

***In Vitro* Comparison of the Cytotoxicity of Acetal Resin, Heat-Polymerized Resin, and Auto-Polymerized Resin as Denture Base Materials**

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Abstract: This *in vitro* study aims to evaluate three different base materials (acetal, heat-polymerized, and auto-polymerized resins) on L-929 mouse fibroblast cells over 1 h-, 1-, 3-, 5-, 7-day periods. The hypothesis was that acetal resin would show higher cytotoxic effect than heat-polymerized and auto-polymerized acrylic resins, as it seems possible that residual formaldehyde might be leaching from the material into the cell culture medium. The samples were produced according to the manufacturer's protocol. Then they were placed in Dulbecco's Modified Eagle Medium/Ham's F12 (DMEM/F12) for 1 h, 1, 3, 5, 7 days. After the incubation periods, cytotoxicity of the extracts to cultured fibroblasts (L-929) was measured by MTT assay. The degree of cytotoxicity of each sample was determined according to the reference value represented by the cells with a control. Statistical significance was determined by one-way ANOVA. Tukey and Tamhane tests were used as a post-hoc method to determine differences among the groups. Statistically significant difference was found among test groups at all time incubation periods ($p = 0.000$). The auto-polymerized resin performed higher cytotoxic effect than heat-polymerized resin and it was statistically significant at 1-day period ($p < 0.05$). The highest cytotoxic effect of acetal resin was observed at 5-day incubation period. In conclusion, the hypothesis was verified, since acetal resin showed more cytotoxic effect on the 3rd, 5th, and 7th days than heat- and auto-polymerized resins. Cell survival rates (% of control) of acetal resin were 58, 54, and 60%, respectively. © 2009 Wiley Periodicals, Inc. *J Biomed Mater Res Part B: Appl Biomater* 91B: 905–909, 2009

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INTRODUCTION

A variety of resins have been introduced into dental treatments for the construction of dental prostheses and their efficacy has been based on physical, chemical, and biological properties.^{1,2} The biocompatibility of these materials is a crucial factor in their clinical use. Biocompatibility can be defined as the ability of a biomaterial to perform its desired function with respect to a medical therapy, without eliciting any undesirable local or systemic effects in the recipient or beneficiary of that therapy, but generating the most appropriate beneficial cellular or tissue response in that specific situation, and optimizing the clinically relevant performance of that therapy.³ Shortly, biocompatibility can be defined as the acceptance of artificial material by the surrounding tissues and by the body as a whole.⁴

The potentially toxic eluates from denture base polymers include formaldehyde, methyl methacrylate, methacrylic acid, and benzoic acid.^{5,6} Many authors have discussed the polymerization process involved in converting monomer to polymer, because adequate polymerization is a crucial factor in maximizing the physical properties and biocompatibility of acrylic denture base resins.^{7,8} The acrylic resins may be classified by polymerization mode and include those that are heat-polymerized, auto-polymerized, microwave-polymerized, and visible light-polymerized resins. Heat-polymerized and auto-polymerized acrylic resins are the most frequently and extensively used materials in daily dental practice.

Polyoxymethylene (POM), also known as acetal resin, has been used as a substitute for acrylic resins as a denture base material and metals as a denture clasp material in many prosthetic applications for more than two decades.⁹ Acetal resin is a product of formaldehyde polymerization.¹⁰ The possible use of polyacetal resins as denture base materials was considered by Smith¹¹ over 40 years ago.

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Several studies have demonstrated the cytotoxicity of various types of acrylic resins according to their polymer to monomer ratio,¹² polymerization methods,^{13–17} and polymerization cycles.^{8,12,18} The reviewed studies indicate that auto-polymerized resins are more cytotoxic than the heat-polymerized denture base resins. On the other hand, acetal resin has been used as an alternative to acrylic resins for more than two decades, but there is limited information about its biocompatibility within the dental literature. Acetal resin has a history of use in animal studies and as an implant material in a variety of medical applications. This has fostered its use in total hip replacements,¹⁹ orthopedic implants,^{20,21} or joint replacement components,²⁰ as an artificial heart valve occluder,²² and as a framework material for removable partial denture for patients have allergic reactions to Co-Cr framework.¹⁰ Penick et al.²³ suggested that the use of acetal resin as a culture-medium wetted component appears to be innocuous for human mesenchymal stem cells (MSCs), after machining and repeated autoclaving. On the contrary, Kusy and Whitley²⁴ concluded that acetal resin should not be used in applications in which patients' appliances or prostheses; it has a propensity to depolymerize into formaldehyde (CH₂O). A study by Laluppa et al.²⁵ suggested that acetal resin had a significant negative effect on cell growth and colony-forming ability of hematopoietic stem cells, whether it was used as a growth substrate or as a medium-wetted component.

The aim of this study was to compare the cytotoxic effects of the most frequently used resins in daily dental practice, heat-polymerized and auto-polymerized acrylic resins; and acetal resin on L-929 mouse fibroblast cells over 1-h, 1-, 3-, 5-, 7-day periods. The hypothesis was that acetal resin would show higher cytotoxic effect than heat-polymerized and auto-polymerized acrylic resins, as it seems possible that residual formaldehyde might be leaching from the material into the cell culture medium.

MATERIALS AND METHODS

Resin Sample Fabrication

The materials used in this study, together with the manufacturer, composition, and mixing proportions of polymer to monomer are listed in Table I. Each resin was fabricated under aseptic conditions in sterile aluminum moulds 10 mm in diameter and 1-mm thick. This sample configura-

tion was selected because it was approximately the minimum thickness that would be present in a complete or removable partial denture, and it fit the experimental system by allowing the medium to completely cover the samples. Seven samples from each resin (acetal, heat-polymerized, auto-polymerized resin) were prepared for every incubation time-period.

The heat-cure acrylic resin specimens (Meliodent, Bayer UK, Berkshire, UK) were fabricated by investing wax patterns in stone moulds within a dental flask as is done in actual denture processing. Packing and processing were carried out in accordance with the manufacturer's instructions (100°C, 20 min). In accordance with the manufacturer's instructions, a 1:3 monomer to polymer ratio, by weight, was used for the heat-polymerized resin. The polymerization of the resin was performed by immersion in boiling water for 20 min. The specimens were cooled at room temperature for 30 min. The acrylic specimens were finished and polished as is done with an actual acrylic resin denture base.

The auto-polymerized resin specimens were prepared by mixing the powder and liquid components for 15 s according to the manufacturer's instructions (powder/liquid ratio of 0.6 g/0.4 g) (Meliodent, Bayer UK, Berkshire, UK).

The acetal resin specimens were prepared in accordance with manufacturer's instructions. As purported by the manufacturer, acetal resin is available in 20 color shades matching the Vita shade guide (Vitapan, VITA Zahnfabrik, Bad Säckingen, Germany). Acetal resin in pink color was chosen, similar to acrylic resin. As the special flask (Muffle-Type 100, Pressing Dental San Marino, Italy) mould with Class IV type plaster (Marble Stone, Pressing Dental San Marino, Italy), the stainless steel mould placed at a distance of about 2.5 cm from the opening of the flask. The flask was closed and hard plaster was poured into the flask through the opening of the special flask cover. The melted wax was removed. One acetal resin cylinder of the selected color was placed into the injection tube and the tube was placed with a special tweezers on the injection machine J-100 (Pressing Dental San Marino, Italy). It was programmed as follows; melting temperature: 220°C, preinjection time with the material kept at the appropriate temperature: 20 min, postinjection time with the temperature maintained at the desired level: 3 min, injection pressure: 4 bar. After processing the specimens were smoothed on both sides with 600 grit silicone carbide papers (Struers

TABLE I. The Manufacturer, Composition, and Mixing Proportions of Polymer to Monomer of the Tested Denture Base Resins

Names of the Resins	Polymerization Conditions	Manufacturers
Acetal resin	Mixing ratio 23.4 g: 10 ml Batch no: 106: injection technique, 220°C, 4 bar J100 injection machine	Pressing Dental San Marino, Italy
Heat-polymerized resin	Heat-polymerized technique, water, 100°C	Meliodent, Dental Bayer Limited Pharmaceuticals, Berkshire, UK
Auto-polymerized resin	Auto-polymerized technique, powder/liquid ratio of 0.6 g/0.4 g	Meliodent, Dental Bayer Limited Pharmaceuticals, Berkshire, UK

TABLE II. The Comparisons of the Cell Survival Rates (% of Optical Density of Extract Treated Cells to the Control Cells) of the Tested Materials and Incubation Time Periods

Test Materials	Incubation Time Period	Minimum	Maximum	Mean	Std. Deviation	Cell Survival Rates
Acetal resin	Hour 1	0.60	0.84	0.69	0.06	92
	Day 1	0.55	0.83	0.67	0.08	90
	Day 3	0.37	0.54	0.44	0.05	58
	Day 5	0.33	0.50	0.41	0.04	54
	Day 7	0.31	0.63	0.45	0.07	60
Heat-polymerized resin	Hour 1	0.60	0.79	0.69	0.06	92
	Day 1	0.54	0.71	0.62	0.05	82
	Day 3	0.53	0.72	0.62	0.05	83
	Day 5	0.55	0.78	0.68	0.07	91
	Day 7	0.58	0.80	0.68	0.06	92
Auto-polymerized resin	Hour 1	0.55	0.75	0.65	0.05	87
	Day 1	0.49	0.66	0.57	0.05	76
	Day 3	0.50	0.66	0.59	0.04	79
	Day 5	0.55	0.77	0.67	0.06	90
	Day 7	0.52	0.74	0.67	0.06	90

waterproof silicone carbide, Struers Scientific, Denmark) to a final thickness of 1.0 mm \pm 0.1 mm. The thickness of specimens was determined with a micrometer set at 0–25 mm: 0.001 mm (Mitotuyo, Japan). This process was repeated seven times, resulting in seven acetal resin specimens for every period.

All materials' specimens were kept for 30 min under ultraviolet light to prevent bacterial contamination.

Cell Culture and MTT Assay

The specimens were placed in DMEM/F12 with 10% FBS and incubated at 37°C and 5% CO₂ in air for 1 h, 1, 3, 5, 7 days. After the incubation periods the extracts were filtered through 0.22- μ m cellulose acetate filters (Milipore; Sigma) to sterile tubes. Medium without disks was also incubated and diluted as above to serve as the negative control.

L-929 mouse fibroblast cells (HÜKÜK 95030802, Şap Enstitüsü, Ankara, Turkey) were used for determining cytotoxic effects of denture base resins. The culture medium was Dulbecco's Modified Eagle Medium Ham's F12 (DMEM/F12) nutrient mixture (1:1 Sigma, St Louis, MO, USA) supplemented with 10% (v/v) fetal bovine serum (FBS, Biochrom, Berlin, Germany) and 1% gentamycin.

The L-929 cell suspension was prepared at a concentration of 4×10^4 cell mL⁻¹ and inoculated onto 96-well cluster cell culture plates (100 μ L per well). The multiwell plates were incubated at 37°C, 5% CO₂ in air for 24 h. After 24 h, the culture medium was removed from the wells and equal volumes (100 μ L) of the extracts were added into each well except control wells. In control wells, 100 μ L—DMEM/F12 with 10% FBS and 1% antibiotic was added. Then 96-well cluster cell culture plates were incubated for 24 h at 37°C. Following removal of the test extracts, 100 μ L DMEM/F12 with 13 μ L of a 5 mg mL⁻¹ MTT (tetrazolium salt 3-[4,5-dimethylthiazol-2-yl]-2,5-

diphenyltetrazolium bromide) solution were added to each well and incubated in a dark environment for 4 h at 37°C. After incubation, 96 wells were checked for formazan crystals with inverted tissue culture microscope. MTT was aspirated and 100 μ L per well of isopropanol (Merck, Darmstadt, Germany) was added to each well. Subsequently, the absorbance at 570 nm was measured using a UV-visible spectrophotometer (LPB Pharmacia, Bromma, Sweden) to evaluate the optical density. Triplicate experiments were performed throughout this study.

Statistical Analysis

Statistical analysis of results was performed using SPSS 11.5.0 (SPSS, Chicago, Illinois 60606, USA) software. Whether the alive cell percentage measurements was normally distributed or not, was determined by using Shapiro Wilk Test. Statistical significance of time dependent changes in cytotoxicity properties of all materials were evaluated by one-way ANOVA. Tukey and Tamhane tests were used as a post-hoc method to determine differences among the groups for each time period and the differences among time periods of each material. All tests were performed at a significance level of $p < 0.05$.

RESULTS

The cytotoxicity percentage of test materials were evaluated relative to the periods of the 1st h, 1st, 3rd, 5th, and 7th day; compared with the control (culture without sample) (Table II). According to one-way ANOVA, statistically significant difference was found among test groups at all time incubation periods ($p = 0.000$). The auto-polymerized resin performed higher cytotoxic effect than heat-polymerized resin and it was statistically significant at 1-day period ($p < 0.05$). At the 3rd, 5th, and 7th day, all materials' eluates demonstrated a decreasing pattern of cytotoxic

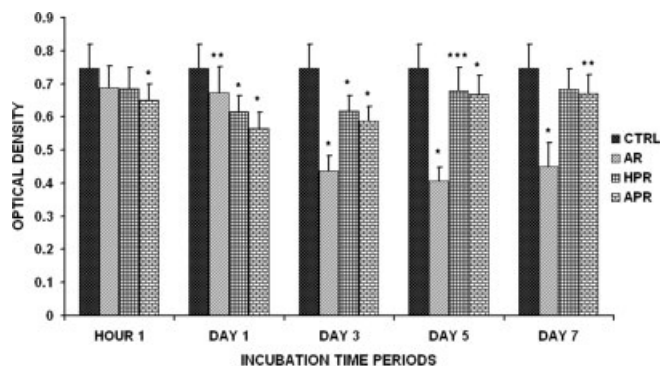


Figure 1. Optical density values and standard deviations of three denture base resins on mouse fibroblast cells(L929). Results were expressed as the mean optical density of MTT absorbance. *** $p < 0.05$, ** $p < 0.01$, * $p < 0.001$ versus control. (CTRL, control; AR: acetal resin; HPR: heat-polymerized resin; APR: auto-polymerized resin).

response. A statistical significant difference was not between 1-h and 1-day period for acetal resin ($p > 0.05$). The highest cytotoxic effect of acetal resin was observed at 5-day incubation period.

In addition, the results showed that auto-polymerized resin showed highest cytotoxic effect at the first day; however acetal resin was more cytotoxic at the 3rd, 5th, and 7th day (Figure 1).

DISCUSSION

There have been reports that denture base acrylic resins have displayed various degrees of *in vitro* cytotoxicity and *in vivo* allergic responses, probably caused by unreacted components remaining after the polymerization process. Huang et al.²⁶ verified that, compared to photo-polymerized and thermo-polymerized resins, auto-polymerized acrylic resins showed a higher cytotoxic effect for fibroblasts and epithelial cell lines. The highest cytotoxic effect was observed on the first day of the test. The same effect was observed in the present study. Sheridan et al.¹³ tested human gingival fibroblasts and reported lower cell viability when in contact with leached components of the resins. They stated that the more a resin is left to elute before contact with cells, the lower the cytotoxic effect exerted. Using the MTT test, Rose et al.²⁷ evaluated orthodontic resins (heat-polymerized, light-polymerized, and auto-polymerized) and reported that heat-polymerized resin was not considered cytotoxic and light-polymerized resins were considered to be of low cytotoxicity. Auto-polymerized resin was considered the most cytotoxic because of its monomer, urethane dimethacrylate, which caused a greater inhibition of cellular growth. The reviewed studies indicate that auto-polymerized resins are more cytotoxic than the heat-polymerized denture base resins. In this study, the same effect was observed, where the auto-polymerized acrylic resin showed more cytotoxic effect and lower cell viability than heat-polymerized acrylic resin.

In this study, the specimens were kept under ultraviolet light for disinfection. Although the resins in the present study were polymerized by thermal energy or chemical activators, UV radiation may have affected the degree of polymerization and the amount of residual monomer.

The cytotoxicity was measured by indirect method using extracts of the specimens. Direct and indirect methods retain advantages and disadvantages. The indirect method requires only one set of specimens for multiple time points, as the specimen can be extracted repeatedly. However, the direct method allows a more legitimate comparison between aging intervals as each set of specimens is statistically independent.²⁸ In the present study, the freshly prepared samples were placed in medium immediately. In *in vitro* studies, it is important for the materials to be tested immediately after mixing/curing to avoid the loss of toxic substances released from the tested materials at this initial stage.

In our study, eluates from three different types of resin were cytotoxic to mouse fibroblast cells (L-929). These results indicated that the leachable substance constantly segregated through time periods 1 h, 1, 3, 5, and 7 days. Auto-polymerized acrylic resin was found to exhibit high cytotoxicity compared to acetal and heat-polymerized resin at the 1st h and on the 1st day. However, our hypothesis was verified, since acetal resin showed high cytotoxicity on the 3rd, 5th, and 7th day. The cause of this contrary may be explained by releasing different toxic substances from unpolymerized resins with time, and different mechanisms of cellular damage from different types of denture base materials. Also, the differences in toxicity patterns at various elution times may be related to the degree of polymerization and amount of filler.

Acetal resin has been used in cell culture environments.^{29,30} Laluppa et al.²⁵ studied the effects of a wide range of plastic materials on the *in vitro* expansion of hematopoietic progenitor cells. They concluded that acetal resin inhibited both colony formation and growth of hematopoietic stem cells, whether it was in direct contact with the cells or in indirect contact by conditioning media. Penick et al.²³ concluded that the use of acetal resin as a culture medium-wetted component appears to be innocuous, at least for human MSCs. The acetal resin samples were steam-autoclaved 1 to 20 times, to assess the possibility of any toxic thermal breakdown product release into the media. As excess heat is known to cause thermal breakdown of acetal resin; products include formaldehyde, carbon monoxide, and carbon dioxide. Because of the toxicity of the first two compounds, it would, therefore, appear advisable to avoid overheating the material. The contrast of these findings to those of Laluppa et al.²⁵ may reflect a cell-type specific sensitivity, due to different handling of the material.

Ozkan et al.³¹ reported the color stability of white and pink acetal resin. According to them, white acetal resin showed less color stability than pink acetal resin. This was

explained as: color change may be associated with porosity caused by overheating or insufficient pressure during polymerization, and the addition of acrylic fibers. It was shown that acetal resin is resistant to temperatures from -40 to $+900^{\circ}\text{C}$. These additional fibers and presence of porosity may be the reason for cytotoxicity of acetal resin.

Acrylic resins are widely used for fabrication, relining, and repair of prostheses even though no biologic testing is required for their use in dental practice because they are considered to be low-risk materials for patients' health.¹¹ In present study, all denture base resins were found cytotoxic at all time periods from control groups. On the other hand, most eluted substances are found to be cytotoxic *ex vivo* and therefore the materials may not necessarily be cytotoxic *in vivo*. It is our opinion that *in vitro* tests are helpful in assaying the biologic effects of dental materials, but they may be limited in their ability to stimulate the clinical condition. From a clinical point of view, there are limitations regarding the correlation between *ex vivo* testing and clinical usage tests. Furthermore, future research is recommended for acetal resins that were responsible for the observed cytotoxicity.

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