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Mechanical and Biological Properties of Acrylic Resins Manipulated and Polished by Different Methods

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This study evaluated the influence of the manipulation technique and polishing method on the flexural strength and cytotoxicity of acrylic resins. Two manipulation techniques and three polishing methods were used in the fabrication of acrylic plates that were divided into 6 groups (n=10). Groups MM, MC and MW: mass technique with mechanical polishing, chemical polishing and without polishing, respectively; and Groups SM, SC and SW: Saturation technique with mechanical polishing, chemical polishing and without polishing, respectively). Flexural strength was tested in a universal testing machine and the cytotoxicity assay used cell cultures (L-929) for periods of 24 h to 168 h. Flexural strength and cytotoxicity data were assessed using two-way and three-way ANOVA, respectively (α=0.05), followed by *post hoc* Bonferroni test for multiple comparisons. The effect of combinations of manipulation techniques and polishing methods on flexural strength showed significant differences only between Group SC and Groups MW, MM and MC ($p<0.01$). Cell viability ranged from 51% (3.9%) to 87,6% (3.2) in the 24-h time interval, and from 87.8% (5.0) to 95.7% (3.1%) in the 168-h time interval. With the increase of cell viability, from the third day (72 h), there was no significant difference among the groups, except between MM and SC ($p<0.01$) at 72 h. In conclusion, the manipulation technique and polishing method had more influence on the cytotoxicity than on flexural strength.

Key Words: acrylic resins, flexural strength, cytotoxicity.

Introduction

The placement of removable orthodontic appliances for tooth movements and splinting in a healthy oral cavity may induce biofilm accumulation (1), alter the normal oral microflora (2), exacerbate periodontal diseases, and consequently cause infections (3).

Since the introduction of acrylic resins began until now, two techniques have been most commonly used: polymerization activated by visible light and chemical polymerization at room temperature (4,5) with the goal of improving their physical properties like occurrence of porosities (5) and reducing the polymerization process time (6). However, conversion of monomers into polymers is never complete, especially in autopolymerized acrylic resins (7).

The residual monomer content varies considerably with the curing conditions and the amount of residual monomer is one of the primary factors affecting the properties of acrylic resins (6,8). Studies (9,10) have reported that variable quantities of residual monomer may be released in the oral cavity during the use of removable orthodontic appliances.

Furthermore, polishing is an important factor for obtaining smooth surfaces of the acrylic resins, which favors the reduction of the amount of released residual monomer (10,11). Chemical polishing has shown higher surface roughness than the mechanic, influencing more the mechanical and biological properties of resins, either for microorganism accumulation or residual monomers release (11), which may be related to greater incorporation of monomers during chemical polishing.

Residual monomers may alter the final physical properties of resins (10) and induce the arise of local and systemic tissue reactions when in contact with saliva and soft tissues (12), causing hypersensitivity, allergic reactions, cell toxicity (13) and systemic involvements (14).

In addition, this material may easily fracture due to its low impact resistance, low flexural strength or low resistance to fatigue (15). Another great disadvantage of acrylic resins is the rapid loss of esthetic, physical and mechanical properties in the oral medium because this material absorbs and releases water (16).

The aim of this study was to verify the influence of the manipulation technique and polishing method on the flexural strength and cytotoxicity of acrylic resins.

Material and Methods

Test Specimen Fabrication

One hundred and twenty specimens were made of colorless acrylic resin (OrtoCril, VIPI, Pirassununga, SP, Brasil), being 60 (25x5x4 mm) for the flexural and 60 (10x1x2 mm) for the cytotoxicity tests. They were fabricated by two different techniques (mass and saturation) and divided into 6 groups (n=10). Groups MM, MC and MW: mass

technique combined with mechanical polishing, chemical polishing and no polishing, respectively; Groups SM, SC and SW: saturation technique combined with mechanical polishing, chemical polishing and no polishing, respectively.

In the mass technique, the powder and liquid were manipulated in a ratio of 3:1, according to the manufacturer's instructions. The specimens were made in a condensation silicone mold (Perfil, Vigodent, Rio de Janeiro, RJ, Brazil) with internal dimensions of 26x6x4 mm, which served as a negative control (7). In the saturation technique, the specimens were fabricated by increments of powder/liquid until saturation, adding small quantities of monomer and polymer to the silicone matrix with a dropper. Polymerization occurred within a resin polymerizer (M-1000; EDG, São Carlos, SP, Brazil), at 20 °C, 25 psi (1.75 kg/cm^2) pressure, for 15 min, according to the manufacturer's instructions. Excess material was removed progressively using abrasive papers with granulations of 150, 400 and 600 in a polishing machine APL-4 (Arotec, Cotia, SP, Brazil), to obtain the desired dimensions, which were measured with a precision caliper (123M-150; Starrett, Itu, SP, Brazil).

For mechanical polishing, a bristle brush with pumice stone was used (Labordent, São Paulo, SP, Brazil) followed by the application of a felt tip, both using a polishing lathe (Nevoni, São Paulo, SP, Brazil), during 1 min each step. The final polishing step was performed with a flannel wheel and white paste of Spain (Labordent), during 1 min each step. For the specimens submitted to chemical polishing a chemical polisher PQ9000 (Termotron, Piracicaba, SP, Brazil) was used associated with Poli-Quim polishing fluid (Clássico Produtos Odontológicos Ltda, São Paulo, SP, Brazil). When the liquid reached the ideal temperature (80 °C), each test specimen was individually immersed and remained in the receptacle for 10 s. All specimens were fabricated and polished by the same operator.

After polishing, all specimens were stored in deionized water at 37 °C (Millipore, Bedford, MA, USA) for 24 h (17) so that the superficial residual monomers could be released, and after this the specimens were submitted to the tests.

Flexural Strength Test

The three-point bending flexural test was performed in a universal test machine (DL 1000; EMIC, São José dos Pinhais, PR, Brazil) at a crosshead speed of 5 mm/min until fracture. The testing device consisted of a base with two 20-mm-high columns with ends in the form of spheres with 5 mm radius and equidistant 20 mm from one another, to support the extremities of the specimen to be tested. Force was applied at the center of the specimen that was placed 10 mm equidistant from each of the two lateral columns from one identical spherical extremity to the support with the same radius of 5 mm in order to avoid differences in the results (7).

Vertical force was applied and the maximum flexural strength was recorded. The flexural strength was calculated from the formula $\alpha=3Fd/2bh^2$, where α is the flexural strength (MPa), F the maximum load exerted on the specimen acrylic (N), d the distance in mm between the supports (20 mm), b the base (5 mm) and h the height of the acrylic specimen (4 mm).

Cytotoxicity Test

Both sides of the acrylic specimens were previously sterilized with ultraviolet light (Labconco, Kansas City, MO, USA) for 30 min (18). To verify the cell response to extreme situations, other three groups were included in the study: Group CC (cell control), consisting of cells not exposed to any material; Group C+ (positive control), consisting of Tween 80 and Group C- (negative control), consisting of PBS solution in contact with the cells.

Cell culture containing L-929 line cells (mouse fibroblast) (American Type Culture Collection - ATCC, Rockville, MD, USA) was maintained in Eagle's minimum essential medium (Cultilab, Campinas, SP, Brazil) by adding 0.03 mg/mL of glutamine (Sigma, St. Louis, MO, USA), 50 µg/mL of garamicine (Schering Plough, Kenilworth, NJ, USA), 2.5 mg/mL of fungizone (Bristol-Myers-Squibb, New York, NY, USA), 0.25% sodium bicarbonate solution (Merck, Darmstadt, Germany), 10 mM of HEPES (Sigma) and 10% bovine fetal serum (Cultilab, Campinas, SP, Brazil) to the growth medium, or no bovine fetal serum to the maintenance medium only. After this, the cell culture medium was incubated at 37 °C for 48 h.

The method for evaluating the cytotoxicity was the "dye-uptake" test (19). This method is based on a neutral red dye incorporated into live cells. It was used in this experiment only for the following periods of evaluation: 24, 48, 72 and 168 h (7 days). These periods represent the time intervals of 24, 48, 72 and 168 h during which the acrylic specimens remained in the cell culture medium before being removed.

Dye Uptake

Volumes of 100 µL of L-929 line cells were distributed into 96-well microplates. After 48 h, the growth medium was replaced with 100 µL of Eagle's minimum essential medium (MEM) obtained after incubation in the different types of acrylic specimens and positive and negative control at 24, 48, 72 and 168 h (7 days). Positive and negative control groups consisted of culture medium in contact with 100 µL of Tween 80 and 100 µL PBS solution, respectively.

After 24-h incubation, 100 µL of 0.01% neutral red dye (Sigma) were added to the culture medium in the 96-well microplates, which were incubated again for 3 h at 37 °C

so that the red dye could penetrate the live cells. After this period of time, 100 µL of 4% formaldehyde solution (Vetec, Rio de Janeiro, RJ, Brazil) in PBS (130 mM of NaCl; 2 mM of KCl; 6 mM of Na_2HPO_4 2 H_2O; 1 mM of K_2HPO_4 1 mM; pH 7.2) were added in order to promote the fixation of the attached cells to the plate. After 5 min, 100 µL of 1% acetic acid (Vetec) and 50% methanol (Vetec) were added in order to remove the dye. After 20 min, a spectrophotometer (BioTek, Winooski, VT, USA) at 492 nm wavelength (λ=492 nm) was used to read the data.

Statistical Analysis

Statistical analysis was performed with BioEstat software program (version 5.0, Belém, PA, Brazil). For each evaluated parameter, descriptive statistical procedures were used, including mean and standard deviation. The homogeneity of the data was verified by the Levene test, and normality of residues verified by the Shapiro-Wilk test. Two-way analysis of variance (ANOVA) and three-way ANOVA were used for flexural strength data and cell viability data, respectively. The level of significance adopted was 5%. *Post hoc* Bonferroni comparisons were performed for multiple comparisons.

Results

Flexural Test

The mass technique presented better performance and higher mean flexural strength values when compared with the saturation technique. The order of groups ranging from the highest to the lowest flexural strength (MPa) was: MM (37.26±2.19), MW (35.13±2.06), MC (34.96±2.32), SM (34.29±1.89), SW (34.18±1.52) and SC (31.02±1.67). There were significant statistically differences only between the group SC with the groups MW, MM and MC ($p<0.01$) (Table 1).

Two-way ANOVA analysis indicated significant differences between the three polishing methods and the two manipulation techniques ($p<0.01$). However, the technical and polishing interaction was not significant ($p=0.127$). The effect of polishing methods on the manipulation technique was not different between the groups.

Cytotoxicity Test

Viability was established by comparison with the viability of control cells, which was arbitrarily set at 100%. Cell viability ranged from 51% (±3.9%) to 87,6% (±3.2%) in the 24-h time interval and from 87.8% (±5.0%) to 95.7% (±3.1%) in the 168-h time interval in comparison with the control group (Table 2).

Table 1. Multiple comparisons test of the flexural strength between the groups

Groups	Intergroup comparison	Mean difference	p
MW	MM	2.13	>0.05
	MC	0.840	>0.05
	SW	0.950	>0.05
	SM	0.830	>0.05
	SC	4.10	<0.01*
MM	MC	2.30	>0.05
	SW	3.08	>0.05
	SM	2.96	>0.05
	SC	6.23	<0.01*
MC	SW	0.780	>0.05
	SM	1.10	>0.05
	SC	3.93	<0.01*
SW	SM	0.110	>0.05
	SC	3.15	>0.05
SM	SC	3.26	>0.05

Two-way ANOVA, followed by Bonferroni Post Hoc multiple comparisons test. *Represent statistically significant difference at $p<0.05$.

Table 2. Descriptive statistics of the cell viability of acrylic resins in the 1-day to 7-day periods

Manipulation technique	Polishing method	1 d (24 h)	2 d (48 h)	3 d (72 h)	7 d (168 h)
		M (SD)*	M (SD)	M (SD)	M (SD)
Mass technique	None	78.7 (6.1)	90.1 (5.2)	91.1 (5.1)	91.2 (4.3)
	M	87.6 (3.2)	91.5 (4.7)	93.8 (3.9)	95.7 (3.1)
	C	70.6 (4.6)	78.2 (5.5)	87.1 (5.6)	90.2 (4.5)
Saturation technique	None	59.1 (5.6)	81.1 (5.1)	86.0 (5.0)	89.9 (7.3)
	M	68.2 (5.7)	83.1 (6.1)	91.6 (5.5)	91.1 (5.6)
	C	51.0 (3.9)	70.3 (7.4)	83.0 (4.1)	87.8 (5.0)
C-		96.9 (2,9)	95.5 (4.4)	96.4 (3.5)	95.0 (3.3)
C+		13.8 (1.1)	10.5 (1.4)	9.6 (1.1)	8.4 (1.1)
CC		100	100	100	100

M(SD): Mean/Standard deviation of cell viability in percentage. Control groups: Group C- (PBS solution), Group C+ (Tween 80) and Group CC (cell control). M: Mechanical. C= Chemical.

Three-way ANOVA indicated a significant effect in the technique and polishing interaction (p<0.01), and technique and time interaction (p<0.01). This variation may possibly be due to the differences between the techniques and polishing methods, and between technique and evaluation times, respectively. However, the time and polishing methods interaction showed no statistical difference (p=0.960). Altogether, these findings indicate that the technique had more influence on cell viability that the polishing method on the evaluated times.

Regarding the technique and polishing interaction, on the first day (24 h) all groups showed statistical difference

Table 3. Multiple comparisons test of the cell viability of acrylic resins between the groups

Group	Comparison with other groups	1 d (24h)		2 d (48h)		3 d (72h)		7 d (168 h)	
		M D*	p	M D	p	M D	p	M D	p
MW	MM	8.90	<0.05	1.40	>0.05	2.70	>0.05	4.50	>0.05
	MC	8.50	<0.05	11.90	<0.01	4.00	>0.05	1.00	>0.05
	SW	19.60	<0.01	9.00	<0.05	5.10	>0.05	1.30	>0.05
	SM	10.30	<0.01	7.00	>0.05	0.50	>0.05	0.10	>0.05
	SC	27.70	<0.01	19.80	<0.01	8.10	>0.05	3.40	>0.05
MM	MC	17.00	<0.01	13.30	<0.01	6.70	>0.05	5.50	>0.05
	SW	28.50	<0.01	10.40	<0.01	7.80	>0.05	5.80	>0.05
	SM	19.40	<0.01	8.40	>0.05	2.20	>0.05	4.60	>0.05
	SC	36.60	<0.01	21.20	<0.01	10.80	<0.01	7.90	>0.05
MC	SW	11.50	<0.01	2.90	>0.05	1.10	>0.05	0.30	>0.05
	SM	2.40	>0.05	4.90	>0.05	4.50	>0.05	0.90	>0.05
	SC	19.60	<0.01	7.90	<0.05	4.10	>0.05	2.40	>0.05
SW	SM	9.10	<0.05	2.00	>0.05	5.60	>0.05	1.20	>0.05
	SC	8.10	<0.05	10.80	<0.01	3.00	>0.05	2.10	>0.05
SM	SC	17.20	<0.01	12.80	<0.01	8.80	>0.05	3.30	>0.05

Three-way ANOVA, followed by Bonferroni post hoc multiple comparisons test. p: Indicate statistically significant difference (p<0.01 or p<0.05). *MD: Mean difference.

Table 4. Multiple-comparison test of cell viability of acrylic resins between the time intervals

Time	Comparison with other time intervals	Groups											
		MW		MM		MC		SW		SM		SC	
		M D*	p	M D	p	M D	p	M D	p	M D	p	M D	p
1 d (24h)	2 d (48h)	11.40	<0.01	3.90	>0.05	7.60	<0.05	22.00	<0.01	14.90	<0.01	19.30	<0.01
	3 d (72h)	12.40	<0.01	6.20	>0.05	16.5	<0.01	26.90	<0.01	23.40	<0.01	26.00	<0.01
	7 d (168 h)	12.50	<0.01	8.10	<0.05	19.60	<0.01	30.80	<0.01	22.90	<0.01	36.80	<0.01
2 d (48h)	3 d (72h)	1.00	>0.05	2.30	>0.05	8.90	>0.05	4.90	>0.05	8.50	>0.05	12.70	<0.01
	7 d (168 h)	1.10	>0.05	4.20	>0.05	12,00	<0.01	8.80	>0.05	8.00	>0.05	17.50	<0.01
3 d (72h)	7 d (168 h)	0.10	>0.05	1.90	>0.05	3.10	>0.05	3.90	>0.05	0.10	>0.05	4.80	>0.05

Three-way ANOVA, followed by Bonferroni post hoc multiple comparisons test. p: Represent statistical difference (p<0.01 or p<0.05). *MD: Mean difference.

between them, except between the groups MC and SM ($p>0.05$). On the second day (48 h) there was an increase in cell viability for all groups, but without statistically significant difference between the group MW and the groups MM and SM; between the group SM and the groups SW, MM and MC; and between the groups MC and SW ($p>0.05$) (Table 3). From the third day (72 h) on, there was no significant difference among all groups, regardless of the manipulation technique and polishing method, except for the groups MM and SC ($p<0.01$) in the 72-h time interval.

In the evaluation of the time intervals, the first day differed significantly from all other evaluated times for the groups MW, MC, SW, SM, SC ($p<0.01$). In the second day, with the increase of cell viability in the groups, there was no statistically significant difference among the groups MW, MM, SW and SM in the comparison with the third and seventh days (168 h) ($p>0.05$). The third day showed no significant difference with the seventh day for all groups ($p>0.05$) (Table 4).

Discussion

In this study, as far as the cytotoxicity test is concerned, the cell culture model used was in monolayer (18-20). This model was used together with the dye-uptake technique (19) because the cytotoxicity of materials can be determined by spectrophotometry (20). L-929 mouse fibroblasts were used in this study because they provide results comparable with those of primary human gingival fibroblasts (21,22).

Considering the variability of the results, one can infer that the association between the saturation technique of fabrication and the chemical polishing method had a more significant influence on the flexural strength results, as shown in the group SC, which differed significantly from the groups MW, MM and MC ($p<0.01$) (Table 1). In the cytotoxicity test, the group SC showed increased cell viability from the first to the seventh day, but it had the lowest cell viability among groups at all evaluated times (Table 2). This can be related to the hypothesis of a greater incorporation of monomer and consequently less condensation of the acrylic mass (17).

In the evaluation of time periods, the first day showed statistically significant difference with all other evaluated times for the groups MW, MC, SW, SM, SC, which shows the greatest cytotoxic potential of the groups at baseline (Table 3). In addition, the group SC, the group SW induced the greatest quantity of cell lysis in 24 h. It may be inferred that the release of cytotoxic substances occurs mainly in the first 24 h, which is consistent with studies (6,9,17) that found an increased release of residual monomers in this period, although it should be noted that on the second day it still showed a lower cell viability in the groups MC, SC and SW compared to the other groups (Table 3). This could have a more relevant negative impact in patients with history of hypersensitivity to acrylic resins (14).

From the third day, there was no significant difference among most groups, regardless of manipulation technique and polishing method that represented respectively decrease of cytotoxic character and increase of cell viability with a similar performance among the groups, which was demonstrated also on seventh day (Table 4). This possibly could mean a decrease until complete absence (third to seventh day) of the inflammatory potential (11,14) of the acrylic resins on gingival tissue.

Taken together, these findings are aligned with the idea that the presence of residual monomer in resin appliances for orthodontic use may influence their clinical performance (23) with regard to their flexural strength and tissue biocompatibility. Although the acrylic resins tested by different methods presented over 50% of cell viability in all the experimental time intervals (Table 2), which does not contraindicate their clinical use, as seen in other studies (20,24) on cytotoxicity of materials (25) for use in dentistry that showed similar performance. However, the findings of this study suggest that the mass technique associated with manual polishing must be encouraged whenever possible, but the saturation technique associated with chemical polishing seems to be less suitable.

These materials are widely employed in the fabrication of appliances for use in orthodontics and pediatric dentistry, so clinical monitoring is necessary to detect cracks and fractures (10) as well as inflammatory processes (12) associated with their use. In patients with gingival hyperplasia and/or potential periodontal disease (20), direct contact of acrylic resins with these gingival tissues may increase their inflammatory (2,11) or allergenic (14) potential. Therefore, after fabrication, the resin appliance must have minimal or none cytotoxicity and, if necessary, avoid the inclusion of acrylic resin or change the appliance proposed for these patients. Within the limits of this *in vitro* study, it can be concluded that the manipulation technique and polishing method had more influence on the cytotoxicity than on flexural strength.

Resumo

Este estudo avaliou a influência da técnica de manipulação e método de polimento sobre a resistência à flexão e citotoxicidade de resinas acrílicas. Duas técnicas de manipulação e três métodos de polimento foram usados na fabricação de placas de acrílico que foram divididas em 6 grupos (n=10). Grupos MM, MC e MW: técnica de massa com polimento mecânico, polimento químico e sem polimento, respectivamente; e Grupos SM, SC e SW: técnica de saturação com polimento mecânico, polimento químico e sem polimento, respectivamente. A resistência à flexão foi testada em uma máquina universal de ensaios e o ensaio de citotoxicidade foi realizada utilizando culturas de células (L929) para os períodos de 24 h a 168 h. Dados da resistência à flexão e de citotoxicidade foram avaliados usando ANOVA dois fatores e ANOVA três fatores, respectivamente (α=0,05), seguido pelo teste post hoc de Bonferroni para comparações múltiplas.

O efeito das combinações de técnicas de manipulação e métodos de polimento na resistência à flexão mostraram diferenças significativas apenas entre Grupo SC e Grupos MW, MM e MC (p<0,01). A viabilidade celular variou de 51,0% (3,9%) para 87,6% (3,2%) no intervalo de tempo de 24 h, e de 87,8% (5,0%) para 95,7% (3,1%) no intervalo de tempo de 168 h. Com o aumento da viabilidade celular, a partir do terceiro dia (72 h), não houve diferença significativa entre os grupos, exceto entre MM e SC (p<0,01) em 72 h. Em conclusão, a técnica de manipulação e o método de polimento tiveram maior influência sobre a citotoxicidade do que sobre a resistência à flexão.

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