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Evaluation of the cytotoxicity of polyurethane and non-latex orthodontic chain elastics

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ABSTRACT

Allergy caused by latex proteins has been well documented. Thus, the study of non-latex materials, is necessary. The aim of this study is to evaluate the cytotoxicity of silver-coloured orthodontic chain elastics, of polyurethane and latex-free. Nine chain elastics from different manufactures (3 latex-free and 6 polyurethane) were divided into 9 groups of 10 elastics each: Group UK (Latex-free, 3M Unitek), Group TP (Látex-free, TP Orthodontics), Group AO (Látex-free, American Orthodontics), Group O (Polyurethane, OrthoSource), Group M (Polyurethane, Morelli), Group TD (Polyurethane, Tecnident), Group UD (Polyurethane, Uniden), Group AZ (Polyurethane, Abzil) and Group AK (Polyurethane, Aditek). The cytotoxicity essay was performed using cell cultures (L-929 line cells, mouse fibroblast) that were submitted to the cell viability test with neutral red ("dye-uptake") at 1, 2, 3, 7 and 28 days. Analysis of variance (ANOVA) with multiple comparisons and Tukey's test were employed ($p<.05$). The results showed no statistically significant differences between Groups UK, TP and AO in all experimental times ($p>.05$), except between the Groups UK and TP at 28 days ($p<.05$). There was significant statistically difference ($p<.05$) between the viability of the cells in the Groups O and TD with the Groups M, AZ, AK at 1 and 2 days. The polyurethane elastics showed lower cell viability in the first 48 hours, with increase on 3rd and 7th day, and viability similar to latex-free elastics at 28 days. It can be concluded that the latex-free chain elastic showed higher cell viability. The OrthoSource and Tecnident trademark showed lower cell viability the initial 48 hours.

Keywords: Cytotoxicity, Elastomers, Cell culture, Orthodontics.

1 INTRODUCTION

Latex has been extensively used in orthodontics since the advent of the specialty, however, the protein content of latex is a known allergen [1, 2]. As alternative to latex, chain elastics of types different as polyurethane and latex-free has been manufactured, in order to decrease the risk of allergic reactions caused by latex orthodontic elastics, as swelling and stomatitits, erythematous oral lesions, respiratory reactions, and even anaphylactic shock, the most severe form of allergy [3, 4], can be cited.

But little is known if polyurethane and latex-free orthodontic ligatures are cytotoxic to oral mucosal cells [1-6]. Cell lines [7], such as L929 mouse fibroblasts[8], have been shown to behave similarly to primary human gingival fibroblasts and, therefore, are a suitable in-vitro model to test the toxicity [9-12] of products used intra-orally during orthodontic treatment [13-15]. In order of evaluate the biological behavior of these materials in cell culture, the Objective of the present in vitro study was to test the cytotoxicity of polyurethane and latex-free Orthodontic elastomeric chains of different manufactures.

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2 MATERIAL AND METHODS

Silver-coloured orthodontic chain elastics (medium) from 9 different manufacturers were selected for cytotoxicity study, being three of latex-free and six containing polyurethane (Table 1). The samples were divided into 9 groups of 10 elastics each: Group UK (Latex-free, 3M Unitek, Monrovia, California, USA), Group TP (Latex-free, TP Orthodontics, Lodi, California, USA), Group AO (Latex-free, American Orthodontics, Sheboygan, Wisconsin, USA), Group O (Polyurethane, OrthoSource, North Hollywood, CA, USA), Group M (Polyurethane, Morelli, Sorocaba, São Paulo, Brazil), Group TD (Polyurethane, Tecnident, São Carlos, São Paulo, Brazil), Group UD (Polyurethane, Uniden, Sorocaba, São Paulo, Brazil), Group AZ (Polyurethane, Abzil, São José do Rio Preto, São Paulo, Brazil) and Group AK (Polyurethane, Aditek, Cravinhos, São Paulo, Brazil).

Table 1: Experimental and control groups used for the assays.

Groups	Trademark	Main Composition	Colour	Reference number
UK	Unitek	Latex-free	Silver	406-669
TP	TP Orthodontics	Latex-free	Silver	389-061
AO	American Orthodontics	Latex-free	Silver	854-299
O	OrthoSource	Polyurethane	Silver	0-0434-420
M	Morelli	Polyurethane	Silver	60-05-217
TD	Tecnident	Polyurethane	Silver	402-001
UD	Uniden	Polyurethane	Silver	000-1495
AZ	Abzil	Polyurethane	Silver	467-816
AK	Aditek	Polyurethane	Silver	0080185
C+	Tween 80 (Polyoxyethylene-20-sorbitan, Sigma, St. Louis, Missouri, USA)			
C-	PBS solution (phosphate-buffered saline, Cultilab, Campinas, SP, Brazil)			
CC	cell control (L-929 line cells, ATCC, Rockville, MD, USA)			

All samples had recent manufacturing dates, were from the same production lot and came in sealed plastic packages. The superficial powder coating of the elastomeric ligatures was removed, in which, all elastics were washed for 15 seconds with current deionized water by using a Milli-Q purification system (Millipore, Bedford, MA, USA) and dried lightly with absorbent paper. Before testing all elastomeric ligatures were sterilized by exposure to ultraviolet light (Labconco, Kansas, Missouri, USA) for 30 minutes [16-17].

The cell culture model used was the monolayer containing L-929 line cells (American Type Culture Collection - ATCC, Rockville, MD, USA) maintained in Eagles' minimum essential medium (Cultilab, Campinas, Brazil) by adding 0.03 mg/ml of glutamine, 50 µg/ml of garamicine, 2.5 mg/ml of fungizone, 0.25% sodium bicarbonate solution, 10 mM of HEPES, and 10% bovine fetal serum for growth medium. Next, the cell culture medium was incubated at 37^{o}C for 48 hours.

To verify the cell response in extreme situations, three additional groups were included in the study: Group CC (cell control), consisting of L-929 cells not exposed to supernatants from the elastomeric ligatures; Group C+ (positive control), consisting of Tween 80 (Polyoxyethylene-20-sorbitan, Sigma, St. Louis, Missouri, USA); Group C- (negative control), consisting of phosphate-buffered saline (PBS) solution (Table 1). The positive and negative controls were incubated in MEM maintenance medium (Eagles' minimum essential medium) for 1, 2, 3, 7 and 28 days and the extracted elutes were added to L-929 line cells incubated in the growth medium.

The cytotoxicity of these orthodontic elastics was determined through the dye-uptake technique [18], which is based on the neutral red absorption by living cells. How these chain elastics are usually maintained in the oral cavity for up to 4 weeks, since patients wearing fixed appliances usually visit the orthodontist once a month, the periods of time: 1, 2, 3, 7, and 28 days were evaluated in this study. These experimental periods represent the time maintenance under cell culture conditions before removal of the chain elastics.

2.1 Dye-uptake

Volumes of 100 µl of L-929 cells were distributed in triplicate for each specimen tested into 96-well microplates. After 48 hours, the growth medium was replaced with 100 µl of Eagles' minimum essential medium (MEM) obtained following incubation in the different types of chain elastics at 1, 2, 3, 7 and 28 days. Eagles' minimum essential medium was employed because it is the same type of material used for cell maintenance, thus not influencing the results.

After 24 hours of incubation, 100 µl of 0.01 per cent neutral red dye (Sigma, St. Louis, Missouri, USA) was added to each well in the microplates and incubated for 3 hours at 37 °C. Following this period of time, 100 µl of 4 per cent formaldehyde solution in PBS (130 mmol of NaCl; 2 mmol of KCl; 6 mmol of Na_2HPO_4 2 H_2O; 1 mmol of K_2HPO_4 1 mmol; pH 7.2) were added to each well to promote cell attachment to the plate. After 5 minutes, 100 µl of 1 per cent acetic acid and 50 per cent methanol were added in order to remove the dye not taken up by the cells. After 20 minutes, a spectrophotometer (BioTek, Winooski, Vermont) set at a wavelength of 492 nm was used to determine the dye taken up by the cells. Because elastomeric ligatures can be in the oral cavity for up to 4 weeks, cell viability was determined after exposure to MEM in which the elastics had been incubated for 1, 2, 3, 7 and 28 days. The cytotoxicity of the materials was determined according to ISO 10993-5 [19] for evaluation and standardization.

For ranking the citoxicity, a post hoc comparison was performed [20, 21]. Statistical calculations were performed with 1-way analysis of variance (ANOVA) followed by the Tukey post hoc test. *P* values less than .05 were considered to indicate significant differences. Each culture well was considered an individual sample.

3 RESULTS

There were statistically significant differences (p=.00) between the viability of the cells of Group CC (Figure 1A) and all other groups at 1, 2, 3, 7 and 28 days. Nor were there any statistically significant differences (p>.05) between the viability of the cells in Groups UK, TP and AO in all experimental times, except between the Groups UK and TP at 28 days (p<.05) (Table 2 and Figure 2).

Table 2: Descriptive statistics for optical density of chain elastics at 1 to 28 days.

	Time (1 day)			Time (2 days)			Time (3 days)			Time (7 days)			Time (28 days)		
G	**M**	**SD**	**VC (%)**	**M**	**SD**	**VC (%)**	**M**	**SD**	**VC (%)**	**M**	**SD**	**VC (%)**	**M**	**SD**	**VC (%)**
CC	.842^{a}	.030	100.0	.890^{a}	.029	100.0	.616^{a}	.025	100.0	.915^{a}	.030	100.0	.519^{a}	.028	100.0
C-	.814	.031	96.7	.862	.022	96.9	.599	.029	97.4	.873	.031	95.5	.499	.022	96.3
C+	.066	.008	7.90	.086	.010	9.70	.060	.009	9.80	.086	.008	9.50	.055	.009	10.6
UK	.691^{be}	.030	82.1	.723^{b}	.030	81.3	.550^{b}	.023	89.4	.841^{bc}	.032	92.0	.488^{b}	.027	94.2
TP	.682^{bde}	.034	81.0	.732^{b}	.031	82.3	.557^{b}	.028	90.5	.839^{bc}	.030	91.8	.473^{c}	.024	91.3
AO	.711^{e}	.037	84.5	.738^{b}	.027	83.0	.550^{b}	.024	89.4	.855^{b}	.036	93.5	.477^{bc}	.024	92.0
O	.597^{f}	.031	71.0	.614^{c}	.024	69.1	.488^{ce}	.027	79.3	.810^{cefg}	.032	88.6	.467^{c}	.020	90.0
M	.651^{dg}	.033	77.4	.661^{d}	.036	74.3	.523^{df}	.027	85.0	.814^{cefg}	.032	89.0	.474^{c}	.028	91.4
TD	614^{cf}	.026	73.0	.605^{c}	.020	68.0	.517^{df}	.029	84.0	.824^{bcef}	.036	90.1	.465^{c}	.029	89.6
UD	.639^{cg}	.028	75.9	.666de	.024	74.9	.535^{db}	.027	87.0	.798de	.028	87.3	.467^{c}	.022	90.0
AZ	.663^{bg}	.031	78.8	.712^{bf}	.028	80.0	.524^{df}	.027	85.1	.808^{df}	.031	88.4	.465^{c}	.026	89.7
AK	.676^{bg}	.034	80.3	.687^{def}	.032	77.2	.511^{ef}	.020	83.0	.786^{dg}	.033	86.0	.472^{c}	.028	91.0

N=10. Analysis of variance ANOVA and Tukey's test were employed (p<.05). Values followed by same letters are not significantly different (p>.05) for the same time. G: Groups. M: Mean. SD: standard deviation. VC: Viable Cells.

There was statistically difference (p<.05) between the viability of the cells in Groups O and TD with the Groups M, UD, AZ, AK at 1 and 2 days, except between the Groups TD and UD at 1 day (p<.05). The polyurethane elastics showed lower cell viability in the first 48 hours, with increase on 3rd and 7th day, and viability similar to latex-free elastics in 28 days (Table 2 and Figure 2).

With 48 hours the percentage of viable cells varied between 83.0 per cent in Group AO (Figure 1B) latex-free chain elastic, to 68.0 per cent in Group TD (Figure 1C) polyurethane chain elastics.

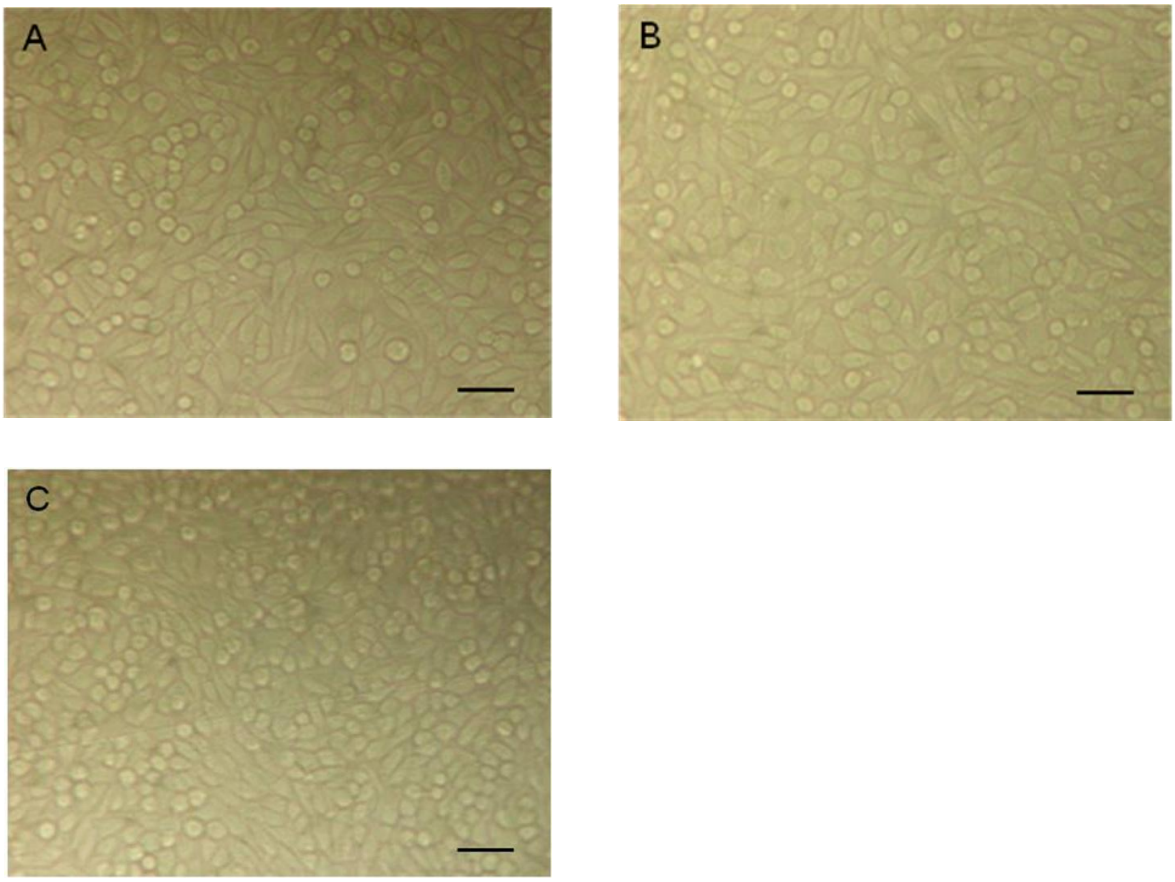

Figure 1: Cell aspect. **A)** cell control; **B)** group AO (American Orthodontics) at 2 days; **C)** group TD (Tecnident) at 2 days. Bar = 50µm.

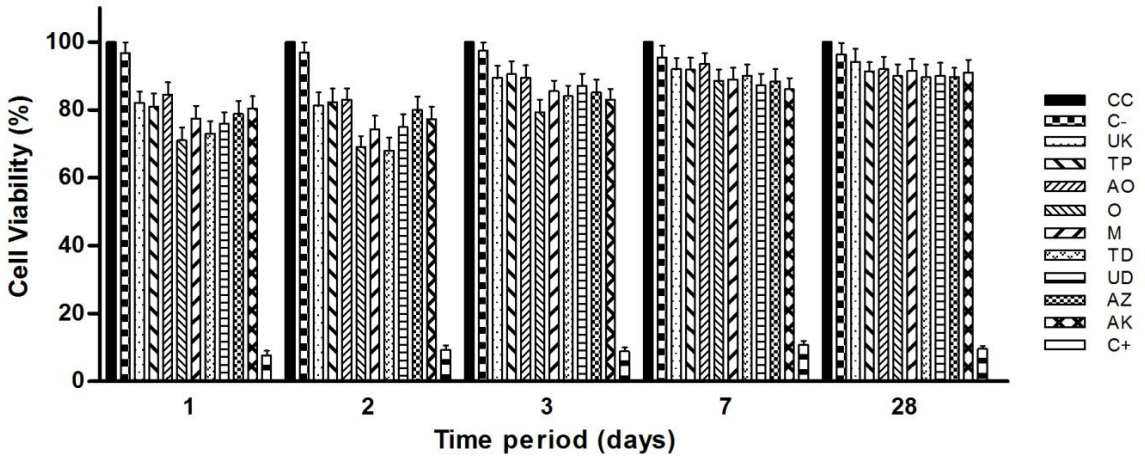

Figure 2: Percentage viability of tested chain elastics obtained by spectrophotometry.

4 DISCUSSION

The use of monolayer cell culture in the present study was based on other studies [17, 22]. This model was used together with the dye-uptake technique [18] because the cytotoxicity of the materials can be determined by spectrophotometry.

Spectrophotometric essay allows rapid and reliable evidence for cell viability to be obtained based on the use of vital stain incorporated by viable cells [16, 17]. In this study, we used neutral red dye as it is largely employed for identification of L-929 cell viability [23, 24]. Dead or damaged cells cannot incorporate vital stain, thus not being recognized on optical reading. Therefore, spectrophotometry does not allow dead cells to be distinguished from the damaged ones [23].

L-929 mouse fibroblasts were used because they have results comparable to primary human gingival fibroblasts [13, 14], but one cannot interpret the cell culture [12] results as a human response.

The percentage of viable cells was obtained by comparing the mean optical density (OD) in the control group (cells with no contact with chain elastics) to that obtained from supernatants of cell cultures that had been in contacted with chain elastics [17, 23].

As sterilization is a prerequisite for cytotoxicity essays, ultraviolet radiation [16, 17] was used in this study for 30 minutes for each elastic surface. It was observed that all elastics exhibited the same colour aspect following sterilization with UV light.

Because natural latex rubber has been increasingly used as dental material, many cytotoxicity issues have been reported as well [5]. Conservants such as sulphur and zinc oxide as well as antioxidants such as di-thio-carbohydrates, N-nitrosodibutylamine, and N-nitrosopiperidine are all known to be cytotoxic substances [6]. The colourants used in the fabrication of coloured latex exhibit low toxicity [25]. What makes her effect to be clinically inoffensive.

As an alternative to latex, front the reports of latex allergy in the literature [26, 27], this study evaluated the cytotoxicity of non-latex materials, as the polyurethane and latex-free orthodontic chain elastics.

Allergy to natural latex occurs because of the presence of many types of proteins, and the powder covering the orthodontic elastics works as a transporter for these proteins. Therefore, the development of non-latex elastics has become increasingly important for clinical usage.

We have assessed Unitek, TP Orthodontics and American Orthodontics, latex-free chain elastics and it was observed that these elastomers induced a lesser amount of cell lysis compared to others polyurethane chain elastics. As the powder covering the chain elastics of all manufacturers was removed before performing the *in vitro* studies, it was not possible to know whether this powder would have any effect. The powder was removed in order to standardize the samples as composition and quantity of powder present in the chain elastics could interfere with the results.

Evidence of this cytotoxic feature was shown following exposition of the chain elastics to cell culture medium. Chain elastics from OrthoSource, Morelli, Tecnident, Uniden, Abzil and Aditek trademarks induced a greater amount of cell lysis at 24 and 48 hours, suggesting a greater release of toxic ingredients at 48 hours, due to release of cytotoxic components, which was shown on days 1 and 2, and decreased on days 3, 7 and 28. However, all chain elastics were found to be biocompatible after the 3rd experimental day.

The latex-free chain elastics presented better performance compared to the polyurethane elastics, suggesting that different processes in the manufacture and materials with different compositions leads to different cytotoxic characteristics of these, despite being reported by manufacturers atoxicity these materials. Latex-free chain elastics showed low capacity of inducing cell lysis regardless of the evaluated time.

The great danger is that potentially cytotoxic intra-oral elastics could release substances that might be ingested by the patient over time, thus causing diseases resulting from a cumulative effect [15].

The chain elastics evaluated in this study showed over 68% cell viability regardless of the experimental period of time. Elastomers of latex and non-latex evaluated in previous study [28] demonstrated that the occurrence of cell lysis was above 50% for latex elastics compared to non-latex ones. However, the authors considered both types of elastics viable for clinical use. Therefore, it is suggested that elastics with cell viability less than 50% should be avoided in order to prevent cumulative effects of the cytotoxic component releasing, into the organism [15].

There seems to be an important relationship between the processes of manufacture these chain elastics and their cytotoxic character. Currently, there is a shortage of clinical studies demonstrating the cytotoxicity of elastomers of orthodontic use. However, as these materials are widely used in clinical orthodontics, care regarding the cytotoxicity of orthodontic chain elastics should be taken, mainly with regard to elastics as they have a very close contact with gingiva.

5 CONCLUSION

Within the limits of this in vitro study, the latex-free chain elastics from Unitek, TP Orthodontics and American Orthodontics trademark induced a lesser amount of cell lysis compared to polyurethane chain elastics. However, chain elastics, of latex-free and polyurethane of all manufacturers were considered suitable for clinical use.

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