Cytotoxicity of Hard Chairside Reline Resins: Effect of Microwave Irradiation and Water Bath Postpolymerization Treatments

Nara Hellen Campanha, DDS[a]/Ana Cláudia Pavarina, DDS, MSc, PhD[b]/
Eunice Teresinha Giampaolo, DDS, MSc, PhD[c]/Ana Lucia Machado, DDS, MSc, PhD[c]/
Iracilda Zeppone Carlos, BPS, MSc, PhD[d]/Carlos Eduardo Vergani, DDS, MSc, PhD[c]

Purpose: To evaluate the influence of water bath and microwave postpolymerization treatments on the cytotoxicity of 6 hard reline acrylic resins. ***Materials and Methods:*** The materials tested were Tokuso Rebase Fast (TR), Ufi Gel Hard (UGH), Duraliner II (D), Kooliner (K), New Truliner (NT), and Light Liner (LL). LL resin was additionally tested with an air-barrier coating (LLABC). Nine disks of each material (10 × 1 mm) were made and divided into 3 groups: group 1 (no postpolymerization treatment); group 2 (postpolymerization in microwave oven); group 3 (postpolymerization in water bath at 55ºC for 10 minutes). L929 cells were cultured in 96-well plates and incubated for 24 hours in Eagle's medium. Eluates prepared from the disks or medium without disks (control) replaced the medium. Cytotoxicity was assessed by both dehydrogenase succinic activity (MTT) assay and incorporation of radioactive ^{3}H-thymidine assay. Tests were carried out in quadruplicate and repeated twice. Differences between groups were determined by analysis of variance with Tukey multiple-comparison intervals (α = .05). ***Results:*** For MTT assay, the postpolymerization treatments had no effect on the cytotoxicity of all materials (P > .05). For ^{3}H-thymidine assay, the postpolymerization treatments significantly decreased the cytotoxicity of UGH (P < .05). The cytotoxicity of K, NT, LL, and LLABC increased after microwave irradiation (P < .05). TR, NT, and LLABC showed an increase in cytotoxicity after water bath (P < .05). ***Conclusion:*** When assessed by MTT assay, the cytotoxicity of the materials was not affected by postpolymerization treatments. ^{3}H-Thymidine assay showed that the cytotoxicity of the resins was not improved by the postpolymerization treatments, with the exception of UGH. *Int J Prosthodont 2006;19:195–201.*

The fit of dentures progressively declines as a result of time-dependent changes in the supporting tissue. Hard chairside reline acrylic resins are proposed for temporary or permanent improvement of denture fit. These autopolymerizing acrylic resins allow the clinician to reline a removable prosthesis directly in the mouth in intimate contact with a large area of oral mucosa. However, with autopolymerizing acrylic resins, the conversion of monomers to polymers may not be complete, and some unreacted monomers could be left in the polymer.[1–3] Residual monomers and other leachable compounds elute from autopolymerized resins at higher concentrations than from heat- and microwave-polymerized denture base resins[4,5] and can induce tissue reactions such as swelling or reddening of the oral mucosa. Therefore, the cytotoxicity of residual monomers and other leachable compounds released from denture base polymers has been a major concern.[6–11]

Methods for reducing the residual monomer contents of polymerized acrylic resins have been described in the literature. Tsuchiya et al[11] recommended the immersion of acrylic resin dentures in hot water (50ºC

[a]*Postgraduate Student, Department of Dental Materials and Prosthodontics, São Paulo State University, Araraquara Dental School, São Paulo, Brazil.*
[b]*Assistant Professor of Removable Prosthodontics, Department of Dental Materials and Prosthodontics, São Paulo State University, Araraquara Dental School, São Paulo, Brazil.*
[c]*Associate Professor, Department of Dental Materials and Prosthodontics, São Paulo State University, Araraquara Dental School, São Paulo, Brazil.*
[d]*Associate Professor, Department of Clinical Analysis, São Paulo State University, Araraquara Pharmaceutical School, São Paulo, Brazil.*

Correspondence to: *Prof Dr Ana Cláudia Pavarina, Faculdade de Odontologia de Araraquara, UNESP Rua Humaitá, no. 1680, Araraquara, São Paulo, Brazil C.E.P. 14801-903. Fax: +55-016-33016406. E-mail: pavarina@foar.unesp.br*

Table 1 Hard Chairside Reline Resins Tested

Code	Brand name	Composition		Manufacturer	Batch number		Power/ liquid ratio	Postpolymerization treatments	
		Liquid	Powder		Liquid	Powder	(g/mL)	Microwave	Water bath
K	Kooliner	IBMA	PEMA	GC America	062900A	080700A	1.4/1	5 min at 550W	10 min at 55ºC ± 2ºC
D	Duraliner II	BMA and EGDMA	PEMA	Reliance Dental	012201	031501	1.0/1	4 min at 650W	10 min at 55ºC ± 2ºC
TR	Tokuso Rebase Fast	MAOP and 1,6-HDMA	PEMA	Tokuyama Dental	094	437	2.05/1	4 min at 550W	10 min at 55ºC ± 2ºC
UGH	Ufi Gel Hard	1,6-HDMA	PEMA	Voco	2210	2210	1.76/1	5 min at 500W	10 min at 55ºC ± 2ºC
NT	New Truliner	IBMA	PEMA	Bosworth	0105-308-X	0105-308-X	1.34/1	3 min at 500W	10 min at 55ºC ± 2ºC
LL	Light Liner	BMA	PEMA	Bosworth	0103-133	0103-133	1.50/1	3 min at 500W	10 min at 55ºC ± 2ºC
LLABC	Light Liner ABC	BMA Polyol polyester	PEMA	Bosworth	0103-133	0103-133	1.50/1	3 min at 500W	10 min at 55ºC ± 2ºC

IBMA = isobutyl methacrylate; PEMA = poly(ethyl methacrylate); BMA = butyl methacrylate; EGDMA = ethylene glycol dimethacrylate; MAO = β-methacryloyl oxyethyl propionate; 1,6-HDMA = 1,6-hexanediol dimethacrylate; IBMA = isobutyl methacrylate; ABC = air barrier coating.

for 60 min) before insertion, especially for autopolymerized resins, to minimize the risk of adverse reactions in patients who wear acrylic resin dentures. Shim and Watts[12] demonstrated that a further heat-polymerization cycle had a significant effect on reducing monomer concentrations in 2 denture base resins. Baker et al[13] concluded that autopolymerized appliances should be immersed for 24 hours in water before being worn to minimize the possibility of residual monomer release. Lee et al[5] concluded from their study that autopolymerization of acrylic resins in water was the key factor for reducing the quantity of residual monomer. According to findings of Yunus et al[14] and Blagojevic and Murphy,[1] lower levels of residual monomer and improved mechanical properties were achieved with microwave irradiation after the initial autopolymerization of acrylic resin repair materials. However, the effectiveness of this procedure is determined by factors such as irradiation time and microwave power.[15,16]

Although a previous study demonstrated that water bath and microwave postpolymerization treatments did not influence the cytotoxicity of 3 heat-polymerizing denture base resins,[7] the effect of these treatments on the cytotoxicity of autopolymerizing reline resins still remains to be investigated. Therefore, in the present study, the cytotoxicity of 6 hard chairside reline resins was assessed in vitro by the cell viability test (MTT) and by the incorporation of radioactive ^{3}H-thymidine. The hypothesis that water bath and microwave postpolymerization treatments could decrease the cytotoxicity of the resins was tested.

Materials and Methods

The product names, codes, compositions, manufacturers, batch numbers, powder/liquid proportions, and postpolymerization treatments of the test materials used in the present study are listed in Table 1.

Specimen Preparation

Nine specimens of each resin were prepared under aseptic conditions. A stainless steel mold with a breakaway compartment (10 mm in diameter by 1 mm thick)[8] was made to fabricate specimens of the various resins. Each material was mixed according to the manufacturer's instructions and applied into the mold, which was placed on an acetate sheet and a glass slab. Another acetate sheet and glass slab were placed over the material, and light pressure was applied to remove excess material from the mold. Photo-curing of LL specimens was performed with a Bosworth Light Cure Unit a short time (3 min) after the polymerization process started. To judge the effect of the oxygen inhibition layer on the cytotoxicity of the visible light–polymerized resin LL, 9 further specimens were polymerized with the air-barrier coating (LLABC) provided by the manufacturer. The air barrier was not removed after light polymerization. After polymerization, the specimens were separated from the molds and the edges were carefully smoothed.

Specimens were divided into 3 test groups. In group 1, specimens were not submitted to any postpolymerization treatment. In group 2, specimens were postpolymerized in a microwave oven (Sensor Crisp 38, Double Emission System, Brastemp) in a dry state (Table 1). The microwave power/time settings used for materials UGH, TR, K, and D were determined in a preliminary study,[16] which evaluated the effect of 9 different power/time combinations on the flexural strengths of these materials. The power/time setting that produced the highest flexural strength value for each material was used in this study. The power/time setting used for postpolymerization of the materials NT and LL was selected based on results from Ilbay et al,[17] who studied the effect of different conditions of power and time on some physical and mechanical properties

of an acrylic resin that was microwave polymerized. In group 3, specimens were postpolymerized in a water bath at 55°C for 10 minutes, following the manufacturer of material D's recommendation to reduce the monomer taste.

Prior to cytotoxicity tests, specimens were ultrasonically cleaned in distilled water for 20 minutes and then exposed to ultraviolet light in dry conditions at room temperature for another 20 minutes to kill any microorganisms that may had contaminated the disks during fabrication.[7,10]

Eluate Preparation

Eluates of the materials were prepared by placing 3 disks into a sterile glass vial with 9 mL of Eagle's minimum essential medium (Instituto Adolfo Lutz) supplemented with 80 mg/mL gentamycin and fetal bovine serum (Instituto Adolfo Lutz); these were then incubated at 37°C for 24 hours. Medium without disks was also incubated as above to serve as the negative control.[7,8,18]

Cytotoxicity Tests

Mossmann's cytotoxicity test (MTT) and ^{3}H-thymidine proliferation test were carried out in L-929 mouse fibroblasts.[19] These assays reflect cellular processes at the protein (mitochondrial metabolism) and the DNA (synthetic) levels, respectively. For this purpose, 104 cells were plated in 96-well plates, and after 24 hours attachment (at 37°C in an atmosphere of 55 CO_2/95% air), cells were re-fed with fresh medium containing appropriate eluates or control medium at a final concentration of 1:1 (v:v).[7,18]

MTT Assay

After 24 hours of cell growth in either the control or the test culture medium, the medium was replaced with 10 mL/well of 5 mg/mL MTT solution (Sigma Chemical) in fresh medium and re-incubated for 3 hours.[6] After the incubation period, the cultures were removed from the incubator, and the resulting formazan crystals were dissolved by adding 100 mL of MTT solubilization solution (Sigma Chemical). Plates were then shaken until crystals were completely dissolved, and the absorbance was spectrophotometrically measured at a wavelength of 540 nm (Labsystems Multiscan Ascent, Thermo Labsystems).[20] The background absorbance was measured at 620 nm and subtracted from the 540-nm measurement. Four wells were fabricated from each experimental group. Tests were repeated twice.[7]

^{3}H-Thymidine Assay

DNA synthesis in fibroblasts was assessed by measuring the incorporation of ^{3}H-thymidine (Amershan Pharmacia Biotech do Brasil).[7,21] Cells were exposed to radioactive DNA labeling at a final concentration of 0.25 mCi/mL^{-1} in the medium concomitantly with eluate or control medium. Cells were released from the bottom of the wells by the addition of 100 mL of trypsin 0.25% at 24 hours from the time of contact of eluates and radioisotope.[21] Cultures were harvested onto glass fiber filter plates using a multichannel automated cell harvester (Unifilter 96 GF/C, Packard Instrument Company). The incorporated radioactivity was measured using a plate scintillation counter (Unifilter 96 GF/C). Cell proliferation was determined from counts per minute (cpm) as the mean value of 4 replicates.[21] Tests were repeated 2 additional times.

Statistical Analysis

Statistical analysis of the data was performed using two-way analysis of variance (ANOVA) and Tukey test ($\alpha = .05$) to determine differences in cytotoxicity based on material and postpolymerization treatment. The extent of DNA synthesis inhibition was calculated based on comparison of the incorporated radioactivity in the experimental cultures and those of the control cultures.

Results

Analysis with two-way ANOVA indicated that the material types and postpolymerization treatments had statistically significant interactions ($P < .05$) in affecting DNA synthesis. Figure 1 shows the effects of eluates from the 6 materials at each postpolymerization group on DNA synthesis. There was a significant inhibitory response of the cells produced by the UGH and D eluates from group 1. In addition, there was significant inhibition of DNA synthesis in cells exposed to group 3 eluates from TR, D, NT, and LLABC resins and to group 2 eluates from K, D, NT, LL, and LLABC resins.

The results of statistical analysis showed that cytotoxicity as assessed by the MTT assay was dependent on the type of acrylic resin. Eluates from disks of the NT, LL, and LLABC resins produced a significant decrease in cell viability compared with the control group (Fig 2).

Discussion

In this study, the cytotoxicity of several hard chairside reline resins was assessed by incorporation of radioactive ^{3}H-thymidine and dehydrogenase succinic activity (MTT). It was suggested that MTT or DNA synthesis activity alone was not very predictive for in vitro

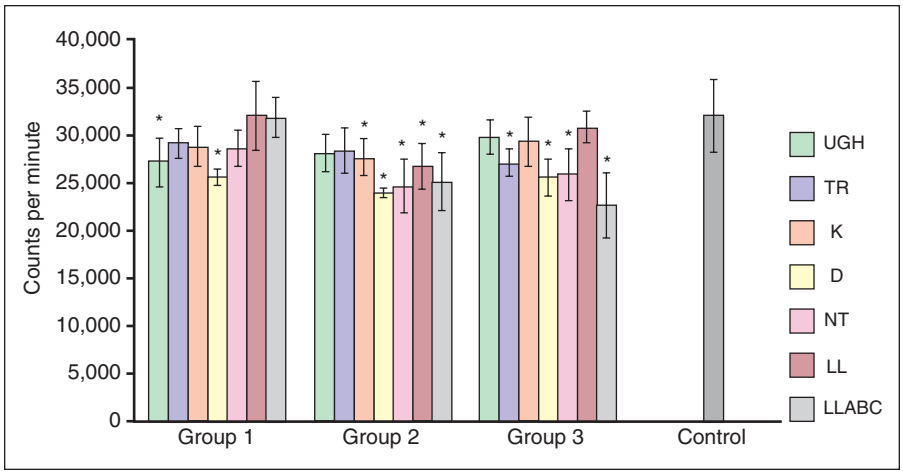

Fig 1 Means and standard deviations of ^{3}H-thymidine incorporation assay results for all experimental and control groups.

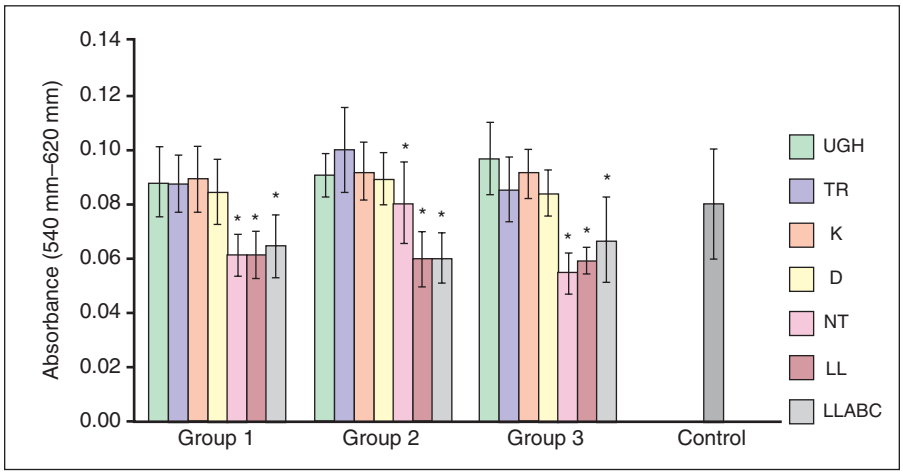

Fig 2 Means and standard deviations of MTT assay results for all experimental and control groups.

cytotoxic effects of dental materials and their components used in dental practice.[22] According to Ciapetti et al,[23] the combination of 2 different methods with specific targets within the structure of the cell provides a more reliable final evaluation of cytotoxicity. In this study, the reline resins that promoted low metabolic activities did not necessarily have low incorporations of radioactive ^{3}H-thymidine. Our results are in agreement with those reported by Schweikl and Schmalz,[19] who found that the L929 cell response was significantly different from those recorded by the MTT and the proliferation assays.

There was a significant inhibitory response of the cell proliferation produced by the D and UGH eluates from group 1 (Fig 1). Self-curing acrylic resins have exhibited high residual monomer levels[3,20] and cytotoxicity to cell culture systems.[6,10] Residual monomers and other leachable compounds elute from autopolymerized resins at higher concentrations than from heat-polymerized resins.[4,5] The biologic features of acrylic resins are highly influenced by the amount of monomer in the mixture ratio.[4,24] Because the liquid/powder ratio recommended by the manufacturer for material D was higher than that of the other materials, it can be assumed that the residual unreacted monomer level is higher in the polymerized resin.[4] In addition, material D contains butyl methacrylate (BMA), which has the effect of hydrophobic interaction with biologic membranes.[25] Therefore, the cytotoxicity of material D might be attributable to the high amount of BMA in the mixture ratio.[4] Eluates of resins TR, K, NT, LL, and LLABC exhibited high measures of radioisotope in group 1 (Fig 1), denoting a noncytotoxic response. However, the MTT assay gave contrasting results. In group 1, there was a significant inhibitory response on cell viability of the cells produced by eluates from NT, LL, and LLABC resins (Fig 2). It seems that the viability assays reacted specifically, depending on the chemical nature of the test material and the different measures used. The incorporation of ^{3}H-thymidine reflects DNA synthesis activity in a dividing cell population, while the MTT reduction assay reflects enzyme activity. Therefore, these

assays yielded different apparent cytotoxicities, and the results of the 2 assays did not overlap. In addition, UGH and D resins may have enhanced mitochondrial activity. The stimulation of cell activity may reflect a compensatory response of cell enzyme activity to resin-associated toxicity.[8] Therefore, it was possible that the enzyme activity (MTT) produced by the D and UGH eluates occurred regardless of the reduced cell proliferation, as assessed by ^{3}H-thymidine incorporation.

An earlier investigation focused on the cytotoxicity of 3 heat-polymerized denture base resins.[7] The cytotoxicity effects of the denture base materials were studied using the same methods of this investigation. However, the results of the controls of both investigations were considerably different (eg, in the earlier investigation, control mean values of ^{3}H-thymidine incorporation were approximately 12,000 cpm, whereas control mean values of the present investigation were approximately 30,000 cpm), suggesting that the results of each experiment should be compared with the matched control culture from that experiment. Differences in the number of culture passages and growth conditions may have accounted for the differences between these controls.[26] In addition, the current study demonstrated that both postpolymerization treatments were able to significantly reduce the cytotoxicity of UGH resin, as assessed by the ^{3}H-thymidine assay. Contrasting results were observed in a previous study,[7] in which the cytotoxicity of 3 heat-polymerized acrylic resins was not decreased by the same microwave and water bath postpolymerization treatments used in the present study. Since there is a considerably higher degree of conversion in heat-polymerized acrylic resins than in autopolymerized acrylic resins,[27] it is possible that the former materials are less affected by postpolymerization treatments.

Contrary to expectations, for most of the reline resins the biocompatibility was detrimentally affected by postpolymerization treatments, as assessed by the ^{3}H-thymidine assay. As assessed by the MTT assay, the biocompatilibility of the reline resins was not influenced by the postpolymerization treatments. These results were surprising, because our hypothesis was that the postpolymerization treatments would decrease the potentially toxic leachable residual monomer of the reline resins and their cytotoxicity. In the case of microwave postpolymerization, the decrease in residual monomer would result from the further polymerization only.[1,14] The use of water bath postpolymerization was intended to decrease the residual monomer by the combination of diffusion into water[3] and the postpolymerization reaction.[2] These mechanisms could be responsible for the favorable results obtained for UGH specimens submitted to microwave and water bath postpolymerization. Although a reduction in monomer level is likely to occur after postpolymerization treatment,[1,2,11,12,14] increased cytotoxicity was observed with the materials K, NT, LL, and LLABC after microwave postpolymerization. Similar results were observed with materials TR, NT, and LLABC after water bath postpolymerization. This could be the result of leaching of compounds such as additives, byproducts from the free radical polymerization reaction, degradation products, impurities, or products such as biphenyl and phenyl benzoate formed from the decomposition of benzoyl peroxide in the initiator system.[9] Also, the cytotoxicity could be the result of formaldehyde formation on the superficial layer of the specimens during postpolymerization.[28] The presence of this substance in dental acrylic resin materials has been well documented.[11,28,29] This substance is known to cause allergic contact dermatitis[30] and to induce DNA damage and delay DNA repair in human skin cells.[31] The formaldehyde molecules possibly eluted from the reline materials to the culture medium, resulting in a cytotoxic effect. Further studies to identify the eluates responsible for the observed cytotoxicity are recommended to confirm these assumptions.

It is important to note that materials K and LL did not become cytotoxic after water bath postpolymerization. Here, it can be supposed that the formation of toxic compounds was hindered by the lower oxygen presence when the specimens were immersed in water. In addition, potentially toxic substances may have eluted from the superficial layer of the specimens into water,[9] thus resulting in lower amounts of these substances in the culture medium during cytotoxicity tests. In the present study, K, NT, LL, and LLABC specimens were submitted to microwave postpolymerization in dry conditions and then became cytotoxic. Therefore, it seems reasonable to assume that immersion of the specimens in water during microwave postpolymerization would lead to more favorable results. Future investigations should be directed toward an improvement in postpolymerization treatments.

Before cytotoxicity tests, specimens were subjected to an ultrasonic bath with distilled water for 20 minutes and then exposed to ultraviolet light for another 20 minutes. Although these are not usual clinical procedures, they were necessary to decontaminate the specimens before the cytotoxicity tests. During the ultrasonic bath, potentially toxic substances from the relining materials and from the air barrier coating may have partially eluted into the distilled water, thus reducing the amounts of these substances in the culture medium during cytotoxicity tests. In addition, it is possible that ultraviolet light exposure to the light-polymerized LL and LLABC specimens may have enhanced their degree of conversion. Therefore, the results from the present investigation should be interpreted with caution,

as the decontamination procedures used may have affected the cytotoxic potential of the relining materials.

Although the purpose of the postpolymerization treatments was to improve the biocompatibility of resins, their usefulness is questionable, because inhibition of most resins' toxicity was not accomplished. However, whereas in vitro tests allow better control of individual variables, oral conditions, such as salivary flow and buffering capacity, are not present. Therefore, direct extrapolation of the toxicity findings from this work to in vivo conditions should not be performed. Further studies are necessary to determine the nature of the chemicals released from hard chairside reline resins, plus their concentrations and relative toxicity.

Conclusion

Within the limitations of this in vitro study, the following conclusions can be drawn:

1. Microwave and water bath postpolymerization treatments had no significant effect on the cytotoxicity of the reline materials, as assessed by MTT assay.
2. As assessed by ^{3}H-thymidine assay, postpolymerization treatments significantly decreased the cytotoxicity of material UGH, whereas the cytotoxicity of material D remained unaffected.
3. A significant inhibition in cell proliferation (^{3}H-thymidine assay) was observed for materials TR, NT, and LLABC after water bath postpolymerization treatment and for materials K, NT, LL, and LLABC after microwave postpolymerization treatment.

Acknowledgments

This research was supported by FAPESP grants 2001-14004-0 and 2002-07616-2. The authors would like to thank the manufactures for the donation of the reline materials.

References

1. Blagojevic V, Murphy VM. Microwave polymerization of denture base materials. A comparative study. J Oral Rehabil 1999;26:804–808.
2. Lamb DJ, Ellis B, Priestley D. The effects of process variables on levels of residual monomer in autopolymerizing dental acrylic resin. J Dent 1983;11:80–88.
3. Vallittu PK, Miettinen V, Alakuijala P. Residual monomer content and its release into water from denture base materials. Dent Mater 1995;11:338–342.
4. Koda T, Tsuchiya H, Yamauchi M, Ohtani S, Takagi N, Kawano J. Leachability of denture-base acrylic resins in artificial saliva. Dent Mater 1990;6:13–16.
5. Lee SY, Lai YL, Hsu TS. Influence of polymerization conditions on monomer elution and microhardness of autopolymerized polymethyl methacrylate resin. Eur J Oral Sci 2002;110:179–183.
6. Huang FM, Tai KW, Hu CC, Chang YC. Cytotoxic effects of denture base materials on a permanent human oral epithelial cell line and on primary human oral fibroblasts in vitro. Int J Prosthodont 2001;14:439–443.
7. Jorge JH, Giampaolo ET, Vergani CE, Machado AL, Pavarina AC, Carlos IZ. Cytotoxicity of denture base resins: Effect of water bath and microwave postpolymerization heat treatments. Int J Prosthodont 2004;17:340–344.
8. Lefebvre CA, Knoernschild KL, Schuster GS. Cytotoxicity of eluates from light-polymerized denture base resins. J Prosthet Dent 1994;72:644–650.
9. Lygre H, Solheim E, Gjerdet NR. Leaching from denture base materials in vitro. Acta Odontol Scand 1995;53:75–80.
10. Sheridan PJ, Koka S, Ewoldsen NO, Lefebvre CA, Lavin MT. Cytotoxicity of denture base resins. Int J Prosthodont 1997;10:73–77.
11. Tsuchiya H, Hoshino Y, Tajima K, Takagi N. Leaching and cytotoxicity of formaldehyde and methyl methacrylate from acrylic resin denture base materials. J Prosthet Dent 1994;71:618–624.
12. Shim JS, Watts DC. Residual monomer concentrations in denture-base acrylic resin after an additional, soft-liner, heat-cure cycle. Dent Mater 1999;15:296–300.
13. Baker S, Brooks SC, Walker DM. The release of residual monomeric methyl methacrylate from acrylic appliances in the human mouth: An assay for monomer in saliva. J Dent Res 1988;67:1295–1299.
14. Yunus N, Harrison A, Huggett R. Effect of microwave irradiation on the flexural strength and residual monomer levels of an acrylic resin repair material. J Oral Rehabil 1994;21:641–648.
15. Araújo PHH, Sayer C, Poço JGR, Giudici R. Techniques for reducing residual monomer content in polymers: A literature review. Polymer Engineering Science 2002;42:1442–1468.
16. Vergani CE, Seó RS, Pavarina AC, Reis JMSN. Flexural strength of auto-polymerizing denture reline resins with microwave postpolymerization treatment. J Prosthet Dent 2005;93:577–583.
17. Ilbay SG, Guvener S, Alkumru HN. Processing dentures using a microwave technique. J Oral Rehabil 1994;21:103–109.
18. Schuster GS, Lefebvre CA, Dirksen TR, Knoernschild KL, Caughman GB. Relationships between denture base resin cytotoxicity and cell lipid metabolism. Int J Prosthodont 1995;8:580–586.
19. Schweikl H, Schmalz G. Toxicity parameters for cytotoxicity testing of dental materials in two different mammalian cell lines. Eur J Oral Sci 1996;104:292–299 [erratum 1996;104:412].
20. Rose EC, Bumann J, Jonas IE, Kappert HF. Contribution to the biological assessment of orthodontic acrylic materials. Measurement of their residual monomer output and cytotoxicity. J Orofac Orthop 2000;61:246–257.
21. Imazato S, Ebi N, Tarumi H, Russell RR, Kaneko T, Ebisu S. Bactericidal activity and cytotoxicity of antibacterial monomer MDPB. Biomaterials 1999;20:899–903.
22. Costa CA, Edwards CA, Hanks CT. Cytotoxic effects of cleansing solutions recommended for chemical lavage of pulp exposures. Am J Dent 2001;14:25–30.
23. Ciapetti G, Granchi D, Verri E, Savarino L, Cavedagna D, Pizzoferrato A. Application of a combination of neutral red and amido black staining for rapid, reliable cytotoxicity testing of biomaterials. Biomaterials 1996;17:1259–1264.
24. Hensten-Pettersen A. Comparison of the methods available for assessing cytotoxicity. Int Endod J 1988;21:89–99.
25. Fujisawa S, Atsumi T, Kadoma Y. Cytotoxicity of methyl methacrylate (MMA) and related compounds and their interaction with dipalmitoylphosphatidylcholine (DPPC) liposomes as a model for biomembranes. Oral Dis 2000;6:215–221.

26. Cress LW, Owen RD, Desta AB. Ornithine decarboxylase activity in L929 cells following exposure to 60 Hz magnetic fields. Carcinogenesis 1999;20:1025–1030.
27. Ruyter IE, Oysaed H. Conversion in denture base polymers. J Biomed Mater Res 1982;16:741–754.
28. Ruyter IE. Release of formaldehyde from denture base polymers. Acta Odontol Scand 1980;38:17–27.
29. Oysaed H, Ruyter IE, Sjovik Kleven IJ. Release of formaldehyde from dental composites. J Dent Res 1988;67:1289–1294.
30. Ravis SM, Shaffer MP, Shaffer CL, Dehkhaghani S, Belsito DV. Glutaraldehyde-induced and formaldehyde-induced allergic contact dermatitis among dental hygienists and assistants. J Am Dent Assoc 2003;134:1072–1078.
31. Emri G, Schaefer D, Held B, Herbst C, Zieger W, Horkay I, Bayerl C. Low concentrations of formaldehyde induce DNA damage and delay DNA repair after UV irradiation in human skin cells. Exp Dermatol 2004;13:305–315.

Literature Abstract

Clinical and radiographic performance of delayed-immediate single-tooth implant placement associated with peri-implant bone defects. A 2-year prospective, controlled, randomized follow-up report

The aim of this randomized, prospective clinical study was to determine the peri-implant and prosthetic success for single tooth replacement using either delayed-immediate or delayed implant placement after 2 years. Forty-six patients (25 women and 21 men) were provided with single implant placement using either a delayed immediate technique (placement within 3 to 15 days after tooth extraction) or a delayed placement (implants placement within 65 to 138 days after tooth extraction). No membranes were placed although grafting was performed in the presence of dehiscence or fenestrations. Second stage surgery was performed after 3 months for both groups and healing was allowed for 4 to 6 weeks. Implants were restored with metal-ceramic crowns on UCLA abutments. Baseline probing depths were measured on the buccal, mesial, distal, and lingual aspects of the implant. Baseline digital radiographs were used to measure marginal bone level, which is the distance from the implant-abutment junction to the first visible bone-to-implant contact, mesial, and distal to the implants using a computer program. Follow-up evaluation was done at 9 months and 2 years after implant placement. Forty patients attended the first recall while 41 attended the second recall. Patients were asked for complaints, and the following were assessed: *(1)* implant mobility; *(2)* screw loosening; *(3)* porcelain fractures; *(4)* exposure of the implant or metal margins of the crown or abutment. Probing depths were again measured. Digitized intraoral radiographs were also repeated to measure marginal bone level. Radiographic evaluations were blinded. Data were analyzed using a Wilcoxon matched-pairs Signed Ranks Test to determine significant differences from baseline to recall 2 between the delayed-immediate and delayed implants. Differences between the 2 groups at baseline, recall 1, and recall 2, and in change over time, were tested by Mann-Whitney U test. Results indicate a 95% success rate for both methods of implant placement, which is in agreement with previous studies.

Schropp L, Kostopoulos L, Wenzel A, Isidor F. *J Clin Periodontol* 2005;32:480–487. **References:** 33. **Reprints:** Lars Schropp, Department of Prosthetic Dentistry, University of Aarhus, Vennelyst Boulevard 9, 8000 Arhus C, Denmark. Email: lschropp@odont. au.dk—*Esquivel-Upshaw, San Antonio, TX*

Literature Abstract

The impact of conventional and implant supported prostheses on social and sexual activities in edentulous adults: Results from a randomized trial 2 months after treatment

The aim of this study was to determine the effect that implant-supported overdentures and conventional complete dentures had on leisure and sexual activities. One hundred and two middle aged (36 to 65) patients were randomly assigned to the implant (n = 54) or the conventional denture group (n = 48) using computer generated random numbers. All subjects had been edentulous for at least 10 years. For the implant overdenture group, 2 Brånemark implants were placed in the interforaminal area of the mandible. Second stage surgery was performed after a 4-month healing period. The abutments were joined using a gold alloy bar and a mandibular overdenture was fabricated. A social impact questionnaire was used to assess the effects of both treatment modalities on social and sexual activity to include avoiding conversations, refusing invitations, feeling uneasy in sexual circumstances such as kissing, and looseness of the prostheses during sports activities. Spearman's rank correlation coefficient was used to assess pretreatment and posttreatment responses. Ratings were recorded on categorical scales at baseline and 2 months after treatment. The Oral Health Impact Profile was used to measure the oral health related quality of life. Analysis of data shows that there were significant improvements in the overdenture group for looseness during eating, speaking, kissing, and yawning ($P < .0001$) and reported increased confidence in performing these activities.

Heydecke G, Thomason JM, Lund JP, Feine JS. *J Dent* 2005;33:649–657. **References:** 49. **Reprints:** Jocelyne S. Feine, Department of Oncology, Faculty of Medicine, McGill University, Montreal, Quebec, Canada. Email: jocelyne.feine@mcgill.ca—*Esquivel-Upshaw, San Antonio, TX*