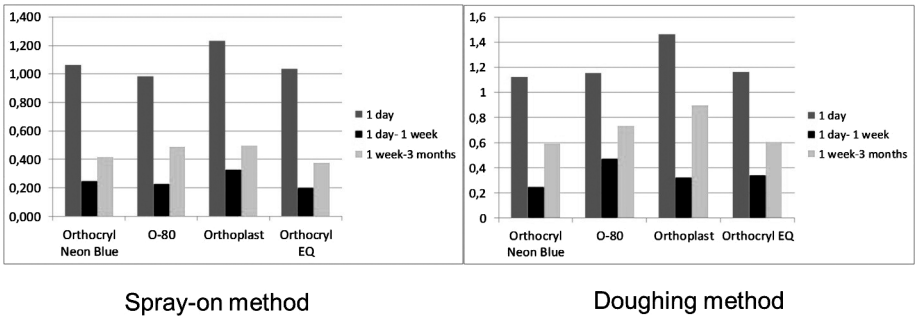

Figure 3. Amounts of monomer released over time.

- Removable appliances should be placed in a water bath after polymerization for at least 24 hours before application in the patient.

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