

Evaluation of the *in vitro* biocompatibility of orthodontic elastics

Matheus Melo Pithon¹; Rogério Lacerda dos Santos¹; Fernanda Otaviano Martins²;
Maria Teresa Villela Romanos³; Mônica Tirre de Souza Araújo⁴

¹ DDS, MS, PhD Student in Orthodontics, Federal University of Rio de Janeiro, Brazil

² BS, Federal University of Rio de Janeiro, Brazil

³ BS, Assistant Professor of Microbiology, Federal University of Rio de Janeiro, Brazil

⁴ DDS, MS, PhD, Assistant Professor of Orthodontics, Federal University of Rio de Janeiro, Brazil

Abstract

Aim: Latex has been extensively used in orthodontics since the advent of the specialty. Natural latex does not fall into the category of materials known to be entirely inoffensive. The objective of the present *in vitro* study is to test the hypothesis that there is no difference in the cytotoxicity between natural latex elastics of different colors. **Methods:** The present article compared different latex intra-oral elastics (5/16 = 7.9 mm, mean load). The samples were divided into six groups of 15 elastics according to their manufacturer: Groups N, Y, V, R, G and P (Uniden, natural latex elastics and colored elastics, namely, yellow, violet, red, green and pink, respectively). Cytotoxicity assays were performed by using cell culture medium containing cells from mouse fibroblast cell line L929. The cytotoxicity was evaluated by using the "dye-uptake" test, which was employed at two different moments (1 and 24 h). Data were compared by ANOVA and Tukey's test ($P < 0.05$). **Results:** There was statistically significant difference ($P < 0.05$) between the groups N, Y, V, R, G, P and the cell control at 1 h. After 24 h, a decrease in cell viability was observed in all groups. There was statistically significant difference ($P < 0.05$) between all test elastics groups and the cell control at 24 h. No statistically significant difference ($P > 0.05$) was found among the test elastics groups at 24 h. **Conclusion:** Latex elastics from natural, yellow, violet and red colors induced a lowest amount of cell lysis compared to the elastics green and pink colors at 1 h, all latex elastics were found to be highly cytotoxic, regardless of their color at 24 h.

Keywords: cytotoxicity, elastics, biocompatibility, orthodontics.

Introduction

Latex elastic has been extensively used in orthodontics since the advent of the specialty. However, natural latex does not fall into the category of materials known to be entirely inoffensive¹⁻².

Allergy caused by latex proteins has been well documented³, including immediate hypersensitivity reactions⁴. Among the allergic reactions caused by orthodontic elastics, swelling and stomatitis, erythematous oral lesions, respiratory reactions, and even anaphylactic shock, the most severe form of allergy⁵⁻⁶, can be cited. Latex allergy occurs in 3-17% of the cases⁷.

Prevulcanized latex is produced by mixing pure natural latex, which has the highest molecular weight⁸, with stabilizers such as zinc oxide and chemically vulcanized materials. The resulting mixture is then heated until 70°C⁹. Although zinc is known to be neurotoxic¹⁰, the amount released by orthodontic elastics can be ingested as research studies show no evidence of harm¹¹. Anti-ozone and anti-oxidant agents are also added to latex during the manufacture of orthodontic elastics⁸. This process has the advantage of producing latex with higher mechanical properties, thus increasing its strength and elasticity^{9,11}.

The use of cell culture medium for testing the toxicity of dental products is a valid way of understanding the biological behavior of such materials¹. The objective of the present *in vitro* study was to test the hypothesis that there is no difference in the cytotoxicity between natural latex elastics of different colors.

Received for publication: May 27, 2009

Accepted: November 11, 2009

Correspondence to:

Mônica Tirre de Souza Araújo
Universidade Federal do Rio de Janeiro - UFRJ
Faculdade de Odontologia -
Departamento de Ortodontia
Av. Prof. Rodolpho Paulo Rocco325,
Ilha do Fundão
Rio de Janeiro - RJ - Brasil - CEP: 21941-617
E-mail: monicatirre@gmail.com

Table 1. Elastic and control groups used for the assays.

Groups	Trademark	Main Composition	Color	Reference number
N	Uniden	Natural Látex	Natural	000-1204
Y	Uniden	Natural Látex	Yellow	000-1206
V	Uniden	Natural Látex	Violet	000-1206
R	Uniden	Natural Látex	Red	000-1206
G	Uniden	Natural Látex	Green	000-1206
P	Uniden	Natural Latex	Pink	000-1206
C+	Tween 80 (Polyoxyethylene-20-sorbitan, Sigma, St. Louis, MO, USA)			
C-	PBS solution (phosphate-buffered saline, Cultilab, Campinas, SP, Brazil)			

Material and methods

Latex intra-oral elastics of different colors (5/16" = 7.9 mm, mean load), were selected for studying their cytotoxicity in cell culture (Table 1). The samples were divided into six groups of 15 elastics according to their manufacturer: Groups N, Y, V, R, G and P (natural latex elastics and colored elastics, namely, yellow, violet, red, green and pink, respectively, Uniden, Sorocaba, SP, Brazil) (Figure 1).

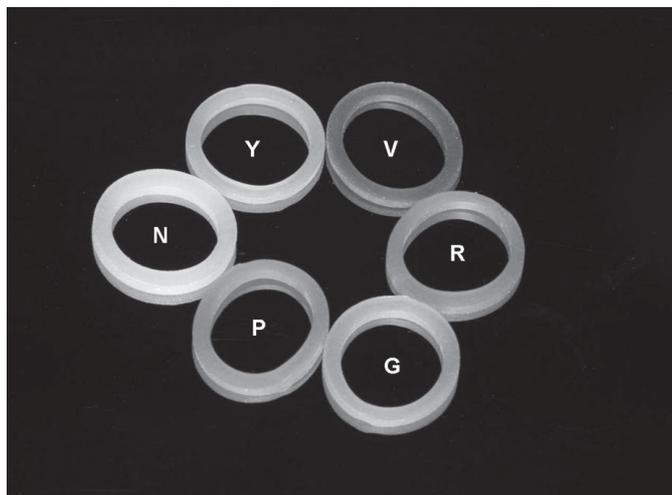


Fig. 1: Latex intraoral elastics evaluated in this study: N, Y, V, R, G and P (Uniden, natural latex elastics and colored elastics, namely, yellow, violet, red, green and pink, respectively).

All samples had recent manufacturing dates, from the same production lot and came in sealed plastic packages. The elastics of experimental and control groups had their both sides previously sterilized with ultraviolet light (Labconco, Kansas City, MO, USA) during 30 minutes¹².

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 (Table 1).

Cells from mouse fibroblast cell line L929 (American Type Culture Collection - ATCC, Rockville, MD) were cultured in Eagles' minimum essential medium (MEM; Cultilab, Campinas, SP, Brazil) by adding 0.03 mg/mL of glutamine (Sigma, St. Louis, MO, USA), 50 µg/mL of gamicine (Schering Plough, Kenilworth, New Jersey, USA), 2.5 mg/mL of fungizone (Bristol-Myers-Squibb, New York, USA), 0.25% sodium bicarbonate solution (Merck, Darmstadt, Germany), 10 mM of HEPES

(Sigma), and 10% bovine fetal serum (Cultilab) for growth medium or no bovine fetal serum for maintenance medium only. Next, the cell culture medium was incubated at 37°C for 48 h.

The method for evaluating the cytotoxicity was the "dye-uptake"¹³ test. This method is based on neutral red dye incorporated into live cells. It was used in this experiment only at two periods of evaluation: 1 and 24 h. The 1-h period represents the maintenance of the elastic in the cell culture medium for 1 h after removal, whereas the 24-h period represents the maintenance of the elastic in the cell culture medium for 24 h after removal.

Dye-uptake

Volumes of 100 µL of L-929 cells were distributed into 96-well microplates. After 48 h, the growth medium was replaced with 100 µL of MEM obtained following incubation in the different types of elastics and positive and negative control at 1 and 24 h. Positive and negative control groups consisted of culture medium put 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. Following 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 Na₂HPO₄ 2 H₂O; 1 mM of K₂HPO₄ 1 mM; pH 7.2) were added in order to promote cell attachment to the plate. After 5 minutes, 100 µL of 1% acetic acid (Vetec) and 50% methanol (Vetec) were added in order to remove the dye. After 20 minutes, a spectrophotometer (BioTek Instruments, Winooski, VT, USA) at 492 nm wavelength (λ492 nm) was used for data reading. This test was repeated 3 different times.

Data were compared by ANOVA, and Tukey's multiple comparison test was used for identifying differences between the groups. Significance level was set at p<0.05.

Results

The results showed significant difference (p<0.05) between the groups N, Y, V, R, G, P and the cell control at 1 h (Table 2). Group G produced the lowest value (34.8% ± 2.9%) and group N produced the more viability (49.8% ± 10.5%), whereas the viability of the Tween 80 was 8.8% ± 10.5% (Table 2). The results showed statistically significant differences (p<0.05) between the groups N, Y, V, R and the groups G and P at 1 h. No significant difference was found between the groups N and Y, N and V, N and R, Y and V, Y and R, V and R, and G and P tested (p>0.05) at 1 h (Table 2).

Table 2. Descriptive statistics for optical density of latex elastics.

Groups	Time							
	1 h			24 h				
	Mean	Median	S. D.	Viable cells	(%)Mean	Median	S. D.	Viable cells (%)
CC	0.874 ^a	0.910	0.062	100.0	0.610 ^a	0.642	0.049	100.0
C-	0.866	0.898	0.007	99.1	0.603	0.634	0.012	98.9
C+	0.076	0.084	0.008	8.8	0.054	0.058	0.007	9.0
N	0.435 ^b	0.458	0.046	49.8	0.115 ^b	0.134	0.008	18.9
Y	0.403 ^{cb}	0.442	0.042	46.2	0.102 ^b	0.127	0.014	16.8
V	0.434 ^{abc}	0.452	0.013	49.7	0.106 ^b	0.129	0.007	17.5
R	0.379 ^{ebcd}	0.402	0.011	43.4	0.101 ^b	0.119	0.006	16.7
G	0.304 ^f	0.338	0.009	34.8	0.096 ^b	0.108	0.005	15.9
P	0.329 ^{gf}	0.346	0.008	35.7	0.100 ^b	0.114	0.007	16.4

N=15. Values followed by same letters are not significantly different ($p>0.05$) for the same time. SD: standard deviation.

After 24 h, a decrease in cell viability was observed in all groups. Viability ranged from 15.9% to 18.9%, relative to the cell control (Table 2). The lowest viability ($15.9\% \pm 5.2\%$) corresponded to group G, whereas the viability of the Tween 80 (positive cytotoxicity control) was $9.0\% \pm 12.9\%$. The results showed statistically significant differences ($p<0.05$) between all test elastics groups and the cell control at 24 h. On the other hand, no statistically significant difference ($p>0.05$) was found among the test elastics groups at 24 h (Table 2).

Discussion

Although case reports on latex allergy is not so frequently seen in the literature, allergic reactions have been relatively prevalent as latex-based products become commercially available. Most of the allergic reactions¹⁴ have been related to the use of orthodontic elastics¹⁵, which is characterized by presence of small vesicles or acute edema and complaints of itching and burning.

The most serious consequence of natural rubber latex allergy commonly takes place during the mucosal absorption of natural rubber latex proteins during intraoperative medical or dental procedures when health care workers or others already sensitized become patients¹⁶.

Because natural latex rubber has been increasingly used as dental material, many cytotoxicity issues have been reported as well¹⁷. Preservatives such as sulfur and zinc oxide as well as antioxidants such as di-thio-carbohydrates, N-nitrosodibutylamine, and N-nitrosopiperidine are all known to be cytotoxic substances¹⁸.

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 usage¹⁷.

As sterilization is a prerequisite for cytotoxicity assay, ultraviolet light was used in the present study for sterilizing both sides of the elastics^{1,12} during 30 minutes. All the elastics were found to have the same color and malleability following UV light sterilization.

It was demonstrated by Franz et al.¹⁹ and Schedle et al.²⁰ that L929 mouse fibroblasts show comparable results to primary human gingival fibroblasts and therefore might represent a model for gingival toxicity *in vitro*²¹⁻²².

The percentage of viable cells was obtained by comparing the mean optical density (OD) of control cells (no contact with the

materials) with that of cell cultures put in contact with different elastics, resulting in 50% toxicity for the cell cultures (CC₅₀) (Table 2).

Evidence of this cytotoxic feature was shown following exposition of the elastics to cell culture medium. It was used in this experiment only two times of evaluation 1 and 24 h, a time that, usually these elastics are changed to each 24 h for the patient.

Natural latex elastics from all colors induced a greater amount of cell lysis at 24 h compared to the time of 1 h. latex elastics from natural, yellow, violet and red colors induced a lowest amount of cell lysis compared to the elastics green and pink colors at 1 h.

After 24 h, a lowest viability was observed in all groups regardless of their color, which is accordance with the findings of Santos, et al.^{12,23}. This suggests a greater release of toxic ingredients at 24 h, due to a possible latex degradation and release of allergenic proteins, which was not shown in 1 h.

Holmes, et al.² have verified whether the colorants used in the fabrication of colored latex could have some toxic effect. Their results showed that these colorants had low toxicity. However, such an effect is clinically inoffensive. Variations occur in the composition of the latex elastics and this could explain the different results between the elastics.

According to Schmalz¹, 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. It is known that latex is not entirely biocompatible as it may interact with foods^{7,24} and medications²⁵.

As these materials are widely used in clinical orthodontics, care regarding the cytotoxicity of orthodontic elastics should be taken, mainly with regard to intraoral elastics as they have a very close contact with gingival and mucosa. Thus, clinically proven biocompatible materials should be used whenever possible.

It is important for the practitioner to know how to manage patients presenting latex allergy and how to deal with this problem²⁶. The safety biocompatibility of silicone has been well proved through the use of mouth guards in dentistry²⁷. An alternative for patients with allergy to latex is to use non-latex elastics, which can be used in orthodontics without jeopardizing the orthodontic treatment²⁸.

It may be concluded that natural, yellow, violet and red latex orthodontic elastics induced less cell lysis than green and pink elastics at 1 h. At 24 h, latex elastics were found to be highly cytotoxic, regardless of their color.

References

1. Schmalz G. Use of cell cultures for toxicity testing of dental materials—advantages and limitations. *J Dent*. 1994; 22 Suppl 2: S6-11.
2. Holmes J, Barker MK, Walley EK, Tuncay OC. Cytotoxicity of orthodontic elastics. *Am J Orthod Dentofacial Orthop*. 1993; 104: 188-91.
3. Palosuo T, Alenius H, Turjanmaa K. Quantitation of latex allergens. *Methods*. 2002; 27: 52-8.
4. Wakelin SH, White IR. Natural rubber latex allergy. *Clin Exp Dermatol*. 1999; 24: 245-8.
5. Everett FG, Hice TL. Contact stomatitis resulting from the use of orthodontic rubber elastics: report of case. *J Am Dent Assoc*. 1974; 88: 1030-1.
6. Tomazic VJ, Withrow TJ, Fisher BR, Dillard SF. Latex-associated allergies and anaphylactic reactions. *Clin Immunol Immunopathol*. 1992; 64: 89-97.
7. Turjanmaa K, Alenius H, Makinen-Kiljunen S, Reunala T, Palosuo T. Natural rubber latex allergy. *Allergy*. 1996; 51: 593-602.
8. Weiss ME, Hirshman CA. Latex allergy. *Can J Anaesth*. 1992; 39: 528-32.
9. Perrella FW, Gaspari AA. Natural rubber latex protein reduction with an emphasis on enzyme treatment. *Methods*. 2002; 27: 77-86.
10. Lobner D, Asrari M. Neurotoxicity of dental amalgam is mediated by zinc. *J Dent Res*. 2003; 82: 243-6.
11. Hanson M, Lobner D. In vitro neuronal cytotoxicity of latex and nonlatex orthodontic elastics. *Am J Orthod Dentofacial Orthop*. 2004; 126: 65-70.
12. Santos RL, Pithon MM, Oliveira MV, Mendes GS, Romanos MTV, Ruellas ACO. Cytotoxicity of intraoral orthodontic elastics. *Braz J Oral Sci*. 2008; 24: 1520-5.
13. Neyndorff HC, Bartel DL, Tufaro F, Levy JG. Development of a model to demonstrate photosensitizer-mediated viral inactivation in blood. *Transfusion*. 1990; 30: 485-90.
14. Snyder HA, Settle S. The rise in latex allergy: implications for the dentist. *J Am Dent Assoc*. 1994; 125: 1089-97.
15. Neiburger EJ. A case of possible latex allergy. *J Clin Orthod*. 1991; 25: 559-60.
16. Sussman GL, Beezhold DH, Liss G. Latex allergy: historical perspective. *Methods*. 2002; 27: 3-9.
17. Hwang CJ, Cha JY. Mechanical and biological comparison of latex and silicone rubber bands. *Am J Orthod Dentofacial Orthop*. 2003; 124: 379-86.
18. Fiddler W, Pensabene J, Sphon J, Andrzejewski D. Nitrosamines in rubber bands used for orthodontic purposes. *Food Chem Toxicol*. 1992; 30: 325-6.
19. Franz A, Konig F, Skolka A, Sperr W, Bauer P, Lucas T et al. Cytotoxicity of resin composites as a function of interface area. *Dent Mater*. 2007; 23: 1438-46.
20. Schedle A, Samorapoompichit P, Rausch-Fan XH, Franz A, Fureder W, Sperr WR et al. Response of L-929 fibroblasts, human gingival fibroblasts, and human tissue mast cells to various metal cations. *J Dent Res*. 1995; 74: 1513-20.
21. Franz A, Konig F, Lucas T, Watts DC, Schedle A. Cytotoxic effects of dental bonding substances as a function of degree of conversion. *Dent Mater*. 2009; 25: 232-9.
22. Schmid-Schwab M, Franz A, Konig F, Bristela M, Lucas T, Piehslinger E et al. Cytotoxicity of four categories of dental cements. *Dent Mater*. 2009; 25: 360-8.
23. Santos RL, Pithon MM, Mendes GS, Romanos MTV, Ruellas ACO. Cytotoxicity of intermaxillary orthodontic elastics of different colors: An in vitro study. *J Appl Oral Sci*. 2009; 4: 326-9.
24. Carey AB, Cornish K, Schrank P, Ward B, Simon R. Cross-reactivity of alternate plant sources of latex in subjects with systemic IgE-mediated sensitivity to *Hevea brasiliensis* latex. *Ann Allergy Asthma Immunol*. 1995; 74: 317-20.
25. Towse A, O'Brien M, Twarog FJ, Braimon J, Moses AC. Local reaction secondary to insulin injection. A potential role for latex antigens in insulin vials and syringes. *Diabetes Care*. 1995; 18: 1195-7.
26. Hain MA, Longman LP, Field EA, Harrison JE. Natural rubber latex allergy: implications for the orthodontist. *J Orthod*. 2007; 34: 6-11.
27. Chauvel-Lebret DJ, Pellen-Mussi P. Evaluation of the in vitro biocompatibility of various elastomers. *Biomaterials*. 1991; 20: 291-9.
28. Gandini P, Gennai R, Bertocchini C, Massironi S. Experimental evaluation of latex-free orthodontic elastics' behaviour in dynamics. *Prog Orthod*. 2007; 8: 88-99.