

In vitro evaluation of cytotoxicity of different brands of artificial teeth

Avaliação *in vitro* da citotoxicidade de dentes artificiais de diferentes marcas comerciais

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ABSTRACT

Aim: The present study aimed to evaluate the cytotoxicity of different brands of stock acrylic teeth against fibroblast cells. **Materials and Methods:** For this experiment, the cell lineage used was the L929 from the American Type Culture Collection (ATCC, Rockville, MD, USA). The brands of stock teeth tested included: Artplus, Biolux, Biotone, Pop Dent, Vipi Dent, Orthosit Ivoclar, Biocler, and Trilux. Each group was placed in contact with the fibroblast cells for the periods of 24, 48, 72, and 168 hours. Control cells, as well as negative and positive controls, were used to compare the extremes. **Results:** The results showed cytotoxicity for all the groups tested in the time periods of 24, 48, and 72 hours during the experiment, which occurred in the Orthosit Ivoclar and Trilux groups even after the experiment had been conducted for 168 hours. **Conclusion:** The researched acrylic teeth developed cytotoxicity against the cells *in vitro* during the time periods of 24, 48, and 72 hours, while Ivoclar Vivodent and Trilux brands of teeth developed cytotoxicity against the tested cells during the time period of 168 hours.

Uniterms: Tooth artificial. Evaluation studies. Cell line.

INTRODUCTION

Similar to that occurring in other countries, the Brazilian population is undergoing a process of aging in the age pyramid. A significant portion of the elderly population is partially or completely edentulous, which can be explained by the absence of preventive health policies geared toward the adult population in Brazil, coupled with inadequate restorative-surgical dental treatments that are far more mutilating than those aimed at maintaining teeth¹.

The dental materials used to manufacture dental prostheses commonly stay in contact with biological structures when functioning in the oral cavity, acting as foreign bodies² capable of causing alterations in the oral cavity tissues³. For this reason, it is necessary to test the possible toxic effect of these dental materials on cells.

Since the 1930s, acrylic resins have been used to manufacture dental prostheses⁴; however, biocompatibility has generally represented the main factor limiting their use⁵. The majority of total and partial removable dentures are manufactured using stock acrylic teeth, the main advantage of which is a firm chemical bond to the denture base acrylic resin, in such a way that there is no need to manufacture

mechanical retainers⁵⁻¹¹. A wide range of commercial brands of teeth are available on the dental product market, which have different aesthetic and functional characteristics, and are mainly chosen in accordance with the patient's financial situation.

Therefore the present study aimed to evaluate the cytotoxicity of different brands of stock acrylic teeth against L929 fibroblast cells.

MATERIALS AND METHODS

The cell lineage used in this experiment was the L929 (mouse fibroblast) from the American Type Culture Collection (ATCC, Rockville, MD, USA), cultivated in minimum essential medium Eagle (MEM) (Cultilab, Campinas, São Paulo, Brazil) and supplemented with 2mM of L-glutamin (Sigma, St. Louis, Missouri, USA), 50mg/ml of gentamicin (Schering Plough, Kenilworth, New Jersey, USA), 2.5mg/ml of fungizone (Bristol-Myers-Squibb, New York, USA), 0.25ml of sodium bicarbonate solution (Merck, Darmstadt, Germany), 10mM of HEPES (Sigma, St. Louis, Missouri, USA), and 10% Fetal bovine serum (FBS) (Cultilab, Campinas, São Paulo, Brazil). This cell lineage was kept at 37°C in an environment containing 5% CO₂.

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To perform this experiment, eight different types of stock teeth were used, divided into eight groups, denominated as follows: Artiplus (Dentsply, Rio de Janeiro, Brazil), Biolux (Vipi, São Paulo, Brazil), Biotone (Dentsply, Rio de Janeiro, Brazil), Pop dent (DentBras, São Paulo, Brazil), Vip Dent (Vipi, São Paulo, Brazil), Orthosit Ivoclar (Ivoclar Vivadent, Schaan, Liechtenstein), Biocler (DentBras, São Paulo, Brazil), and Trilux (Vipi, São Paulo, Brazil).

To verify the cell response against the extremes, another three groups were inserted into the evaluations: Group CC (cell control), in which the cells were not exposed to any material; Group C+ (positive control), consisting of one amalgam test specimen; and Group C- (negative control), consisting of stainless steel wire, all of which remained in contact with the cells.

The materials were sterilized prior to exposure to UV light (Labconco, Kansas, Missouri, USA) for 1 hour. After, three samples of each material were placed in 24-well plates containing culture medium (MEM) (Cultilab, Campinas, São Paulo, Brazil). After every 24h time period, the culture medium was replaced with a new medium. The supernatants were collected after 24, 48, 72, and 168 hours and were evaluated with regard to toxicity against L929 cells. The supernatants were placed in triplicate in a 96-well plate containing a confluent monolayer of L929 cells and incubated for 24 hours at 37°C in an environment containing 5% CO₂, totaling nine wells to be evaluated (n=9). After the incubation time, the effect on cell viability was determined by means of the dye-uptake technique, described by Neyndorff et al.¹², with slight modifications.

After 24 hours of incubation, 100ml of 0.01% neutral red (Sigma, St. Louis, Missouri, USA) was added to the culture medium in each well of the miniplates and was incubated at 37°C for 3 hours for the dye to penetrate into the live cells. After this time interval had elapsed, and after discarding the dye, 100ml of 4% formaldehyde solution (Reagen) was added to the PBS (NaCl 130 mM; KCl 2 mM; Na₂HPO₄ 2H₂O 6mM; K₂HPO₄ 1mM, pH7.2) for 5 minutes, to promote cell fixation to the plates. Next, to extract the dye, 100ml of 1% acetic acid solution (Vetec, Rio de Janeiro, Brazil) with 50% methanol was added (Reagen, Rio de Janeiro, Brazil). After 20 minutes, the reading was measured by a spectrophotometer (BioTek, Winooski, Vermont, USA) at a wavelength of 492nm (λ = 492 nm).

Statistical analyses were performed using the SPSS 13.0 program (SPSS Inc., Chicago, Illinois, USA). Descriptive statistical analyses, including mean and standard deviation, were calculated for the evaluated groups. The values for the quantity of viable cells were first submitted to an analysis of variance (ANOVA) to determine if there was any statistical difference among the groups. After, the Tukey test was performed.

RESULTS

After 24h of incubation, no statistical difference could be observed regarding the number of viable cells for the tested groups of teeth. By contrast, the extremes did present a statistical difference among the groups, while the cell control and negative control presented no statistical difference between them. After 48 hours, Artiplus, Biotone, Pop Dent, and Trilux presented similar results when the groups were compared. A similarity in results also occurred when comparing the Biotone with the Orthosit Ivoclar groups. However, when comparing the Vip Dent with the Biocler groups, different results could be observed. Statistical difference could be identified among all the tested groups when compared to the control groups. After 72 hours, the Artiplus, Biolux, Biotone, Pop Dent, and Vip Dent groups presented no statistical difference among them; the Orthosit Ivoclar and the Trilux groups presented statistically similar results; while the Biocler group presented results that were different from all the other tested groups. Results from the tested groups were statistically different from those of the control groups. After 168 hours, the Artiplus, Biolux, Biotone, Pop Dent, Vip Dent, and Biocler groups presented statistically similar results and presented no statistical difference when compared to the cell control and the negative control. The Trilux and Orthosit Ivoclar groups, although similar to one another, presented a statistically significant difference when compared to the other groups and controls. The positive control proved to be statistically different from all the groups (Table 1).

The results of the experiment showed that during the time periods of 24, 48, and 72 hours, all the tested groups of teeth presented statistically different results from those of the cell control and negative control. In addition, based on the number of viable cells, a cytotoxic effect on the tested cells could be observed. In the time period of 168 hours, only the Trilux and Orthosit Ivoclar groups still developed cytotoxic effects on the tested cells.

Table 1 - Mean, standard deviation and statistical analysis of the groups evaluated

GROUPS	MEAN (SD)	EST	MEAN (SD)	EST	MEAN (SD)	EST	MEAN (SD)	EST
	24 hrs		48 hrs		72 hrs		168 hrs	
Artiplus	503.3 (236)	A	822.2 (103.5)	AE	630.4 (45.8)	A	984.7 (144)	A
Biolux	378.2 (68.6)	A	616 (127.6)	AD	612.6 (80.6)	A	977.2 (93)	A
Biotone	402.8 (88.4)	A	809.1 (269.5)	AE	607 (149.6)	A	950.4 (142.2)	A
Pop dent	421.2 (139.5)	A	782.4 (141)	AE	616.4 (94.9)	A	919.8 (96.9)	A
Vip Dent	362.5 (104.3)	A	763.4 (186.9)	A	613.7 (97.8)	A	975.4 (76.2)	A
Orthosit Ivoclar	284.6 (89.9)	A	706.1 (91.5)	AD	517.3 (44.9)	B	632.6 (119.3)	B
Biocler	510.6 (178.7)	A	894.1 (127.9)	BE	663.3 (73.8)	C	952.7 (122)	A
Trilux	414.6 (59.6)	A	843.5 (90.2)	AE	544.1 (153.1)	B	692.7 (60.5)	B
CC	871.3 (37.1)	B	1190 (119.1)	C	692 (4.3)	C	1006.4 (98.8)	A
C+	72.6 (7.3)	C	491.3 (67.1)	D	215.6 (41.2)	D	164.3 (4.76)	C
C-	826.8 (31.2)	B	998.3 (63.9)	CE	655 (67.2)	CA	1018.6 (96.5)	A

Est= statistical analysis in which equal letters correspond to the absence of statistical differences ($p < 0.05$)

SD = Standard Deviation

DISCUSSION

Dentures made of acrylic resin, or which contain this resin in their composition, are widely used, such as: conventional complete dentures, partial removable dentures with clips or retained by connections, fixed implant supported dentures (Branemark protocols), and implant retained removable dentures (overdentures). As these artificial structures remain in constant contact with an individual's oral cavity cells, which may occur throughout one's life, it is thus pertinent to evaluate the possible toxic effects on cells developed by acrylic resin.

Acrylic resins used to manufacture artificial teeth for prosthetic purposes are similar to those used to make complete denture bases. The difference that exists is the much higher number of cross links present in the material that the teeth are made of, making them apt to perform the function for which they are intended, namely incision and food grinding¹³.

Despite the subject's importance, no studies evaluating the cytotoxicity of stock teeth made of acrylic resin can be found in the literature, which makes this study relevant from a scientific point of view. The studies from the literature with closely related methodologies have studied acrylic resin for complete denture and partial removable denture bases¹⁴⁻²². The majority of these studies present unfavorable results regarding the behavior of acrylic resin, which develops a cytotoxicity against the tested cells.

In the present study, during the three initial periods of the experiment, a toxic effect on the fibroblast cells could be identified for all the groups of teeth tested. Shin and Watts¹⁹ observed that after a prolonged polymerization cycle of the denture base acrylic resin, a reduction in the amount of residual

monomer released could be observed. Based on this result, it is possible to conclude that residual monomer released by the groups of teeth had occurred in the three initial periods of the experiment.

Moreover, within the limits of this study, the results allow us to suppose that the acrylic resin constituent of the majority of tested teeth had no relevant cytotoxic effect on the cells, since only the Orthosit Ivoclar and Trilux groups, among the eight tested groups, still presented cytotoxicity in the time period of 168 hours and even thereafter. However, it was not possible to affirm whether or not the cytotoxic effect remained for an undetermined period of time in the Orthosit Ivoclar and Trilux groups. Therefore, further studies are warranted concerning these groups.

CONCLUSION

In the light of this experiment, it could be concluded that:

The researched acrylic teeth did in fact develop a cytotoxicity against the cells in vitro in the time periods of 24, 48, and 72 hours during the experiment, and only the Orthosit Ivoclar and Trilux brands of teeth developed cytotoxicity against the tested cells in the time period of 168 hours.

RESUMO

Objetivo: O objetivo deste estudo foi avaliar a citotoxicidade de diferentes marcas de dentes de estoque contra células fibroblásticas. **Materiais e Métodos:** Para esta experiência a linhagem celular utilizada foi L 929 American Type Culture Collection (ATCC, Rockville, MD). As marcas de dentes de estoque testadas foram: Artplus, Biolux, Biotone, Pop Dent, Vipi Dent, Orthosit Ivoclar, Biocler Trilux. Cada grupo foi colocado em contacto com as células fibroblásticas nos períodos de 24, 48, 72 e 168

horas. Controles positivo e negativo foram usados para comparação com os extremos. **Resultados:** Os resultados mostraram citotoxicidade para todos os grupos testados nos períodos de 24, 48 e 72 horas da realização do experimento, o que ocorreu para os Grupos Orthosit Ivoclar e Trilux ainda após 168 horas de condução do experimento. **Conclusão:** Os dentes de acrílico estudados pesquisadas desenvolveram citotoxicidade contra as células fibroblásticas *in vitro*, nos períodos de 24, 48 e 72 horas e Vivodent Ivoclar e Trilux desenvolveram citotoxicidade contra as células testadas até o período de 168 horas.

Descritores: Dente artificial. Estudos de avaliação. Linhagem celular.

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