

Cytotoxicity of separation orthodontic elastics.

Rogério Lacerda dos Santos¹, Matheus Melo Pithon², Fernanda Otaviano Martins³, Maria Teresa Villela Romanos⁴

Objective: To test the hypothesis that there is no difference in cytotoxicity between separating elastics of different manufacturers.

Methods: The present article compared latex elastics (4.0 mm, 4.4 mm and 4.8 mm) of four different manufacturers. The sample was allocated to seven groups of 9 elastics: Group A (American Orthodontics, green color, modules), Groups M1 and M2 (Morelli, blue color, modules and free in pack respectively), Groups M3 and M4 (Morelli, green color, modules and free in pack respectively), Group U (Uniden, blue color, free in pack) and Group T (Tecnident, blue color, free in pack) regarding their possible cytotoxic effects on oral tissues. Cytotoxicity assays were performed using cell culture medium containing epithelioid-type cells (Hep-2 line) derived from human laryngeal carcinoma and submitted to the methods for evaluating the cytotoxicity by the “dye-uptake” test, at time intervals 24, 48, 72 and 168 h. Data were compared by analysis of variance (ANOVA) and Tukey’s test ($p < 0.05$).

Results: Results showed statistically significant difference ($p < 0.05$) between group U and all the other Groups (A, M1, M2, M3, M 4 and T) at 24 and 48 hours.

Conclusions: Uniden elastics evoked more cell lysis at 24 and 48 h, although, all brands showed biocompatibility from 72 h onwards.

Keywords: Cytotoxicity. Elastics. Biocompatibility. Orthodontics.

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Citotoxicidade de elásticos ortodônticos de separação

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Objetivo: o propósito do presente estudo foi testar a hipótese que não existe diferença de citotoxicidade entre elásticos de diferentes marcas.

Métodos: foram comparadas entre si 4 marcas de elásticos de separação (4,0mm, 4,4mm e 4,8mm) intrabucais de látex quanto ao possível efeito citotóxico nos tecidos bucais, divididos em 7 grupos de 9 elásticos cada: grupo A (cor verde – modular, American Orthodontics), grupos M1 e M2, (cor azul – modular e a granel, respectivamente, Morelli), grupos M3 e M4 (cor verde – modular e a granel, respectivamente, Morelli), grupo U (cor azul – a granel, Uniden) e grupo T (cor azul – a granel, Tecnident). O ensaio de citotoxicidade foi realizado utilizando-se cultura de células da linhagem HEP-2 (do tipo epitelioide, que tem origem em carcinoma de laringe humana), sendo submetido o material ao teste para células viáveis em vermelho neutro (“dye-uptake”), no tempo de 24, 48, 72 e 168 horas. A análise de variância e comparação múltipla (ANOVA) e o teste de Tukey foram utilizados ($p < 0,05$).

Resultados: os resultados evidenciaram diferença estatisticamente significativa dos grupos A, M1, M2, M3, M4 e T com o grupo U nos tempos de 24 e 48h ($p < 0,05$).

Conclusão: pôde-se evidenciar que os elásticos da marca Uniden causaram alta quantidade de lise celular em 24 e 48h, porém, todas as marcas mostraram-se biocompatíveis a partir de 72h.

Palavras-chave: Teste de materiais. Látex. Ortodontia. Toxicidade.

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INTRODUCTION

Orthodontic elastics are widely used in orthodontic practice with the purpose of helping orthodontic treatment, and therefore need to be inert to oral tissues. Elastics in contact with the oral mucosa for several hours a day is a situation that may continue for months. Therefore, the question arises about the possibility of toxic substances being released by elastomers, which may be capable of harming the cells.

Latex is constituted of chains of cis-1, 4-poly-isoprene. After obtaining the liquid from latex, it is preserved by the addition of conservants (usually ammonia). When it is manufactured, various substances are added in order to achieve the final properties.²²

There are variations in the composition of latex elastics, and consequently, there are differences in their properties. This may be one of the reasons why companies produce various sizes to compensate for the variations in physical properties.¹ Depending on how latex is stored, alterations may occur in its composition, as its major limitation is sensitivity to ozone or other systems generating free radicals, such as sunlight which weakens the latex polymer chain.²² Pre-vulcanized latex production involves the mixture of latex from the purest and highest molecular weight natural rubber with stabilizers, such as zinc oxide and vulcanized chemical products. The mixture is heated to a temperature of 70°C.¹⁴ Zinc is known to be neurotoxic.⁸ Although the zinc released from orthodontic elastics may be swallowed, the results suggest that the use of latex elastics in orthodontics is appropriate.⁵

Natural latex is not in the category of materials generally considered safe.^{6,16} Allergy caused by proteins from latex has been well documented,¹³ and may present immediate hypersensitivity reactions.²¹ Among the reactions caused by orthodontic elastics, there have been reports of the development of stomatitis with swelling, erythematous oral lesions, in addition to respiratory, and systemic reactions, and in extreme cases, anaphylactic shock.^{3,18} The prevalence of latex allergy is between 3% and 17%.²⁰

Cell culture tests for the evaluation of dental material toxicity are a valid method to enable understanding of their biologic behavior.¹⁶ The aim of the present study was to test the hypothesis that there is no difference in cytotoxicity between separating elastics of different brands.

MATERIAL AND METHODS

Samples of intraoral latex separating elastics (4.0 mm, 4.4 mm and 4.8 mm) (Fig 1) of 4 different brands and colors were selected (Table 1), and divided into 7 groups containing 9 elastics each: Group A (Green color- modular, American Orthodontics, Sheboygan, Wisconsin, USA), Group M1 (Blue color- modular, Morelli, Sorocaba, Brazil), Group M2 (Blue color- in bulk, Morelli, Sorocaba, Brazil), Group M3 (Green color- modular, Morelli, Sorocaba, Brazil), Group M4 (Green color- in bulk, Morelli, Sorocaba, Brazil), Group U (Blue color- in bulk, Uniden, Sorocaba, Brazil) and Group T (Blue color- in bulk, Tecnident, São Carlos, Brazil) with regard to the possible cytotoxic effect on oral tissues. The elastics used in this research were from the same production lot for each tested color. Copper amalgam was used as positive control (Vigodent, Rio de Janeiro, Brazil), standardized by size and weight, and as negative control, stainless steel wire (American Orthodontics, Sheboygan, Wisconsin, USA) (Table 1).

To conduct this study, HEP-2 (human carcinoma of the larynx) cell culture was used, maintained in Eagle's Minimum Essential Medium (MEM-Eagle) (Cultilab, Campinas, Brazil) with the addition of 0.03 mg/ml glutamine (Sigma, St. Louis, Missouri, USA), 50 µg/ml gentamicin (Schering Plough, Kenilworth, New Jersey, USA), 2.5 mg/ml fungizone (Bristol-Myers-Squibb,

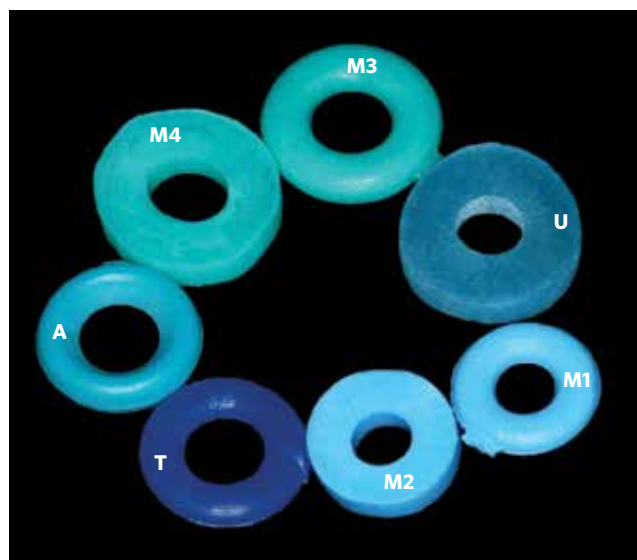


Figure 1 - Separating elastics evaluated in this study: A (green modular, American Orthodontics), M1 (blue, modular, Morelli), M2 (blue, in bulk, Morelli), M3 (green, modular, Morelli), M4 (green, in bulk, Morelli), U (blue, in bulk, Uniden) and T (blue, in bulk, Tecnident).

New York, USA), 0.25% sodium bicarbonate solution (Merck, Darmstadt, Germany), 10 mM HEPES (Sigma, St. Louis, Missouri) and 10% fetal bovine serum (Cultilab, Campinas, Brazil) (growth medium) or without fetal bovine serum (maintenance medium) and incubated at 37°C for 48 hours.

The elastics were previously sterilized by ultraviolet radiation (Labconco, Kansas, Missouri) for 30 minutes on each surface of the elastic. To determine the cytotoxicity of the orthodontic elastics, the technique denominated “dye-uptake” was used,¹¹ which is based on the incorporation of neutral red dye by live cells. The time intervals of 24 h, 48 h, 72 h and 168 h were used, as these elastics are usually maintained in the oral cavity for up to 168 h (7 days) to separate the teeth. This time represents maintenance of the elastic in the cell culture medium for 24 h, 48 h, 72 h, 168 h, and later removal.

Dye-uptake

Volumes of 100 µl of HEp-2 cell suspension were distributed into 96-well microplates. After 48 hours, the growth medium was replaced by 100 µl of culture medium (MEM-Eagle) obtained after incubation with different elastics for time intervals of 24 h, 48 h, 72 h and 168 h. As positive and negative controls the culture media obtained after contact with amalgam and stainless steel wire respectively were used. The experiment was conducted in quadruplicate.

After 24 hours of incubation, 100l of 0.01% neutral red (Sigma, St. Louis, Missouri, USA), were added to culture medium in each well of the miniplate, and these were incubated at 37°C for 3 hours for the dye to penetrate into the live cells. After the elapse of this time interval, and after disregarding the dye, 100µl of 4% formaldehyde solution (Vetec, Rio de Janeiro, Brazil) was added to PBS (NaCl 130 mM; KCl 2 mM; Na₂HPO₄ 2H₂O 6 mM; K₂HPO₄ 1mM, pH7.2) for 5 minutes, to promote cell fixation to the plates. Next, in order to extract the dye, 100µl of 1% acetic acid solution (Vetec, Rio de Janeiro, Brazil) with 50% methanol was added (Vetec, Rio de Janeiro, Brazil). After 20 minutes the readout was taken in a spectrophotometer (BioTek, Winooski, Vermont, USA) at a wavelength of 492nm ($\lambda = 492$ nm).

Data were compared by analysis of variance (ANOVA) and afterwards by Tukey’s test for evaluation among groups, with reliability at a level of significance of 0.05.

RESULTS

The results showed statistically significant difference among Groups A (Green color- modular, American Orthodontics), M1 (Blue color- modular, Morelli), M2 (Blue color- in bulk, Morelli), M3 (Green color- modular, Morelli), M4 (Green color- in bulk, Morelli) and T (Blue color- in bulk, Tecnident) with Group U (Blue color- in bulk, Uniden) at the time intervals of 24 h and 48 h ($p < 0.05$) (Table 2). There was no statistically significant difference between Groups M2, M3, U and T at 72 h ($p > 0.05$) (Table 2). At 72 h, the Morelli and Tecnident brands of elastics caused a larger quantity of cell lysis in comparison with the time intervals of 24, 48 and 168 h. This may mean greater release of toxic substances by these elastics in 3 days.

Discussion

In this study, the option was taken to use copper amalgam as positive control and stainless steel wire as negative control (Table 1) as they have been proved to be adequate for this test.^{9,12} The cytotoxic potential of dental amalgam comes from the presence of mercury, however, the amalgam contains other substances that

Table 1 - Elastics and control groups used in the tests.

Groups	Brand	Color	Diameter (mm)	Reference
A	A. Orthodontics	Green	4.44	854-251
M1	Morelli	Blue	4.00	60.04.201
M2	Morelli	Blue	4.00	60.04.200
M3	Morelli	Green	4.80	60.04.401
M4	Morelli	Green	4.80	60.04.400
U	Uniden	Blue	4.80	000-1320
T	Tecnident	Blue	4.40	A-007
Positive Control	Copper Amalgam. Pratic NG 2. Vigodent			
Negative Control	Stainless steel wire American Orthodontics. 0.019 x 0.025-in			

Table 2 - Dye-uptake technique. Statistical description for optical density of elastic evaluated.

Groups	n	Time (24 h)			Time (48 h)			Time (72 h)			Time (168 h)		
		Mean	S.D.	Viable cells (%)	Mean	S.D.	Viable cells (%)	Mean	S.D.	Viable cells (%)	Mean	S.D.	Viable cells (%)
CC	9	0.672 ^a	0.139	100.0	0.557 ^a	0.139	100.0	0.690 ^a	0.149	100.0	0.778 ^a	0.124	100.0
C-	9	0.644	0.149	95.9	0.539	0.149	96.8	0.681	0.131	98.8	0.747	0.120	96.1
C+	9	0.200	0.126	29.8	0.197	0.126	35.4	0.268	0.129	38.9	0.295	0.127	38.0
A	9	0.640 ^b	0.135	95.3	0.526 ^b	0.135	94.5	0.660 ^b	0.131	95.7	0.749 ^b	0.131	96.4
M1	9	0.617 ^{cb}	0.133	91.9	0.518 ^b	0.133	93.1	0.598 ^c	0.113	86.8	0.728 ^{cb}	0.130	93.7
M2	9	0.606 ^{dc}	0.116	90.2	0.517 ^b	0.116	92.9	0.532 ^d	0.126	77.2	0.729 ^{dbc}	0.120	93.8
M3	9	0.612 ^{ecd}	0.119	91.1	0.515 ^b	0.119	92.6	0.521 ^d	0.128	75.6	0.719 ^{ecd}	0.112	92.5
M4	9	0.611 ^{fcde}	0.123	91.0	0.509 ^b	0.123	91.4	0.489 ^e	0.121	70.9	0.718 ^{fcde}	0.117	92.4
U	9	0.114 ^e	0.096	17.1	0.068 ^c	0.096	12.3	0.542 ^d	0.089	78.6	0.724 ^{ecdef}	0.115	93.1
T	9	0.620 ^{bcddef}	0.122	92.3	0.516 ^b	0.122	92.7	0.544 ^d	0.112	78.9	0.721 ^{cdefg}	0.119	92.7

Values followed by equal capital letters present no statistically significant difference ($p > 0.05$). CC: Control of cells. C-: Negative Control (stainless steel wire). C+: Positive Control (copper amalgam) and S.D.: Standard Deviation.

may also be neurotoxic, depending on its composition and manufacturer.⁸

Sterilization is a pre-requisite for the cytotoxicity test. Autoclave sterilization may be used, however, elastics have been shown to darken and harden after this type of sterilization due to the heat liberated,²³ which may cause degradation and the release of substances that are toxic to cells. In this study, sterilization by ultraviolet radiation was used¹⁵ for 30 minutes on each side of the elastic. In this study the elastics were shown to have the same aspects of color and malleability after UV light sterilization.

With the increasing use of rubber latex as dental material, many cytotoxic factors have been reported.⁷ Sulphur and zinc oxide, as conservants, exhibit cytotoxicity, and dithiocarbates, N-nitrosodibutylamine, and N-nitrosopiperidine, which act as antioxidants, are also known to be cytotoxic substances.⁴ Holmes and cols⁶ verified whether coloring agents used in colored latex manufacture may have any toxic effect. Their results showed that they have low toxicity. Clinically therefore, this effect is harmless.

Although case reports on allergy to latex do not appear frequently, allergic reactions have become somewhat more prevalent with the increase in latex-based products. The majority of allergic reactions¹⁷ have been related to the use of latex gloves, but only 2 cases

were related to the use of orthodontic elastics.¹⁰ In the cases related to orthodontic elastics, the presence of small vesicles or acute edema occurred, and the patients complained of burning and itching.

Allergy to natural latex occurs because it contains many types of proteins, and the powder present in the coating of orthodontic elastics function as a vehicle for these proteins. Therefore, from a clinical point of view, the development of elastics without latex is increasingly important.

In this study, the talc was removed before the in vitro studies were conducted, and it is not known whether the talcum powder would have made any difference.

According to Schmalz,¹⁶ the great danger with the use of intraoral elastics with cytotoxic potential would be the fact that the substances released by these would be ingested by the patient, and over the course of time, cause diseases resulting from the cumulative effect of toxic substances. It is known that latex is not a completely biocompatible substance. It may cause allergic reactions^{20,22} and generate cross-reactions with foods^{2,20} and medications.¹⁹

As they are widely used materials in the orthodontic clinic, one must be concerned about the cytotoxicity of elastics, particularly the intraoral type that comes into intimate contact with the mucosa, and opt

for materials that have been proved to be biocompatible from this aspect. Previous studies on the toxicity of latex orthodontic elastics used for separating the teeth, both clear and neon, have been shown to be cytotoxic to gingival fibroblasts.⁶

The cytotoxic nature was evidenced after the elastics were exposed to the culture medium. The Uniden brand of separating elastics caused the greatest quantity of cell death in comparison with the other brands evaluated in the time intervals of 24 and 48 h, which suggests the release of toxic ingredients in the first 48 h for this elastic, however, as from the 3rd day, all the elastics demonstrated a less cytotoxic nature.

The percentage of viable cells was obtained by means of comparison of the mean optic density (OD) of the control cells (without coming into contact with the elastics) with the means of OD obtained from the

supernatant of the cell cultures that were placed in contact with the elastics, and toxicity was calculated for 50% of the cell cultures (CC₅₀) (Table 2).

Variations occur in the composition of the latex elastics and this may explain the difference in the results obtained among the brands. Although the *in vitro* evaluation does not simulate the oral medium, it is necessary to consider that the elastics are not clinically inert.

CONCLUSION

It could be concluded that:

- 1) The separating elastics of American Orthodontics, Morelli and Tecnident brands caused a small quantity of cell lysis.
- 2) The Unident brand of separating elastics cause a large quantity of cell lysis in the time intervals of 24 and 48 h.

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