Leachability of Degradation Products from Hard Chairside Reline Resins in Artificial Saliva: Effect of Water-Bath Post-Polymerization Treatment

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ABSTRACT: The effect of a post-polymerization treatment on the leaching of methacrylic acid (MA) and benzoic acid (BA) from the reline resins [Kooliner (K), New Truliner (N), Ufi Gel hard (U), and Tokuso Rebase Fast (T)] was evaluated. Specimens of each material were divided into two groups: Group C (control) – left untreated; Group WB (water-bath) – immersion in water at 55 ± 1°C for 10 min. Specimens were placed in artificial saliva at 37 ± 1°C and, after 1-, 3-, 5-, 24-h and 3-, 7-, 14-, and 30-day intervals, aliquots were removed and analyzed using high performance liquid chromatography. Data were analyzed by using Wilcoxon, Mann–Whitney or Kruskal–Wallis tests ($\alpha = 0.05$). At 1 h, the concentration of MA released from U control specimens was higher than those of the other ones, and decreased after 3 h. WB specimens released lower amounts of MA than control specimens only for material U, at the 1- and 3-h periods. For all control specimens, concentrations of leached BA progressively decreased within 5 h and from 24 h to the end. WB specimens released significantly lower amounts of BA than did the control groups. The highest concentration of MA was leached from control specimens of Ufi Gel hard. Water-bath post-polymerization treatment caused a significant reduction in elution of BA. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 123: 732–739, 2012

Key words: dental polymers; HPLC; resins; crosslinking

INTRODUCTION

Methacrylate-based polymers have been widely used for the fabrication and relining of removable denture bases.[1] It is known that the polymerization reaction does not result in complete conversion of the initial methacrylate moiety, and certain amounts of unreacted monomers remain in the polymer.[2,3] In addition, during clinical use, polymeric materials may undergo degradation due to mechanical, chemical, and thermal stresses.[4] Degradation can be caused by different mechanisms, such as oxidation, decomposition, and hydrolysis,[4–6] and may result in the formation of several by-products, among them methacrylic acid (MA)[5,7] and benzoic acid (BA).[7] MA is probably formed via hydrolysis or esterification of methacrylate groups.[6,7] BA is ascribable to the decomposition product of benzoyl peroxide used as polymerization initiator.[7]

Components eluted from acrylic resins into saliva may diffuse to the oral mucosa around the dentures. MA and BA[5,7] are potential sensitive/irritant agents, and may induce irritant and/or delayed allergic reactions.[8,9] It is therefore important to identify and quantify substances released from polymeric biomaterials,[10] and *in vitro* and *in vivo* studies have evaluated the release of leachable components from denture-based acrylic resins.[4,5,7] However, although hard chairside reline resins are widely used in prosthodontics, the leachability of degradation products from these materials has not been reported in the literature.

The elution of components from polymers has been shown to be dependent upon the degree of double bond conversion, size of the leachable species, hydrophilicity of the monomers, sorption/solubility behavior of the material, degree of crosslinking, and storage time.[5,6,10] Highly crosslinked polymers can be more resistant to degradative reactions, due to the more limited space and pathways available for molecules to diffuse within the structure.[6] The composition of the hard chairside

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TABLE I
Materials Evaluated in This Study

Product	Code	Batch number	Manufacturer	Powder (P)/ Liquid (L) ratio (g/mL)	Composition		Polymerization conditions
					Powder	Liquid	
Kooliner	K	0208282 (P) 0206251 (L)	Coe Laboratories, Inc., York, Chicago, IL	1.4/1.0	PEMA	IBMA	10 min at room temperature
New Truliner	N	0212-703 (P) 0401-050 (L)	Bosworth Company, Skokie, IL	1.34/1.0	PEMA	IBMA and DBNP	15 min at room temperature
Ufi Gel hard	U	531727 (P) 531172 (L)	Voco GmbH, Cuxhaven, Germany	1.77/1.0	PEMA	1,6-HDMA	7 min at room temperature
Tokuso Rebase Fast	T	4641 (P) 176B1 (L)	Tokuyama Dental Corp., Tokyo, Japan	2.056/1.0	PEMA	MAOP and 1,6-HDMA	8 min at room temperature

PEMA, poly(ethyl methacrylate); IBMA, isobutyl methacrylate; DBNP, dibutyl-*n*-phthalate; 1,6-HDMA, 1,6-hexanediol dimethacrylate; MAOP, β-methacryloyl oxyethyl propionate.

reline resins differs significantly from that of the poly(methyl) methacrylate (PMMA) denture-based acrylic resins, and varies considerably among different commercial products.[11] Moreover, some of the current available autopolymerizing reline resins are highly cross-linked.[11] This may influence water sorption and solubility[1,12] and, consequently the leachability of residual compounds.

Water-bath post-polymerization treatment has shown effectiveness in reducing amount[2] and leachability in artificial saliva[13] of residual monomer of polymerized acrylic resins. It can be assumed that a more complete polymerization could diminish the leaching of residual compounds, thus minimizing the risk of adverse reactions induced by methacrylate-based polymers.

Therefore, the aim of this *in vitro* study was to use a validated high performance liquid chromatographic (HPLC) method to quantify the leaching of MA and BA from hard chairside reline resins in artificial saliva. It was also evaluated the effect of water-bath post-polymerization treatment on the leaching process.

EXPERIMENTAL

The materials used in the current study, batch number, along with the manufacturer's compositions, powder/liquid ratios and polymerization conditions are listed in Table I. These materials were selected because they contain different types of monomer. In addition, Kooliner (K) and New Truliner (N) are noncrosslinked, whereas Ufi Gel hard (U) and Tokuso Rebase Fast (T) are crosslinked reline resins.

Specimen preparation

Specimen disks (50 mm in diameter and 2-mm thick) were prepared for each hard chairside reline resin using a stainless steel mold. The materials were mixed in accordance with the manufacturer's instructions (Table I) and were inserted into the metal mold. The specimens were covered with a polyester sheet and a glass plate.[13] The set was maintained under compression in a hydraulic bench press at room temperature (23 ± 2°C) for the polymerization time recommended by the manufacturers (Table I). After polymerization, excess material was carefully removed using 360-grit silicon carbide paper.[13]

Specimens of all materials were then divided into two groups (n = 6) as follows: Group C (control), in which the specimens were left untreated; Group WB (water-bath), in which the specimens were submitted to post-polymerization treatment by immersing in water-bath at 55 ± 1°C for 10 min. This treatment was based on the recommendations of one of the manufacturers of autopolymerizing reline resins to reduce the monomer taste. Moreover, in recent works, this treatment significantly reduced the amount[2] and leachability of residual monomer from hard chairside reline resins in artificial saliva.[13]

HPLC analysis

The HPLC system consisted of a Shimadzu LC-10AD pump (Shimadzu Corp., Kyoto, Japan) equipped with a system controller (CBM-10A), a UV–vis detector (SPD-10A) and an auto-injector (SIL-10F). The HPLC chromatograms were recorded by Shimadzu Class LC10 software. An LC-18 column (5 μm particle size, 100 Å pore size, 0.46 cm I.D. × 15 cm length) was used to achieve the chromatographic separations. The mobile phase (acetonitrile : water 20/80 at pH 3.0, adjusted by the addition of glacial acetic acid) eluted at a flow rate of 0.8 mL/min under isocratic mode for 20 min. The elution then changed to gradient mode of acetonitrile (20–100% during 3 min), and returned to 20% during 5 min. Thereafter, the mobile phase eluted under isocratic mode for additional 5 min for

column conditioning. Absorbance readings were performed at 230 nm.

Standard solutions were prepared in artificial saliva using appropriate stock solution in triplicate at the concentrations ranging from 1.0 to 128.0 μg/mL for MA and from 0.5 to 64.0 μg/mL for BA. Calibration curves were built by plotting the peak area against the concentration of each acid evaluated (MA and BA).

The HPLC method was developed and validated as described previously.[14] Briefly, the validation parameters for MA and BA were as follows: linearity, 1.0–128.0 μg/mL ($R^2 = 0.99791$) and 0.5–64.0 μg/mL ($R^2 = 0.99844$); accuracy, 96.1–112% and 95.2–106.0%; intra- and interday precision, 0.3–8.1% and 0.4–7.1%; limits of quantification, 1.0 and 0.5 μg/mL; limits of detection, 0.75 and 0.1 μg/mL, respectively. After that, the method was used to quantify the leached compounds.

Each specimen was individually placed in closed plastic containers with 20 mL of artificial saliva at 37 ± 1°C, protected from light during 30 days. Artificial saliva was composed of NaCl (0.4 g), KCl (0.4 g), $CaCl_2{\cdot}2H_2O$ (0.795 g), $NaH_2PO_4{\cdot}2H_2O$ (0.78 g), $Na_2S{\cdot}9H_2O$ (0.005 g), urea (1.0 g), and distilled water (1000 mL), at neutral pH as previously described.[13,15] To determine the amount of MA and BA released from the hard chairside reline resins, aliquots (200 μL) from each immersion solution were analyzed 1, 3, 5, and 24 h and 3, 7, 14, and 30 days after specimen preparation. This 30-day period is longer than those used previously[5,7] and was selected to allow the diffusion of residual compounds and by-products that could be located in the inner part of the resin. For the periods up to 24 h, the liquid in the container was replaced with fresh artificial saliva (20 mL) after each aliquot was taken. After 24-h interval, the artificial saliva was daily replaced for 30 days.[13] For the saliva replacements, each specimen was washed with deionized water, dried with absorbent paper and immersed into the fresh artificial saliva. The aliquots were subjected to HPLC analysis immediately after sampling. For each reline resin, the concentration (μg/mL) of MA and BA in each sample solution was calculated using the respective linear regression equation from the calibration graphs.

Statistical analysis

The amount of released MA and BA was calculated based on the calibration graphs by taking the area under the chromatographic bands and expressing it in μg/mL. Differences in the amount of leached compounds among intervals within each material were tested for significance using Wilcoxon's signed rank sum test. For each material, Mann–Whitney two-sample test was used to evaluate the effectiveness of the post-polymerization treatment on the leaching of degradation products at each immersion period. Kruskal–Wallis test was used to investigate the differences between materials within the same experimental condition at each time interval. All tests were performed at 95% confidence level.

RESULTS AND DISCUSSION

In this study, a HPLC method was developed and validated for the quantification of the degradation products MA and BA, which were detected leaching out of all reline resins. Figure 1 illustrates typical chromatograms of standards and reline resins. To the author's knowledge, to date, no studies concerning the leachability of degradation products from hard chairside reline resins have been conducted.

MA is probably formed via hydrolysis[6,7] or esterification of methacrylate groups (Fig. 2). Although the results of a previous study[2] have demonstrated that Ufi Gel hard material had lower residual monomer content than New Truliner and Kooliner hard chairside reline resins, this material released significantly higher ($P < 0.05$) amount of MA (17.9 μg/mL) than the others at the first hour of immersion (Table II). For the other time intervals, no significant difference was observed among the control groups of the four reline resins ($P > 0.05$). As 1,6-hexanediol dimethacrylate is a diester monomer, two MA molecules can be formed by its hydrolysis (Fig. 2). This might explain the higher percentages of the MA formed when Ufi Gel hard material was tested. However, although the liquid composition of Tokuso Rebase Fast also comprises 1,6-hexanediol dimethacrylate monomer, this reline resin released less MA in comparison with the other materials, including Ufi Gel hard. This difference could be related to a number of factors. Firstly, Ufi Gel hard demonstrated significantly higher residual monomer content[2] and lower degree of conversion[16] than Tokuso Rebase Fast. Accordingly, a previous study on the leachability of these materials showed that higher amount of 1,6-hexanediol dimethacrylate was released from Ufi Gel hard.[13] In addition, this material undergoes rapid polymerization and quick solidification.[17] Therefore, during the mixture between powder and liquid components, air voids are likely to be more entrapped, thus resulting in high porosity, as previously observed.[18,19] Taking all these results into consideration, it can be assumed that the crosslink density of Ufi Gel hard is lower than that of Tokuso Rebase Fast and this may have facilitated the release of MA through the matrix.[6]

The amount of MA released from Ufi Gel hard control specimens significantly decreased ($P < 0.05$) from 1 h (17.9 μg/mL) to 3 h (2.57 μg/mL) of

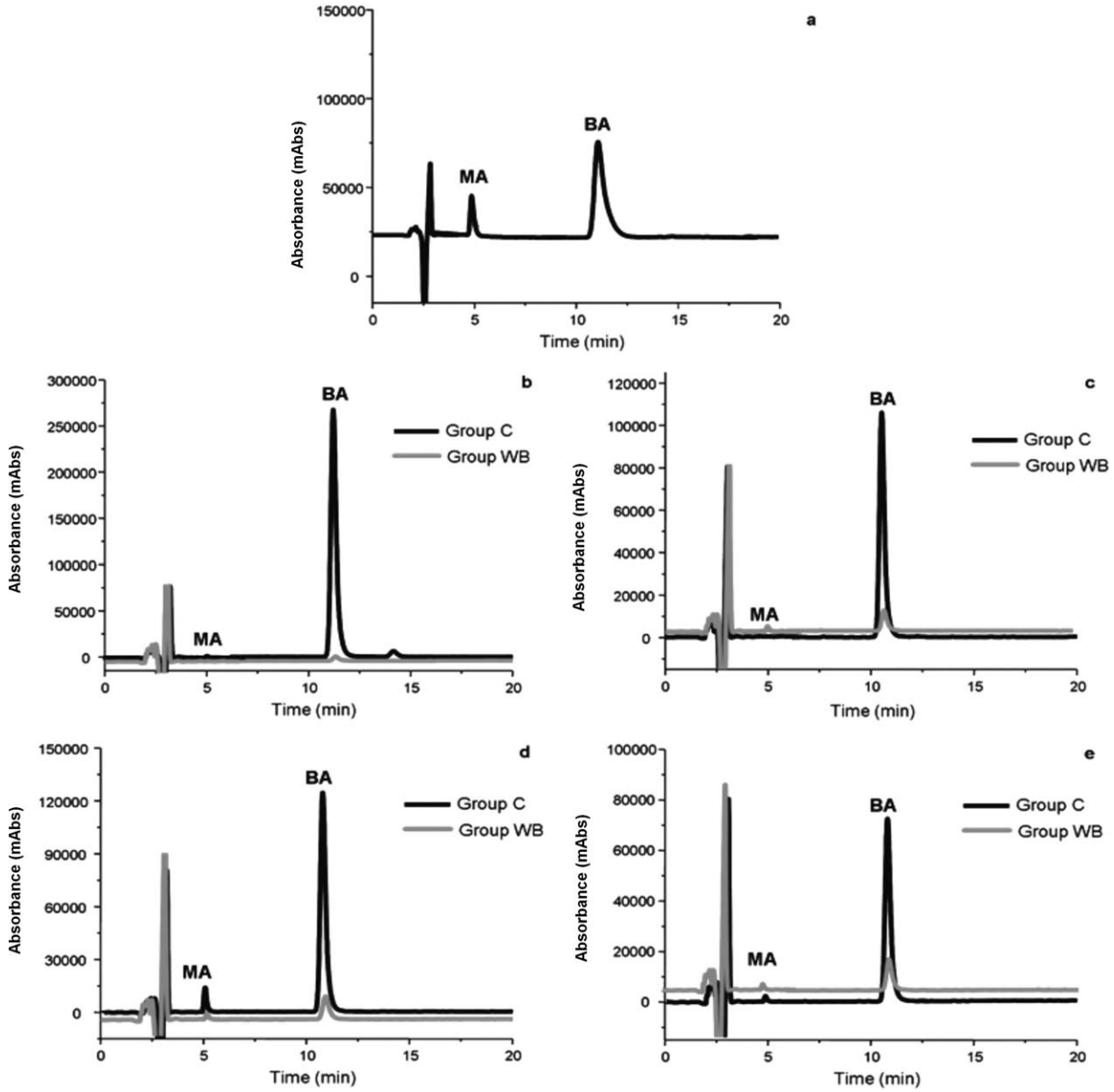

Figure 1 HPLC chromatograms obtained from a standard solution (a) and from the hard chairside reline resins [(b) Kooliner; (c) New Truliner; (d) Ufi Gel hard; and (e) Tokuso Rebase Fast] immersed in artificial saliva for 1 h. Group C, control; Group WB, water-bath post-polymerization treatment.

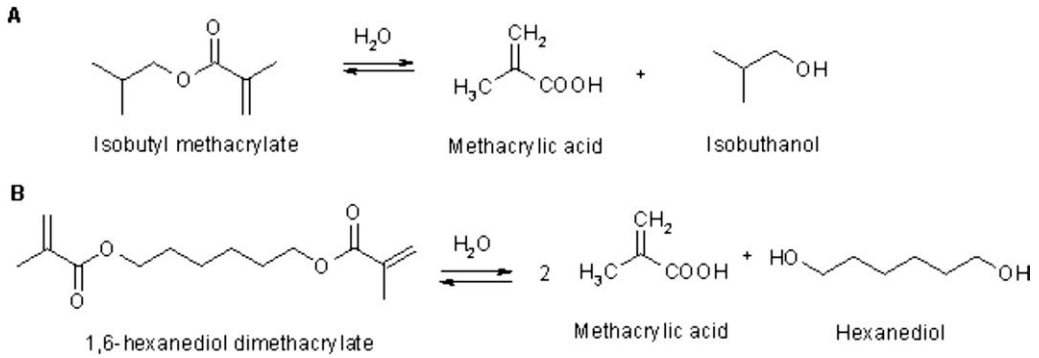

Figure 2 Mechanism for MA production by esterification of isobutyl methacrylate (IBMA) (a) and 1,6-hexanediol dimethacrylate (1,6-HDMA) (b).

TABLE II
Median (Maximum–Minimum) values (μg/mL) of MA Released from Each Reline Material for Control (C) and Treated (WB) Groups at Each Time Interval

			Time interval							
Material	Group	Concentration	1 h	3 h	5 h	24 h	3 d	7 d	14 d	30 d
K	C	Median	–	–	–	–	3.76[a]	–	1.25	–
		Maximum	2.91	2.55	2.67	28.8	15.8	–	3.11	–
		Minimum	–	–	–	–	–	–	–	–
	WB	Median	–	–	–	–	–	–	–	2.16[A]
		Maximum	–	1.98	–	1.88	4.39	4.13	–	3.49
		Minimum	–	–	–	–	–	–	–	–
N	C	Median	–	–	2.60[a]	–	–	–	–	–
		Maximum	–	2.76	4.52	–	4.97	2.62	–	–
		Minimum	–	–	–	–	–	–	–	–
	WB	Median	–	–	–	–	–	–	–	2.19[A]
		Maximum	–	3.26	2.19	5.52	2.36	–	–	6.06
		Minimum	–	–	–	–	–	–	–	–
U	C	Median	17.9	2.57[a]*	1.95[a]	2.14[a]	–	–	–	–
		Maximum	21.9	4.95	3.59	5.03	29.71	2.11	1.73	–
		Minimum	8.64	–	–	–	–	–	–	–
	WB	Median	1.16	1.04[A]	–	2.22[A]	–	2.08	–	–
		Maximum	3.58	2.43	2.45	4.32	15.97	3.25	2.33	7.05
		Minimum	–	–	–	–	–	–	–	–
T	C	Median	–	1.60[a]	–	2.00[a]	3.05[a]	–	–	–
		Maximum	–	3.97	2.21	5.17	10.08	4.20	–	–
		Minimum	–	–	–	–	–	–	–	–
	WB	Median	–	0.89[A]	–	0.99[A]	3.04	–	–	–
		Maximum	2.92	4.49	2.63	3.46	8.74	3.30	–	4.08
		Minimum	–	–	–	–	–	–	–	–

"–" Concentrations below the detection limit. Within the rows, "*" denotes a significant difference compared with the immediately preceding time interval. For each material, vertical bars indicate significant between-group (control and water-bath) differences. Within the columns, identical superscript small letters indicate no significant between-materials differences, when control specimens (group C) were compared. Within the columns, identical superscript capital letters indicate no significant between-materials differences, when treated specimens (group WB) were compared.

immersion in artificial saliva (Table II), whereas no significant changes were detected throughout the immersion period for the other reline resins ($P > 0.05$). The findings related to Ufi Gel hard control specimens are in agreement with previous studies, which showed that residual monomer values decreased with increasing time.[5,20] However, as one of the mechanisms involved in the reduction of residual methacrylate monomer is its hydrolysis to MA, an increase in the release of MA with increasing time would be expected.[7] The fact that MA is unstable and may polymerize[21] may help to explain the decrease observed for material Ufi Gel hard. In addition, MA undergoes oxidation to CO_2 and H_2O and a smaller part escapes as methylmalonate, succinate, and possibly as β-hydroxy-isobutyrate[22] or is promptly converted into pyruvic acid. Hence, the decrease in the concentration of MA released from material Ufi Gel hard may be also due to degradation of this unstable compound.

Compared with control specimens, water-bath post-polymerization treatment significantly reduced ($P < 0.05$) the amounts of MA released from material Ufi Gel hard after 1 h (1.16 μg/mL) and 3 h (1.04 μg/mL) of immersion (Table II). During this treatment, the specimens were immersed in water at 55 ± 1°C, so the increased temperature of the water probably allowed the water molecules to diffuse more rapidly into the polymer.[23] As a result, most of the MA may have leached out into water during the post-polymerization treatment. In previous studies, it was also observed that the residual monomer content and the residual monomer leached out from some reline resins were significantly reduced by this treatment.[2,13] This reduction has been attributed to both diffusion of residual monomer into water[13,20] and post-polymerization.[2,13,20] Thus, it can be assumed that less residual monomer molecules were available to be hydrolyzed to form MA after Ufi Gel hard specimens were heat-treated. The water-bath post-polymerization treatment had no significant effect on the amounts of MA released from the other reline resins ($P > 0.05$), which showed low leachability of MA at the earlier stage of immersion.

When the specimens submitted to the post-polymerization treatment were compared, no significant differences were observed among materials at all

TABLE III
Median (Maximum–Minimum) Values (μg/mL) of BA Released from Each Reline Material for Control (C) and Treated (WB) Groups at Each Time Interval

			Time interval							
Material	Group	Concentration	1 h	3 h	5 h	24 h	3 d	7 d	14 d	30 d
K	C	Median	11.68^{a}	5.88^{ab}	2.78^{a}*	9.83^{a}*	3.51^{a}*	1.46^{a}*	1.02^{a}	1.03^{a}
		Maximum	41.17	39.17	11.20	37.37	5.95	3.54	2.87	1.45
		Minimum	5.63	4.96	1.85	–	0.84	–	–	–
	WB	Median	1.69^{AB}	1.54^{A}	1.02^{A}	7.10^{A}*	1.35^{A}*	1.53^{A}	–	–
		Maximum	1.86	1.94	5.26	12.08	4.47	2.48	1.02	0.82
		Minimum	1.39	0.90	–	–	–	–	–	–
N	C	Median	25.03^{b}	11.83^{b}	4.30^{a}*	32.33^{b}*	7.10^{a}*	2.94^{a}*	2.27^{a}	1.44^{a}
		Maximum	39.40	24.26	8.41	44.15	10.00	6.62	5.06	2.67
		Minimum	9.24	9.05	1.04	30.63	1.20	–	–	–
	WB	Median	3.20^{B}	2.93^{A}	2.09^{A}	10.60^{A}*	4.94^{A}*	0.50^{A}	0.45^{A}	–
		Maximum	4.64	5.55	4.09	21.87	7.50	6.00	2.23	0.80
		Minimum	1.17	–	0.86	7.05	–	–	–	–
U	C	Median	17.77^{b}	8.56^{ab}*	4.94^{a}*	17.76^{ab}*	5.36^{a}*	3.10^{a}*	2.32^{a}	1.46^{a}*
		Maximum	20.40	17.66	9.13	24.76	7.17	3.55	2.89	1.71
		Minimum	13.97	4.94	1.73	9.59	0.84	–	0.78	–
	WB	Median	3.58^{B}	2.53^{A}*	2.00^{A}	6.58^{A}*	2.87^{A}	0.43^{A}*	0.44^{A}	–
		Maximum	5.42	4.46	3.66	13.65	4.89	1.34	1.07	–
		Minimum	1.03	–	–	0.93	–	–	–	–
T	C	Median	6.36^{a}	5.77^{a}	1.19^{a}*	13.10^{a}*	3.39^{a}*	0.42^{a}*	–	0.78^{a}
		Maximum	9.19	10.35	3.79	16.26	5.65	2.60	2.14	1.11
		Minimum	4.60	2.39	–	7.43	0.87	–	–	–
	WB	Median	1.01^{A}	1.17^{A}*	1.00^{A}*	4.87^{A}*	0.41^{A}*	–	–	–
		Maximum	2.68	6.11	3.83	12.85	1.80	1.79	–	–
		Minimum	–	0.80	0.77	0.96	–	–	–	–

"–" Concentrations below the detection limit. Within the rows, "*" denotes a significant difference compared with the immediately preceding time interval. For each material, vertical bars indicate significant between-group (control and water-bath) differences. Within the columns, different superscript small letters indicate significant between-materials differences, when control specimens (group C) were compared. Within the columns, different superscript capital letters indicate significant between-materials differences, when treated specimens (group WB) were compared.

time intervals ($P > 0.05$). Nevertheless, concentrations of the MA were detected for all materials even after 30 days of immersion in artificial saliva (Table II). This may be attributed to the slow hydrolysis of entrapped unreacted monomer molecules within the heat-treated specimens. If the additional conversion expected with heating resulted in a more crosslinked polymer matrix, MA formed in the polymer would be much more difficult to diffuse from these specimens.[6] However, further investigation is required to clarify such hypothesis. In addition, the long-term release of MA from these materials should be considered in future studies.

For all control specimens, the concentrations of BA significantly decreased ($P < 0.05$) within the first 5 h of immersion (Table III). BA is ascribable to the decomposition product of benzoyl peroxide used as polymerization initiator.[7] It is known that, in the first hours after processing, the polymerization reaction still proceeds at the sites of active radicals,[20] resulting in a decrease in benzoyl peroxide availability, which may have accounted, in part, for the fall in concentration of BA released. When the specimens were heat-treated, the further polymerization reaction may have been accelerated and the peroxide consumed more quickly.[24] As a result, significantly lower BA was released from the treated specimens compared with controls in the initial periods of immersion (Table III).

Concentrations of BA leached from all specimens (control and treated) increased ($P < 0.05$) at the first day of immersion and then gradually decreased during the monitoring period. A previous study found that a high amount of residual monomer was released from Kooliner, New Truliner, Ufi Gel hard, and Tokuso Rebase Fast up to 24 h of immersion in artificial saliva and the elution was significantly reduced after this time.[13] Thus, in the present investigation, it is likely that, at the 24-h period, the residual monomer content was significantly reduced. In addition, the residual monomer molecules were probably entrapped in the formed polymer network, decreasing their mobility within the matrix. These may have limited the further polymerization process. Therefore, it is possible that the remaining peroxide was less consumed. Concurrently, if the water absorption increases during the 24 h,[25] the diffusion of the formed BA is then facilitated, as observed at

the 24-h interval. The diffusion of the BA from the reline resins to artificial saliva may also help explain the decrease in the amount of this degradation product with time.

At the 1-, 3-, and 24-h interval analysis, significant differences were found between control specimens of the materials ($P < 0.05$). Tokuso Rebase Fast resin leached less BA than New Truliner. These results are reasonably consistent with the chemical formulation of these reline resins (Table I). Tokuso Rebase Fast has 1,6-hexanediol dimethacrylate as monomer, whereas New Truliner includes isobutyl methacrylate in its liquid composition. The use of dimethacrylates, such as 1,6-hexanediol dimethacrylate, can enhance the polymerization, by increasing the reactivity with the second methacrylate group,[26] and consequently, the consumption of benzoyl peroxide. In addition, Tokuso Rebase Fast also contains β-methacryloyl oxyethyl propionate.[11] Probably, after the initiation period, the presence of this bifunctional monomer along with 1,6-hexanediol dimethacrylate resulted in a more complete polymerization. This result corroborates the results from previous studies, in which Tokuso Rebase Fast had the lowest amount of residual monomer and also released less residual monomer when compared with the other evaluated reline resins.[2,13]

After 1 h of immersion, WB specimens of all materials released significantly lower ($P < 0.05$) amounts of BA than did the control groups (Table III). Water-bath post-polymerization treatment also significantly decreased BA release at 3 h of immersion for materials Kooliner, New Truliner, and Ufi Gel hard, and at 24 h of immersion for materials New Truliner, Ufi Gel hard, and Tokuso Rebase Fast ($P < 0.05$). In addition, there were no significant differences among treated materials at all time intervals ($P > 0.05$). The only exception was observed at the first hour-period for material Tokuso Rebase Fast, which showed lower BA release than materials New Truliner and Ufi Gel hard. As stated before, when specimens are treated with water-bath, the post-polymerization at the sites of active radicals[20] was probably accelerated,[24] so the remaining peroxide is consumed more quickly than in the untreated specimens. In addition, particularly for BA, the extraction rate is directly dependent on temperature: the higher the temperature, the higher the rate of extraction.[5] Thus, the decrease in BA leachability could also be related to the heating of acrylic resins during the post-polymerization treatment, which may have enhanced the diffusion of this by-product.

Results from a previous study[27] showed that Ufi Gel hard enhanced the mitochondrial activity of L929 fibroblasts, which may reflect a compensatory response of cell enzyme activity to resin-associated toxicity.[28] Furthermore, Campanha et al.[27] observed an increased cytotoxicity of some reline resins even after the water-bath post-polymerization treatment. It has been previously demonstrated that Ufi Gel hard presents low residual monomer content compared with other reline resins and that the water-bath post-polymerization treatment significantly reduced the residual monomer content of reline acrylic resins.[2] On the other hand, Campanha et al.[27] also have suggested that the cytotoxicity may be related to the leaching of other compounds, such as additives, by-products from the free radical polymerization, degradation products, impurities, or products formed from the decomposition of benzoyl peroxide.[4] Thus, cytotoxicity assays evaluating the effects of the concentrations of MA and BA acids obtained in the current study are being undertaken to determine if such concentrations of these acids may induce cytotoxic responses.

CONCLUSIONS

Within the limitations of this *in vitro* study, the following conclusions were drawn:

1. MA and BA acids were detected leaching out of all reline resins;
2. The highest concentration of MA was leached from control specimens of Ufi Gel hard.
3. For all control specimens, the leachability of BA progressively decreased with increasing immersion time.
4. The water-bath post-polymerization treatment reduced the amounts of MA released from Ufi Gel hard after 1 and 3 h of immersion. The concentrations of MA released from the other reline resins were not significantly changed by the post-polymerization treatment.
5. The water-bath post-polymerization treatment decreased the amounts of BA leached from all materials after 1 h of immersion. This treatment also reduced BA release at 3 h of immersion for Kooliner, New Truliner, and Ufi Gel hard, and at 24 h of immersion for New Truliner, Ufi Gel hard, and Tokuso Rebase Fast.

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References

1. Seó, R. S.; Vergani, C. E.; Giampaolo, E. T.; Pavarina, A. C.; Reis, J. M. S. N.; Machado, A. L. J Appl Polym Sci 2008, 107, 300.
2. Urban, V. M.; Machado, A. L.; Oliveira, R. V.; Vergani, C. E.; Pavarina, A. C.; Cass, Q. B. Dent Mater 2007, 23, 363.
3. Lung, C. Y.; Darvell, B. W. Dent Mater 2005, 21, 1119.
4. Lygre, H.; Solheim, E.; Gjerdet, N. R. Acta Odontol Scand 1995, 53, 75.

5. Sofou, A.; Tsoupi, I.; Emmanouil, J.; Karayannis, M. Anal Bioanal Chem 2005, 381, 1336.
6. Ferracane, J. L. Dent Mater 2006, 22, 211.
7. Koda, T.; Tsuchiya, H.; Yamauchi, M.; Ohtani, S.; Takagi, N.; Kawano, J. Dent Mater 1990, 6, 13.
8. Bohnenkamp, D. M. J Prosthet Dent 1996, 76, 113.
9. Ruiz-Genao, D. P.; Moreno de Veja, M. J.; Sanchez Perez, J.; Garcia-Diez, A. Contact Dermatitis 2003, 48, 273.
10. Örtengren, U.; Wellendorf, H.; Karlsson, S.; Ruyter, I. E. J Oral Rehabil 2001, 28, 1106.
11. Arima, T.; Murata, H.; Hamada, T. J Prosthet Dent 1995, 73, 55.
12. Arima, T.; Murata, H.; Hamada, T. Oral Rehabil 1996, 23, 346.
13. Urban, V. M.; Machado, A. L.; Vergani, C. E.; Giampaolo, E. T.; Pavarina, A. C.; De Almeida, F. G.; Cass, Q. B. Dent Mater 2009, 25, 662.
14. Urban, V. M.; Cass, Q. B.; Oliveira, R. V.; Giampaolo, E. T.; Machado, A. L. Biomed Chromatogr 2006, 20, 369.
15. Fusayama, T.; Katayori, T.; Nomoto, S. J Dent Res 1963, 42, 1183.
16. Urban, V. M.; Machado, A. L.; Vergani, C. E.; Jorge, E. G.; Santos, L. P. S.; Leite, E. R.; Canevarolo, S. V. Mater Res 2007, 10, 191.
17. Yannikakis, S.; Polychronakis, N.; Zissis, A. Eur J Prosthodont Restor Dent 2010, 18, 84.
18. Bulad, K.; Taylor, R. L.; Verran, J.; McCord, J. F. Dent Mater 2004, 20, 167.
19. Novais, P. M. R.; Giampaolo, E. T.; Vergani, C. E.; Machado, A. L.; Pavarina, A. C.; Jorge, J. H. Gerodontology 2009, 26, 65.
20. Lamb, D. J.; Ellis, B.; Priestley, D. J Dent 1983, 11, 80.
21. Morawetz, H.; Rubin, I. D. J Polym Sci 2003, 57, 687.
22. Bratt, H.; Hattway, D. E. Br J Cancer 1977, 36, 114.
23. Braden, M. J Prosthet Dent 1964, 14, 307.
24. Araújo, P. H. H.; Sayer, C.; Poço, J. G. R.; Giudici, R. Polym Eng Sci 2002, 42, 1442.
25. Joshi, N. P.; Sanghvi, S. J. J Pierre Fauchard Acad 1994, 8, 97.
26. Ruyter, I. E.; Svendsen, S. A. J Prosthet Dent 1980, 43, 95.
27. Campanha, N. H.; Pavarina, A. C.; Giampaolo, E. T.; Machado, A. L.; Carlos, I. Z.; Vergani, C. E. Int J Prosthodont 2006, 19, 195.
28. Lefebvre, C. A.; Knoernschild, K. L.; Schuster, G. S. J Prosthet Dent 1994, 72, 644.