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High Performance Liquid Chromatographic Determination of Residual Monomer Released from Heat-Cured Acrylic Resin. An In Vivo Study

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Abstract

Purpose: Heat-polymerized acrylic resins are used in dentistry for complete denture fabrication. Despite the polymerization method, conversion of monomer into polymer is often incomplete with free or unreacted residual monomer remaining in the polymerized resin. The aim of this study was to determine the amount of residual monomeric methyl methacrylate (MMA) leaching in the saliva of patients wearing complete dentures in their postinsertion period.
Materials and Methods: Thirty edentulous participants as first-time complete denture wearers (age 60 to 65 years) were selected. All the prostheses were fabricated using a similar standard technique with a heat-cured acrylic resin denture base material. Saliva samples were collected at time intervals of 1 hour, 1 day, and 3 days postdenture insertion. Participants were asked to discharge saliva every 30 seconds into a preweighed screw-capped container for a 5-minute period. MMA levels were measured using high performance liquid chromatography. Data were analyzed by ANOVA and Tukey-HSD.
Results: The maximum concentration of monomer released into saliva peaked 1 day after insertion of the complete dentures. The mean (SD) MMA content was 0.04 ± 0.01 (μg/ml) 1 hour after insertion, and 0.3 ± 0.09 (μg/ml), and 0.05 ± 0.01 (μg/ml) on the first and third days postinsertion, respectively.
Conclusions: Although the released monomeric MMA was not at toxic levels, it could potentially sensitize complete denture patients or elicit an allergic reaction. The risk of the residual material as a primary irritant for a sensitizing reaction could be minimized by immersion of the denture in water for 24 hours before insertion.

There has been a constant search to obtain a denture base material of lifelike appearance that will not deteriorate in service. Several difficulties exist in producing a satisfactory denture material or designing a technique useful for its application. Acrylic resin polymers are the most commonly used materials for dental prostheses. These materials are supplied in powder/liquid form. The powder contains polymethyl methacrylate beads along with benzoyl peroxide (initiator), dibutyl phthalate (plasticizer), pigments, and opacifiers. The liquid contains methyl methacrylate (MMA) monomer with hydroquinone (inhibitor), glycol dimethacrylate (cross-linking agent), and plasticizers. Decomposition of the initiator (mainly dibenzoyl peroxide) into radicals under heat initiates the setting of heat-polymerizing products.[1] Numerous researchers have tried to identify the components that generally leach from polymerized resin. Most of the information regarding biocompatibility of these materials is the result of various in vitro studies.

Conditions in the oral cavity are different, difficult, and seem almost suited to annihilation. Biting stresses on dentures can be extremely high, temperatures may fluctuate between 25° and 45°C, and pH may change instantaneously from acidic to alkaline. The warm and moist oral environment, which is also enzyme- and bacteria-rich, is conducive to further decay.[2] The soft tissues and structures of the oral cavity in contact with the denture may be injured from the toxic leaching or breakdown of the material.[3] Despite their excellence, some problems,

including allergic and/or local inflammatory and biochemical reactions, have been reported in the literature regarding denture base materials.[3] Clinical signs and symptoms most frequently reported include erythema, erosion of oral mucosa, and a burning sensation on the mucosa and tongue. A majority of published studies refer to the elution of unbound components mainly as consequences of material biodegradation.[4]

MMA monomer, phthalate esters, and additives like benzoyl peroxide, formaldehyde, bisphenyl, and phenyl benzoate are main components leaching from acrylic resins.[5,6] The cellular compatibility of solid specimens, aqueous resin extracts, and MMA were investigated in permanent cells and primary cultures as well.[7] Irritation of the oral mucosa beneath or adjacent to PMMA dentures is certainly the most severe local clinical adverse effect.[8] Allergic reactions in patients are also associated with leached-out monomer. In an experimental clinical study, Austin and Basker documented a clear association between irritation of the mucosa beneath dentures and the release of residual monomers.[8] There are many reports on in vitro release studies, but experiments using human saliva are rare, and few in vivo studies have been reported.[4] Hence, this study was performed to evaluate human saliva for monomer leaching from resin-based denture base materials at different time intervals using high performance liquid chromatography.

Materials and methods

Thirty edentulous patients (age 60 to 65 years) who visited the Department of Prosthodontics were included in the study group, after receiving approval from the ethics committee of King George's Medical University UP (Lucknow, India; process number: 5498/R.cell-10). The selection criteria for the study were as follows: patients requiring complete dentures for the first time and no deleterious habits such as smoking or tobacco chewing. All participants were healthy and not taking any medication that could affect salivary flow rate. After history and thorough examination, written consent was taken from each participant.

All complete dentures were fabricated using a similar standard technique with a heat-cured acrylic resin denture base material (Trevalon, Dentsply India Pvt Ltd., Delhi, India) (monomer/polymer ratio: 0.417 ml/g). The polymerization was carried out according to Indian standard specification (ISI) No. 6887-1973 for denture base polymers using the compression molding technique. After heat curing, the clamped flasks were bench cooled for a period of 30 minutes and then kept under tap water for 15 minutes. Dentures were finished, polished, and inserted accordingly. Postinsertion instructions were given to the participants. A similar process followed for all participants.

Saliva sample collection

Patients were asked to discharge saliva every 30 seconds into pre-weighed screw-capped containers for a 5-minute period. The containers were reweighed to estimate the salivary flow rate, assuming that 1 ml of saliva weighed 1 g. Saliva samples were taken after denture insertion at the following three stages:

Stage 1: 1 hour-sample x.
Stage 2: 1 day-sample y.
Stage 3: 3 days-sample z.

All the saliva samples were collected at the same time every day. Three samples were taken for each participant at different time intervals. Samples were stored in a refrigerator at -20°C before starting the analysis.

Estimation of monomer in saliva

No further preparation of the saliva sample was done. High-performance liquid chromatography [HPLC 515, Waters, Milford, MA; with 2487 UV and visible detector] was used for determination of monomer in saliva. Monomer was detected by UV absorbance at 230 nm, on a Nucleosil C18 (5 μm particle size, 100 Å pore size, 15 $\times$ 0.46 cm i.d.) column. Monomer concentration was estimated by comparing the area under the peak on the graph with that of standard for mono-MMA.

Statistical analysis was done using SPSS version 15.0 statistical analysis software (SPSS Inc., Chicago, IL). The values were represented in number (%) and mean $\pm$ SD. ANOVA and Tukey-HSD was employed. ANOVA was used to compare the within-group and between-group variances among the study groups (i.e., the three time intervals at which saliva samples were collected). ANOVA provided an "F" ratio, where a higher "F" value depicted a higher intergroup difference ($p < 0.05$ was considered significant; $p < 0.01$ was highly significant).

Results

This study comparatively evaluated the leaching from acrylic resin dentures in saliva at different time intervals. Leaching of monomer was highest at day 1 (0.40 μg/ml) and lowest at 1 hour (0.03 μg/ml) postdenture insertion (Table 1, Fig 1). ANOVA shows a significant difference in the mean value of leaching at different time intervals (Table 2). The values at 1 hour and day 3 postdenture insertion intervals were of lower order, whereas the same values at the day 1 interval were of higher order. An overlap in inter-quartile values of 1 hour after and day 3 after insertion was observed.

Comparison between groups using Tukey HSD test revealed statistically significant differences between day 1 versus day 3 and day 1 versus 1 hour postdenture insertion (Table 3). There was no significant difference found between 1 hour vs. day 3 postdenture insertion intervals ($p > 0.05$). Thus, the order of leaching observed at different time intervals was as follows: day 1 $>$ day 3 $\simeq$ 1 hour after insertion.

Discussion

Although allergic and inflammatory reactions from dentures have been ascribed mainly to elutes (e.g., residual monomer from acrylic resins) leaching from denture base material, for sensitization to occur, these elutes must presumably be released from the prosthesis into saliva, in which it is conveyed to the oral mucosa or, after being swallowed, to the gastrointestinal tract.[9] This study was undertaken as an attempt to evaluate saliva of complete denture wearers for leaching from heat-cured denture base material. The limitations of the current study include the limited time period and restricted sample size, which could be unduly influenced by a peculiarity in the sample. The amount

Table 1 Leaching of monomer (MMA) from heat-cured acrylic denture base

	Mean (n = 30)	Standard deviation	Minimum (μg/ml)	Maximum (μg/ml)
1 hour after acrylic denture insertion	0.0410	0.01101	0.03	0.06
1 day after acrylic denture insertion	0.3100	0.08756	0.20	0.40
3 days after acrylic denture	0.0510	0.00994	0.04	0.07

of residual monomer may be influenced by materials, curing cycle, and processing method. Different denture base acrylic resins and processing methods need to be evaluated for the residual monomer level.

Edentulous patients were selected to estimate MMA leaching, as most of the reports of irritation are associated with these patients.[10] A prosthesis was inserted on the same day of curing to minimize any changes (physical and chemical) occurring due to storage of the prosthesis.[11] Saliva samples were collected three times (1 hour, 1 day, and 3 days after insertion of denture, as these timings coincide with postinsertion recall visits of the patients).

Heat-cured PMMA-based acrylic resin of a standard brand was used for fabrication of dentures for participants included in study. Dentures were cured in a water bath acrylizer using a curing cycle as per manufacturer's instructions in accordance with ISI No. 6887–1973 for denture base polymers, as both the monomer/polymer ratio and time and temperature of curing cycle affects the residual monomer concentration of dentures.[12]

Although the curing cycle used in the study was a short curing cycle, curing of dentures at 100°C for 20 minutes can effectively reduce the residual monomer content because this high temperature increases the mobility and hence reactivity of monomer chains in the curing denture.[12,13] In previous studies, it has been found that the residual monomer content of injection-molded denture base resins was higher than that of compression-molded heat-cured resin.[14]

A variety of methods, such as chemical method,[15] gas liquid chromatography,[9] UV spectroscopy,[16] and high performance liquid chromatography,[17] have been used to determine the amount of leached MMA in saliva from resin dentures. HPLC has been used in this study to estimate MMA monomer both qualitatively and quantitatively.[17] This method was chosen because it is simple, accurate, and has high resolution and rapid analysis.

The maximum mean value of monomer (0.3 μg/ml) leaching in saliva was seen 1 day after denture insertion (Table 1, Fig 1). This correlated with various in vitro studies.[18] Though the concentration was lowest 1 hour after insertion (0.04 μg/ml), it may be because the in vivo conditions may differ from those of in vitro experiments of diffusion.[16] Acrylic dentures in the mouth are bathed in a current of continuously replaced saliva. The acquired salivary pellicle could also form a barrier to diffusion. Oxidative enzymes such as myeloperoxidase in saliva might also be involved in the degradation of MMA, which occurred more rapidly in saliva than in water.[9] So, monomer

Table 2 ANOVA for monomer leaching at different time intervals

	Mean square	F	Sig.
Between groups*	0.233	88.467	<0.001
Within groups	0.003		

*Group x-1 hr after insertion, Group y-1 day after insertion, Group z-3 days after insertion.

Table 3 Comparative evaluation of monomer leaching at different time intervals (Tukey HSD)

S.No.	Comparison	Mean difference	SE	*p*
1.	1 hr vs. Day 1	−0.269	0.229	< 0.001
2.	1 hr vs. Day 3	−0.010	0.229	0.901
3.	Day 1 vs. Day 3	0.259	0.229	< 0.001

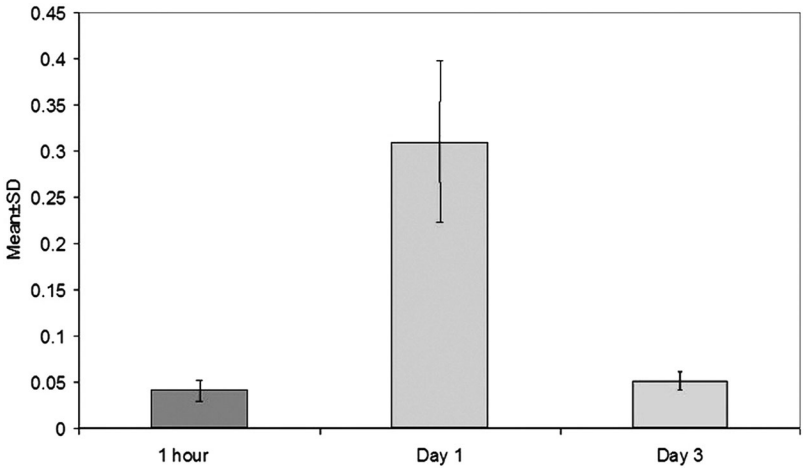

Figure 1 Leaching of monomer (MMA) from heat-cured acrylic denture base at different time intervals.

concentration may be higher in salivary film intimately in contact with the denture than in the whole saliva.[9]

The change in monomer concentration was significant over the period of 3 days (Table 2), making time an effective variable for monomer leaching over the period of denture use and, hence, indicating that monomer concentration will decrease during denture use.[19] Comparing leaching of monomer in saliva at different time intervals (Table 3) showed a significant increase in monomer concentration in saliva over the first 24 hours of denture wearing. It may be because once the leaching of MMA has started, equilibrium needs to be established between dentures and saliva until MMA leaching reaches a maximum value.[20] The concentration decreases significantly further over 72 hours of denture use, as not all of the free residual monomer in the acrylic dentures may be available for external diffusion.[15] Smith and Bains referred this to "nonextractable" components of residual monomer trapped in the polymer chains.[15] These observations also suggest that immersion of dentures in water for 24 hours before insertion will help further decrease the leachable monomer in saliva.[16,18]

The present study showed that the highest concentration of MMA found in saliva was 0.3 μg/ml (Table 1). This concentration is too small to directly inflict an irritation/allergic reaction in oral mucosa.[9] Although sensitization could occur from this dose, the literature says that patch test has been performed in patients suspected of MMA allergy.[21] Therefore, this low dose can sensitize patients who could show allergic reaction in the future.[21]

Further long-term follow-up studies on larger patient populations are required. Because of biodegradation of denture base materials over time, certain elutes not found in saliva initially may become detectable with further use of the prosthesis.

Conclusion

This study found most of the MMA release within 24 hours of insertion of the dentures in the mouth. It may be concluded, however, that according to the present investigation, the amount released for ingestion from a denture seems comparatively small and is quickly degraded. The risk of the residual material as a primary irritant for a sensitizing reaction could be minimized by immersion of denture in water for 24 hours before insertion.

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