Cytotoxicity of Two Autopolymerized Acrylic Resins Used in Orthodontics

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ABSTRACT

Objective: To test in vitro the null hypothesis that the toxic effect of different acrylic resins used in orthodontics cannot be reduced when a prior elution is performed.

Materials and Methods: Three established cell lines were used (HeLa, NIH3T3, and Hep2) and cultured under standard conditions. Resin segments were immersed in a culture medium and left to elute for 24 and 48 hours. Cells were exposed to medium containing eluates for 24 or 48 hours. The 3-(4,5 dimethyl-2-thiazolyl)-2.5-diphenyl-2H-tetrazolium bromide (MTT) assay was used as the cytotoxicity test. Control cells contained standard medium with no eluate. Analysis of variance and Tukey test were used for statistical analysis.

Results: Fibroblastic viability was not affected when the elution time was 24 hours, but treatments showed higher cell viability than controls when the elution time was 48 hours. When left to elute for 24 hours, both resins had a cytotoxic effect on epithelial cells, but this effect was not observed when the elution time was 48 hours.

Conclusions: The hypothesis was rejected as both tested materials showed lower cytotoxic effect when treated with 48-hour elutes compared with 24-hour elutes, which indicates that a longer elution time reduces resin toxicity.

KEY WORDS: Cytotoxicity; Acrylic resins; Removable appliances

INTRODUCTION

Acrylic resin is composed of high molecular weight polymers, and it polymerizes in an addition reaction. Although there are thermopolymerized and photopolymerized acrylic resins, autopolymerized resins remain the most popular material for use in orthodontics because of their low cost and ease of use.¹

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These materials can be responsible for hypersensitivity and allergic reactions, which can lead to systemic involvement.^{2–7} Such reactions are linked to the elution of toxic components from the resin, generally called haptens.⁸ These haptens can include formaldehyde, benzyl peroxide, plasticizers such as dibutyl phthalate, and especially its own residual methyl methacrylate monomer. In addition, the dental staff can also be affected by frequent contact with acrylic resin.⁹

Many authors agree that residual monomer leaching into the oral environment is a cause of hypersensitivity and allergic reactions; therefore, its concentrations have been widely investigated. Stafford and Brooks¹⁰ verified that residual monomer content is usually around 1.5% to 4.5% in self-curing acrylic resins, but for heat curing it is only around 0.3% when submitted to the cycle proposed by Huggett et al.¹¹ Davy and Braden¹² even found levels of residual monomer between 0.045% and 0.18%. Harrison and Huggett¹³ referred to the British Standard Specifications for selfcuring orthodontic resins, which establishes 3.5% as a limit to residual monomer concentration. Yilmaz et al14 mentioned the international patterns of ISO 1567 as a reference. This reference limits the level of residual monomer to 2.2% for heat curing and 4.5% for self curing acrylic resins.

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Residual monomer leaches from the resin to the oral environment. When in contact with the saliva and the oral mucosa it can cause not only local but also systemic reactions.⁴ It has been claimed that acrylic resins are cytotoxic,¹⁵ especially the chemically activated ones, because of their high levels of residual monomer.¹⁶

Schuster et al¹⁷ assessed the cytotoxicity of the acrylic resins for denture base through in vitro evaluation of the lipid metabolism of epithelial cells from hamsters. The components that leached from the tested acrylic resins adversely affected lipid metabolism and possibly led to membrane alterations. In addition, new lipids were produced in high concentrations when cells were exposed to resins with different polymerizing activation.

Sheridan et al¹⁸ also evaluated the cytotoxicity of microwave, thermopolymerized, and autopolymerized acrylic resins to evaluate the effect of the leached products from the resins on fibroblasts. Resin discs were immersed in culture medium that was used on incubated cells. The authors¹⁸ reported that all resins had cytotoxic effects on cells and that the longer the resin is left to leach its products, the lower the cytotoxic effect.

The tetrazolium salt 3-(4,5 dimethyl-2-thiazolyl)-2.5diphenyl-2H-tetrazolium bromide (MTT) cytotoxicity test has been used by some authors to estimate cell viability.^{16,18–20} Kedjarune et al¹⁶ reported that human fibroblasts grown in culture medium containing methyl methacrylate leached from acrylic resin and were cytotoxic. Also using the MTT test, Rose et al¹⁹ evaluated orthodontic resins (thermopolymerized, photopolymerized, and autopolymerized) and reported that thermopolymerized resin was not considered cytotoxic and autopolymerized resins were considered to be of low cytotoxicity. Photopolymerized resin was considered the most cytotoxic because of its monomer, urethane dimethacrylate, which caused a greater inhibition of cellular growth.

Huang et al²⁰ used the MTT test to evaluate the cytotoxicity of denture base materials on fibroblasts and epithelial cells and noted that, although all materials showed effects on cell viability, the autopolymerized resins were highly cytotoxic for both cells. Lai et al²¹ tested the cytotoxicity of autopolymerized acrylic resins and resins with lower levels of free monomer on fibroblasts and periodontal ligament cells through the MTT test. All tested materials showed a certain cytotoxic effect.

The aim of this study was to evaluate the cytotoxicity of two autopolymerized acrylic resins used in orthodontics with different exposure time, in three different established cell lines to test whether a prior elution could reduce toxic effects.

MATERIALS AND METHODS

Resin Sample Preparation

A metallic matrix (10 mm \times 10 mm \times 2 mm) was impressed with silicon and followed by exposure to ultraviolet light for 20 minutes to reduce adherence of microorganisms. Molds were filled with one of the two acrylic resins tested (Orto Class, Clássico, São Paulo, Brazil and JET, Clássico, São Paulo, Brazil). No grinding or polishing was performed in the resin samples.

Elute Preparation

Elutes were prepared by placing four resin segments into sterile vials with 9 mL of Dulbecco modified Eagle's medium (DMEM; GIBCO, Grand Island, NY) supplemented with antibiotics (gentamicin, 10 μ g/mL, GIBCO) and incubated for 24 or 48 hours at 37°C. No serum supplementation was used to avoid degradation of heat-labile components during the incubation period. The medium containing eluted components from the resin was then diluted with an equal volume of fresh medium containing antibiotics and 10% fetal bovine serum (FBS), leading to a final concentration of 5% FBS. A control (with no resin segment) was also used.

Cell Culture

Established cell lines used in this study were NIH3T3 (mouse fibroblasts), Hep2 (epithelial cells from human larynx carcinoma), and HeLa (human cervical cancer epithelial cells). All cultures were performed in DMEM supplemented with 10% FBS (GIB-CO) and gentamicin (10 μ g/mL), at 37°C and 5% carbon dioxide (CO₂; Sanyo MCO-15A, Osaka, Japan).

Cells were seeded in 96-well microplates (TPP, Trasadingen, Switzerland) at a density of 5 \times 10⁴ cells per well, complete with medium. After 24 hours of incubation, the culture media was replaced by 100 μ L of medium containing 24- or 48-hour resin eluates. After 24 or 48 hours of incubation, cells were observed under a light microscope before cell viability testing. Cells treated with no eluate were used as controls. All conditions were tested as triplicates.

MTT Assay

Cell viability was evaluated by the MTT assay, which is based on the ability of the mitochondrial enzyme succinate dehydrogenase to convert the yellow water-soluble tetrazolium salt (MTT) into formazan crystals in metabolically active cells. This water-insoluble, dark blue product is stored in the cytoplasm of cells, and is soluble afterwards, generating a blue color. The color intensity is directly proportional to the amount of viable cells.

Elution Time/			
Treatment	Orto Class		
Time	Resin	JET Resin	Control
NIH3T3 cells			
24h/24h	0.56 ^A (± 0.09)	0.57 ^κ (± 0.07)	0.59 ^{s,T} (± 0.06)
24h/48h	0.57 ^A (± 0.12)	0.50 ^κ (± 0.10)	0.66 ^s (± 0.08)
48h/24h	1.27 ^B (± 0.20)	1.18 [∟] (± 0.17)	0.54 [⊤] (± 0.05)
48h/48h	1.06 ^c (± 0.20)	1.00 ^м (± 0.15)	$0.60^{S,T} (\pm 0.07)$
HeLa cells			
24h/24h	1.17 ^D (± 0.21)	1.14 ^ℕ (± 0.31)	2.18 ^u (± 0.26)
24h/48h	0.33 ^E (± 0.29)	1.05 ^N (± 0.08)	2.00 ^{U,X} (± 0.17)
48h/24h	1.99 ^F (± 0.34)	1.69°(± 0.22)	2.52 ^v (± 0.21)
48h/48h	1.17 ^D (± 0.53)	1.45°(± 0.19)	1.78×(± 0.41)
Hep2 cells			
24h/24h	0.41 ^G (± 0.10)	1.57 [₽] (± 0.41)	3.11 ^v (± 0.22)
24h/48h	2.21 ^H (± 0.24)	2.01 ^{QR} (± 0.21)	2.71 ^z (± 0.21)
48h/24h	2.53 ⁽ ± 0.27)	2.29 ^R (± 0.17)	$2.56^{z}(\pm 0.40)$
48h/48h	1.61 ^J (± 0.35)	1.73 ^{P,Q} (± 0.40)	2.56 ^z (± 0.28)

Table 1. Cell Viability of Cells Exposed to Resins Elutes in Different Times; Average Optical Density OD 550nm $(\pm \mbox{ SD})^a$

^a For each cell line in the same column, averages followed by the same letter do not differ from each other; * P = .01.

After treatment periods of 24 or 48 hours, 200 μ L of MTT (Sigma, St Louis, Mo) was added to each well of tested cells, followed by 4 hours of incubation at 37°C and 5% CO₂. The medium was then removed and formazan crystals were dissolved with 120 μ L per well of dimethyl sulfoxide (Henrifarma, São Paulo, Brazil), generating a blue color. Optical density was read at 550 nm (microplate reader, Biorad, Tokyo, Japan).

Statistical Analysis

Statistical analysis was performed using SPSS version 10.0 (SPSS, Chicago, IL). The results were analyzed by one-way analysis of variance and Tukey test. Statistical significance was considered for values of P < .01 (99% confidence interval).

RESULTS

The NIH3T3 cells showed a peculiar behavior (Table 1, Figure 1). When left to elute for 24 hours, there was no significant difference between the cell viability of the controls and both treatments. But when resins were left to elute for 48 hours, the treatments showed higher cell viability than the controls.

When HeLa cells and Hep2 cells were evaluated (Table 1; Figures 2 and 3) for all dilution and treatment times, the controls showed higher cell viability than the treatments. For NIH3T3 cells, when the resins were left to elute for 24 hours there was a slightly cytotoxic effect that did not happen when the materials were eluted for 48 hours (Table 1). The Orto Class resin showed a higher cytotoxic effect when left to elute for



Figure 1. Cell viability of NIH3T3 cells exposed to elutes; average optical density OD 550 nm.

24 hours. The HeLa cells showed significant lower cell viability when treated with this resin for 48 hours, but the Hep2 cell showed significant lower cell viability when treated with this resin for 24 hours (Table 1).

DISCUSSION

Acrylic resins are widely used in dentistry. In the past decades concern regarding the biocompatibility of this material grew because of reactions to acrylates described in the literature.^{2–7} Some studies were published about the cytotoxicity of acrylates, but in general most reported on testing prosthodontic materials.

For this study, established cell lines were used, whereas other studies^{16,18,20,21} have used primary human cells. Schmalz and Browne²² stated that permanent cell lines should be used in a standard assay for toxicity screening once they are well defined and generally available for good reproducibility of results, because they are rather simple replicating systems. According to the authors²², this is the philosophy of ISO 10993 part 5 on the standardization of cell culture experiments. For these reasons, established cell lines were used in our study.

In this study, the MTT assay was used because of its simple execution, accessible costs, and objective results. The optical density read at the end of the test



Figure 2. Cell viability of HeLa cells exposed to elutes; average optical density OD 550 nm.



Figure 3. Cell viability of Hep2 cells exposed to elutes; average optical density OD 550 nm.

is proportional to the cellular viability: the higher the optical density, the higher the number of viable cells and the lower the toxicity of the tested product. The MTT test has been frequently used for evaluating acrylic resin.^{16,18–20}

Cells from cervical and oral mucosa have histologic similarity. Hep2 cells are of epithelial origin and HeLa cells are from cervical carcinoma, and both resemble oral mucosa.²³ NIH3T3 cells have a fibroblast lineage similar to that of cells of the lamina propria of the oral mucosa and are used as test systems.²⁴ Considering that acrylic appliances remain in intimate contact with epithelial cells from the oral mucosa, the results for epithelial cells would be more significant in this aspect. On the other hand, the oral mucosa has a keratinized cover that provides a natural defense, contributing to the low reported occurrence of hypersensitivity and allergic reactions to acrylic resins.

Kedjarune et al¹⁶ concluded that the MMA leached from acrylic resins was cytotoxic. Tsuchiya et al¹⁵ also found that, besides MMA, formaldehyde was leached from acrylic resins, especially from autopolymerized resins. Both MMA and formaldehyde were shown to be toxic to L cells in the leached concentrations.¹⁵ In our study, the cytotoxic effect of all the leached products from the resin were tested, not only the monomer alone. The autopolymerized acrylic resins used showed cytotoxic effects on the cell lines tested, especially for epithelial cell lines. Rose et al¹⁹ tested autopolymerized and photopolymerized orthodontic acrylic resins and evaluated residual monomer and cytotoxicity on established cultured fibroblasts. They stated that all orthodontic materials had a low cytotoxicity and that thermopolymerized resins were not considered cytotoxic. This is in accord with our findings of low cytotoxic effect of both autopolymerized resins tested. Although in some countries the use of photopolymerized acrylic resin has increased in the past few years, self-cured resin is still the most popular and least expensive material.

Huang et al²⁰ verified that, compared to photopoly-

merized and thermopolymerized resins, autopolymerized acrylic resins showed a higher cytotoxic effect for fibroblasts and epithelial cell lines, although all resins showed some cytotoxic effect on both cell lines. The highest cytotoxic effect was observed in the first day of the test. This is in accord with the findings of the present study, where a shorter elution time led to higher cytotoxic effect, especially for the epithelial cell lines. In our study no difference was observed for NIH3T3 fibroblasts when resins were left to elute for 24 hours. Higher cell viability was observed when NIH3T3 cells were treated with the 48-hour elute. This may be explained by the fact that in this cell line overconfluence could lead to apoptosis, resulting in a lower cell number in controls than in treated cells. Sheridan et al¹⁸ 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. The same effect was observed in the present study where the autopolymerized acrylic resin showed cytotoxic effect and lower cell viability; in addition, the longer the elution time, the lower the cytotoxic effect observed.

Lai et al²¹ studied the cytotoxicity of relining dental polymers and its monomers on human gingival fibroblasts and periodontal ligament and reported that all materials had cytotoxic effects in a dose-dependent manner. Possibly, when the elution time is longer, some toxic components are eliminated and the dosedependent cytotoxic effect is lower. As stated by Kedjarune et al,¹⁶ the amount of residual monomer is dependent on the amount of liquid in the mixture ratio. In this study, samples of acrylic resin left to elute in culture media were prepared following the manufacturer's instructions, including powder to liquid ratio. It must be considered that in practice, the preparation of the orthodontic appliances generally follow the saltand-pepper technique, leading to a different powder to liquid ratio. In these cases, the cytotoxic effect would possibly be higher.

Schmalz and Browne²² stated that for biocompatibility of dental materials an appropriate host response is necessary. In dentistry, this means no adverse reaction or a tolerable adverse reaction of a living system to the material. Therefore, toxicity may be only one reason for nonbiocompatibility of a dental material. Acrylic resins may sometimes cause toxicity and allergic reactions, but these do not occur frequently. Thus, it is considered a safe product once it qualifies for the other properties.

Once a cytotoxic effect of an acrylic resin is detected, some measures should be taken in an attempt to reduce the occurrence of allergic reactions. Many authors suggest maneuvers to reduce residual monomer Downloaded from https://prime-pdf-watermark.prime-prod.pubfactory.com/ at 2025-05-14 via free access

after the appliance is ready: hot water storage for at least one hour after confection or water immersion for 24, 36, or even 72 hours before delivery.^{2,10,15,16,19} Our study confirms that the longer the resin is left to elute, the lower the cytotoxic effect observed. These data further support the need for treatment of the acrylic resin, such as polymerization in water or under pressure,^{25–27} as well as allowing additional polymerization cycles.^{5,25–29} During the preparation of the acrylic appliance, it is also important to keep the right proportion of powder and liquid.^{16,25} All these procedures can reduce the frequency of hypersensitivity reactions and help guarantee the patient's well-being.

CONCLUSIONS

- Both tested materials showed cytotoxic effect on tested cells, especially for epithelial cell lines.
- The longer the resins were left to elute leachable components in culture media, the lower the cytotoxic effect observed.

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