

Research Article

In Vitro Comparison of the Effect of Three Types of Heat-Curing Acrylic Resins on the Amount of Formaldehyde and Monomer Release as well as Biocompatibility

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Background/Purpose. The biocompatibility and cytotoxicity of formaldehyde and monomer are essential in resin-based denture's byproducts. This present study was performed to compare the release of formaldehyde and monomer and biocompatibility of three brands of heat-curing acrylic resins, including Ivoclar, Bayer, and Acropars, with different mixing properties and the same processing methods. **Materials and Methods.** In this experimental in vitro study, 18 samples were fabricated from Ivoclar, Bayer, and Acropars heat-curing acrylic resins (each group consisting of 6 samples). The released formaldehyde and monomer level were measured and registered for 1, 7, and 30 days. Also, methyl methacrylate release from samples was used to test cell cytotoxicity using L-929 murine fibroblast. The data were analyzed with repeated measures, one-way ANOVA, and Kruskal–Wallis tests. **Results.** For formaldehyde release of 1 day, Ivoclar acrylic resin showed the lowest level, followed by Bayer and Acropars acrylic resins ($P < 0.05$). On 7 and 30 days, Bayer acrylic resin released the lowest formaldehyde, followed by Ivoclar and Acropars acrylic resins ($P < 0.05$). Acropars showed the weakest and most significant results regarding biocompatibility and monomer release in all three points of time, respectively ($P < 0.05$). **Conclusion.** Acropars acrylic resin showed the most significant formaldehyde and monomer release and least biocompatibility compared to Bayer and Ivoclar for 1, 7, and 30 days; however, after 30 days, all three resins displayed the same amount of formaldehyde release.

1. Introduction

Over the last decade, advances in dental material science have promoted the expectations of dentists and patients about the safety and efficiency of dental products [1]. Nowadays, there are several kinds of acrylic resins in the

dental market, and each of them shows some advantages and disadvantages [2]. Most denture bases are made from heat-cured acrylic resins [3]. Polymethyl methacrylate (MMA) resin has been widely used to manufacture many dental materials for a long time [2]. On the contrary, many research studies have shown that heat-polymerized

acrylates have many advantages over cold-polymerized materials due to almost complete polymerization and better biological properties [3, 4]. One of the significant concerns about using MMA resins for denture base fabrication is the release of byproducts such as formaldehyde and monomer into the oral environment [5]. The residual monomer is the primary substance eluting from the acrylate denture base [6].

On the contrary, formaldehyde is formed as a product of oxidation of the residual MMA in the layers with a low degree of conversion [7, 8]. Investigations have shown a correlation between formaldehyde release and residual free monomer after polymerization [7, 9]. Various factors could affect the content of residual monomer including polymerization temperature and duration, the thickness of acrylic resin [10, 11], the measurement method [12, 13], length of the polymerization cycle, and the type of acrylic resin [12, 14]. Some studies have shown the lack of biocompatibility and cytotoxicity of this material [11, 12]. MMA monomer is an allergen and a contributing factor in the sick building syndrome (SBS), and even some studies of humans have suggested that MMA exposure is linked with certain types of cancers [4]. Long-term exposure to this substance causes a headache, burning eyes, and mutational consequence respiratory system irritation [2, 3, 5, 8]. According to the International Agency for Research on Cancer, formaldehyde has been introduced as a potential carcinogen for animals, but the carcinogenic effect of formaldehyde on humans is controversial [4]. However, the toxicity effects of formaldehyde on man and animals in some studies have been reported [13, 14]. Formaldehyde is cytotoxic in smaller quantities in comparison with the MMA monomer. It can irritate the mucosa even at low concentrations of 0.63–1.2 ng/ml [15, 16]. MMA molecules can be associated with saliva's proteins making large molecules responsible for oral tissue allergic reactions [17, 18]. Cheilitis, stomatitis, burning mouth, and painful sensation are some side effects of resin acrylic materials reported in people who have received a complete or partial denture [19]. All cells in the human body contain some amount of formaldehyde, which is produced due to the metabolism of serine, glycine, methionine, and other amino acids in the human body [4]. In the recent years, Ivoclar, Bayer, and Acropars heat-curing acrylic resins have been introduced for fabrication of removable prostheses in the market. The present study was conducted with the aim of comparing the effect of the three types of heat-curing acrylic resins on the amount of formaldehyde and monomer release as well as biocompatibility within 30 days.

2. Materials and Methods

In this *in vitro* study, all samples were fabricated using the conventional flasking method for denture base fabrication from Ivoclar (Ivoclar Vivadent, Schaan, Liechtenstein), Bayer (Meliodent; Bayer Dental Ltd., Newbury, UK), and Acropars (Marlic, Tehran, Iran) heat-curing acrylic resins, according to the manufacturer's instructions (Table 1).

2.1. Assessment of the Release of Formaldehyde

2.1.1. Specimen Preparation. Thirty cubic samples with $3 \times 3 \times 2 \text{ mm}^3$ dimension of each brand were provided and divided into three groups of 10 samples and weighted with a digital scale with 0.05 accuracy. The samples were incubated at 37°C and 100% humidity in 100 ml of double-distilled water in 3 closed glass containers. Assessment of formaldehyde release and calibration curve preparation was performed by Gigante et al.'s method [20]. In brief, 1 ml of formaldehyde solution (Merck KGaA, Darmstadt, Germany) with predetermined concentrations was poured in a 50 cc glass beaker. 300 microliters of chromotropic acid 5% (Sigma-Aldrich, Germany) was added to the solution with 3 ml phosphoric acid (H_3PO_4) 85% and 70 microliters of hydrogen peroxide (H_2O_2) 2.5% (Scharlau Chemie S.A., Spain) simultaneously. The glass beaker was covered with a glass block and was heated in an electric microwave oven with 1100 W power for 35 seconds. Afterward, the solution was cooled gradually at room temperature (25°C). Finally, the level of absorbance of the solution at the wavelength of 570 nm was measured using the UV-Vis spectrophotometer (Agilent Cary 60, USA) to provide the calibration curve. 1 ml of water in each closed glass jar was picked up. The molar concentrations of formaldehyde in distilled water were assessed using the previously described method according to the calibration curve for 1, 7, and 30 days. The remaining water was discarded and replaced with fresh distilled water each time.

2.2. Assessment of the Release of the MMA Monomer

2.2.1. Specimen Preparation. A total of 30 cubic samples measuring $10 \times 50 \text{ mm}$ with 1.5 mm thickness fabricated each denture base acrylic resins' brand. The samples were weighted with a digital scale with 0.05 accuracy, placed in closed glass containers containing 100 mL of double-distilled water, and incubated at 37°C and 100% humidity. To assess monomer release and preparation of the calibration curve, 1 ml of MMA monomer solution (Merck KGaA, Darmstadt, Germany) with predetermined concentrations was prepared, and the level of absorbance of the solution at the wavelength of 240.5 nm was measured using the UV-Vis spectrophotometer (Agilent Cary 60, USA) to provide the calibration curve. 1 ml of water of each container, as mentioned above, was collected for 1, 7, and 30 days after incubation, and the remaining water was discarded and replaced with fresh distilled water each time. The collected water was read in the spectrophotometer. The numerical absorbance values were recorded, and the corresponding concentration was extracted from the calibration curve and reported in $\mu\text{g/mL}$.

2.3. Assessment of Cell Viability (MTT Assay). Thirty cubic samples with $3 \times 3 \times 2 \text{ mm}^3$ dimension of each brand were provided and divided into three groups of 10 samples and weighted with a digital scale with 0.001 accuracies. To perform the MTT assay for each time interval (1, 3, and 7

TABLE 1: Physical properties of three brands of heat-cured acrylic resins.

	Water absorption ($\mu\text{g}/\text{mm}^3$)	Powder/liquid ratio	Processing method	Methyl methacrylate content (%)
Ivoclar	21.6	20.5 g/10 ml	Boiling water 35 min	95.9
Bayer	23.2	35 g/14 ml	Boiling water 20 min	>90
Acropars	30	24 g/10 ml	Boiling water 30 min	>90

days), ten samples of each brand were placed in a well (in a 96-well plate) in direct contact with L-929 fibroblasts (20,000 cells in each well). The samples and medium on cells were discarded at the test time intervals, washed with PBS, and replaced by the MTT-containing medium. Then, the 96-well plate was incubated for 4 h. To solve the formazan crystals reduced from tetrazole by viable cells, the MTT solution was discarded, and without washing, DMSO was added. The plates were left for 20 min and then transferred to the ELISA reader to measure the absorbance at 570 nm. The results were expressed as a percent of viable cells according to positive and negative control groups.

2.4. Statistical Analysis. The data were analyzed with repeated measures, one-way ANOVA, and Kruskal–Wallis tests. The data were statistically analyzed using SPSS version 22, and $P < 0.05$ was considered statistically significant.

3. Results

3.1. Formaldehyde Release. The formaldehyde release (mol/L) level was assessed according to follow-up intervals of a day, a week, and a month (Figure 1). Statistical analysis by the two-way repeated-measure ANOVA revealed a significant difference between Acropars and two other groups considering the effect of time and resin on formaldehyde release for 1 and 7 days ($P < 0.05$). However, the results failed to reveal any significant effect of time and type of resin after 30 days ($P > 0.05$). One-way ANOVA showed that Acropars resin exhibited the highest level of formaldehyde release for 1, 7, and 30 days. The difference of Acropars formaldehyde release compared to Bayer and Ivoclar was significant ($P < 0.05$) for 1 and 7 days, while the difference between 3 groups was not significant ($P > 0.05$) for 30 days. Also, no significant difference was recognized between Ivoclar and Bayer resins for all intervals ($P > 0.05$). During the 30 days of the experiment, formaldehyde release lasted from all three acrylic resins; however, it decreased significantly compared to the first day after processing the resin.

3.2. Monomer Release. During 30 days, the highest and the lowest amount of monomer were released from Acropars and Bayer, respectively (Figure 2). According to ANOVA, this difference was statistically significant ($P < 0.05$), while the difference between Bayer and Ivoclar was not significant in three points of time ($P = 0.8$, $P = 0.6$, and $P = 0.8$).

3.3. Cell Viability (MTT Assay). The cell viability results at different time points are presented in Figure 3. The highest percentage of cell viability for 1, 3, and 7 days belonged to

Ivoclar and Bayer, while the lowest was reported in Acropars resin compared to the control group. According to the Kruskal–Wallis test, this difference was statistically significant ($P < 0.05$). On the contrary, no significant difference was noted between Bayer and Ivoclar acrylic resins compared to the control group during one week.

4. Discussion

In our study, the amount of monomer and formaldehyde release and cell viability were investigated in three brands of heat-curing resins, which made our study unique and comprehensive compared to other studies conducted in this field. To the best of our knowledge, no article in this field evaluated these three features simultaneously in acrylic resin materials. Biocompatibility and cytotoxicity of dental materials should be taken care of when materials are used to fabricate dental applications to prevent their toxic effect on the surrounding tissue. Based on previous studies which recommended heat-curing resins as the lowest harmful dental material, three popular heat-curing acrylate resin brands in the dental market were chosen to investigate in this study [15, 18, 21].

The present study showed that the difference in formaldehyde release from Acropars acrylic resin was significant compared to Bayer and Ivoclar acrylic resins for 1 and 7 days, but the difference among these three groups was not statistically significant for 30 days. Furthermore, Acropars monomer release was statistically significant all the time compared to Bayer and Ivoclar.

Formaldehyde is one of the certain toxic chemicals, which is released gradually from acrylic resins resulting in irritation of the surrounding tissue [11]. It is one of the byproducts generated due to the oxidation of methacrylic groups or degradation of the oxide-methacrylic copolymer [22]. MMA concentration and polymerization rate play an essential role in releasing formaldehyde from acrylic resin materials, one of the many factors responsible for cytotoxicity and irritating effects of acrylic resins [10]. As shown in our study, there was a direct relationship between MMA and formaldehyde released from the three heat-cured acrylate resins tested. It means how much the leaching of MMA rises, and formaldehyde will enhance directly. This finding was by other previous studies [10, 21].

Tsuchiya et al. [21] assessed the amount of leaching and cytotoxicity of formaldehyde and methyl methacrylate monomer from three denture base resins into saliva, including autoresinizing, heat-curing, and microwave resins, for the first day until one month later. They observed that the level of these substances in saliva was high for the first day, and the leaching continued for one month; however, it decreased gradually. Also, the level of cytotoxicity of formaldehyde was higher than that of methyl methacrylate,

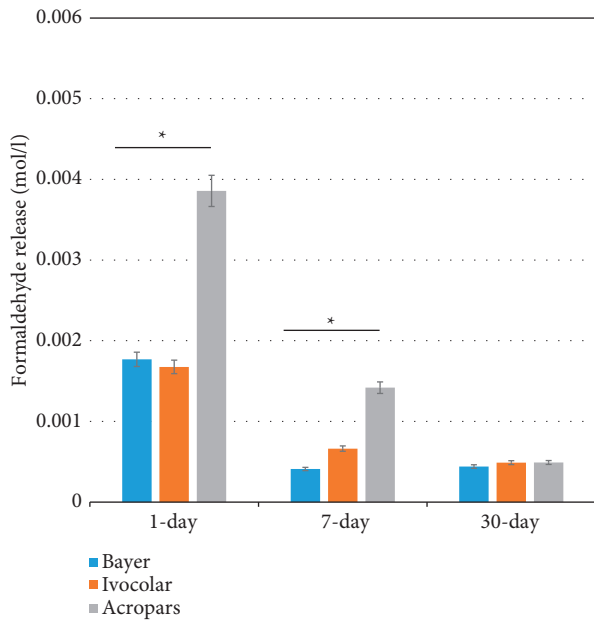


FIGURE 1: The amount of formaldehyde release (mol/L) is categorized by the type of acrylic resin according to follow-up intervals.

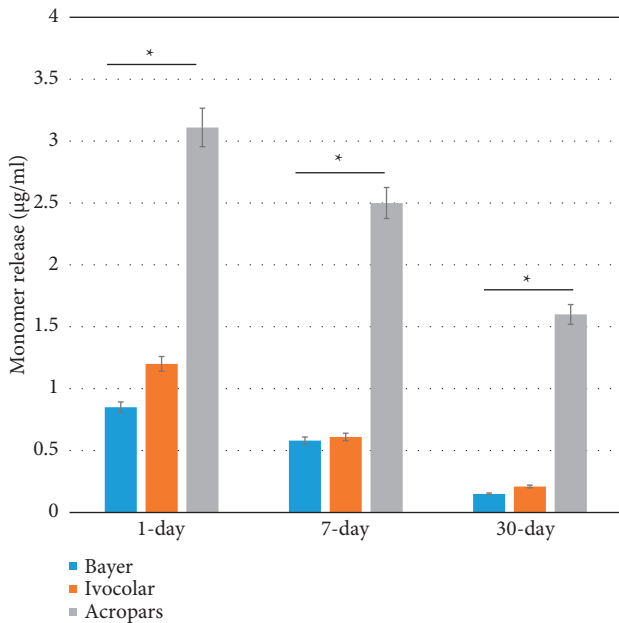


FIGURE 2: The amount of monomer release ($\mu\text{g/ml}$) is categorized by the type of acrylic resin.

even at lower concentrations. Furthermore, in heat-curing resins, the amount of leaching of the mentioned substances was significantly lower than other forms of acrylic resin polymerizations. Cytotoxicity might be related to the amount of formaldehyde released from resins, which was reduced over time [22]. In the present study, it was observed that, during the 30 days of the experiment, formaldehyde and monomer release lasted from all three heat-curing resins; however, it decreased significantly in comparison with the first day after acryl processing that confirmed

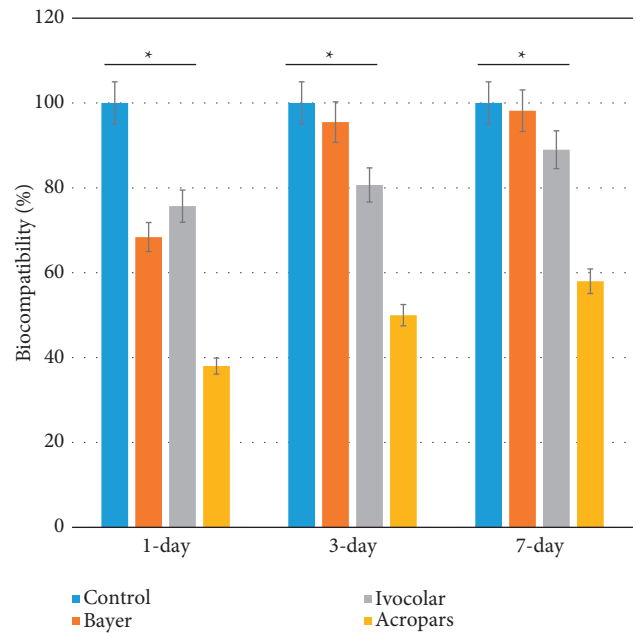


FIGURE 3: Amount of biocompatibility (%) according to follow-up intervals and categorized by acrylic resin type.

Tsuchiya findings. In 2012, Kostic et al. [23] assessed the cytotoxicity of three groups of denture base acrylic resins, including soft and hard autopolymerizing and heat-curing resins, by using a direct contact test. They concluded that autopolymerizing acrylic resins (soft or hard) had a mild inhibitory effect on cell growth. On the contrary, heat-curing acryl exhibited no signs of cell growth inhibition. Since the cell growth inhibition effect of acrylic resins was attributed to the monomer and formaldehyde released as byproducts of the acrylic resins [18], it was concluded that the heat-cured resin showed the smallest amount of formaldehyde and monomer release compared to other acrylic resins. In contrast, in our study, we concluded that even heat-cured acrylic resins could produce the noticeable amount of formaldehyde and monomer resulting in cytotoxicity of these materials. Thus, it seems that more research through heat-cured resins is necessary in order to find the most biocompatible materials. Ebrahimi Saravi et al. [24] in 2012 assessed the biocompatibility of three acrylic polymers by the use of L-929 murine fibroblasts, and the MTT assay revealed the superior biocompatibility of Bayer to Futura Gen and GC Reline at one hour, 24 hours, and one week which was similar to our findings. The present study chose three different heat-cured resins to determine the least harmful heat-cured acrylic resins. Formaldehyde and monomer release from Acropars acrylic resin for 1 and 7 days was beyond the permissible limit declared by the official website of the Iran Food and Drug Administration, which is under supervision of the Ministry of Health which reached the limit for 30 days. Besides, MTT assay revealed that Acropars and Bayer acrylate resins showed the least and most biocompatibility, respectively. On the contrary, in our study, like many previous studies, the MTT cytotoxicity test was used to investigate the toxicity of MMA. It has been

shown that MMA was not the only substance released from acrylic denture bases. Other substances could be detected, including methacrylic acid, benzoic acid, phthalates dibutylphthalate, dicyclohexyl phthalate, phenyl benzoate, and phenyl salicylate, as well as formaldehyde. Also, Sadamori et al. [25] reported that residual monomer content in acrylic dentures could be detected for up to several years after use. It appeared that complete loss of the residual monomer content might take more than 30 days that we have detected. Hence, more research in this field is mandatory either to evaluate the amount of monomer release for a longer time or to detect other cytotoxic substances released from the denture base. Sheridan et al. [26] reported that, during the first 24 hours, the cytotoxic effect of acrylic resins, which was associated with the rate of polymerizations and chemical composition of the resin, was greater and moderated after a week. Our study resembling Sheridan et al.'s report showed that the peak formaldehyde release, which was one of the most important factors of cytotoxicity, was observed during the first 24 hours and subsided over time. Except for Tsuchiya et al. [21], who determined formaldehyde release similar to the present study, other researchers have evaluated just free methyl methacrylate monomer [19, 22, 24, 27]. However, formaldehyde is more cytotoxic than methyl methacrylate, even at lower concentrations [21]. Therefore, substances with the probability of high formaldehyde release should not be used. Also, since formaldehyde is formed due to the oxidation of free monomer mostly at the superficial layers [28], acrylic resins should be carefully processed and immersed in water for 60 minutes after deflasking the level of free monomer and formaldehyde [29]. For many years, heat-cured acrylic resins have been the most commonly used denture base materials [29, 30]. Still, it is preferable to choose a type that releases the least formaldehyde and monomer due to its adverse effects on the oral mucosa. Using Acropars, acrylic resin showed the greatest formaldehyde and monomer release for all intervals compared to Bayer and Ivoclar acrylic resins. Also, Acropars acrylic resin was less compatible than Bayer and Ivoclar acrylic resins. On the contrary, the difference between Bayer and Ivoclar acrylic resins was not significant at 30-day intervals; however, Bayer displayed the fewer formaldehyde release.

Data Availability

All the data generated or analyzed during this study are included within this published article, and also, the datasets analyzed to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare no conflicts of interest.

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