Evaluation of the in vitro biocompatibility of orthodontic elastics

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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 weight4, with stabilizers such as zinc oxide and chemically vulcanized materials. The resulting mixture is then heated until 70° C8. Although zinc is known to be neurotoxic9, the amount released by orthodontic elastics can be ingested as research studies show no evidence of harm10. 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 elasticity11.

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.
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).

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

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

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 minutes.

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 gentamicine (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" 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 (λ) 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 and P, and the control groups at 1 h (Table 2). Group G produced the lowest value (34.8% ± 2.9%) and group N produced the most 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, 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 V, Y and R, V and R, and G and P tested (p>0.05) at 1 h (Table 2).
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% ± 5.2%) corresponded to group G, whereas the viability of the Tween 80 (positive cytotoxicity control) was 9.0% ± 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 are not so frequently seen in the literature, allergic reactions have been relatively prevalent as latex-based products become commercially available. Most of the allergic reactions have been related to the use of orthodontic elastics, 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 rubbers latex proteins during intraoperative medical or dental procedures when health care workers or others already sensitized.

Because natural latex rubber has been increasingly used as dental material, many cytotoxicity issues have been reported as well. 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 substances.

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.

As sterilization is a prerequisite for cytotoxicity assay, ultraviolet light was used in the present study for sterilizing both sides of the elastics 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. and Schedle et al. that L929 mouse fibroblasts show comparable results to primary human gingival fibroblasts and therefore might represent a model for gingival toxicity in vitro.

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 (CCP) (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. 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. 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 Schmalz, 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 and medications.

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 problem. The safety biocompatibility of silicone has been well proved through the use of mouth guards in dentistry. An alternative for patients with allergy to latex is to use non-latex elastics, which can be used in orthodontics without jeopardizing the orthodontic treatment.

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.

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean</th>
<th>Median</th>
<th>S. D.</th>
<th>Viable cells (%)</th>
<th>Mean</th>
<th>Median</th>
<th>S. D.</th>
<th>Viable cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>0.874a</td>
<td>0.910</td>
<td>0.062</td>
<td>100.0</td>
<td>0.610a</td>
<td>0.642</td>
<td>0.049</td>
<td>100.0</td>
</tr>
<tr>
<td>C-</td>
<td>0.866</td>
<td>0.898</td>
<td>0.007</td>
<td>99.1</td>
<td>0.603</td>
<td>0.634</td>
<td>0.012</td>
<td>98.9</td>
</tr>
<tr>
<td>C+</td>
<td>0.076</td>
<td>0.084</td>
<td>0.008</td>
<td>8.8</td>
<td>0.054</td>
<td>0.058</td>
<td>0.007</td>
<td>9.0</td>
</tr>
<tr>
<td>N</td>
<td>0.435b</td>
<td>0.458</td>
<td>0.046</td>
<td>49.8</td>
<td>0.115b</td>
<td>0.134</td>
<td>0.008</td>
<td>18.9</td>
</tr>
<tr>
<td>Y</td>
<td>0.434c</td>
<td>0.442</td>
<td>0.042</td>
<td>46.2</td>
<td>0.102c</td>
<td>0.127</td>
<td>0.014</td>
<td>16.8</td>
</tr>
<tr>
<td>V</td>
<td>0.379d</td>
<td>0.452</td>
<td>0.013</td>
<td>49.7</td>
<td>0.106d</td>
<td>0.129</td>
<td>0.007</td>
<td>17.5</td>
</tr>
<tr>
<td>R</td>
<td>0.304e</td>
<td>0.402</td>
<td>0.011</td>
<td>43.4</td>
<td>0.101e</td>
<td>0.119</td>
<td>0.006</td>
<td>16.7</td>
</tr>
<tr>
<td>G</td>
<td>0.304f</td>
<td>0.338</td>
<td>0.009</td>
<td>34.8</td>
<td>0.096f</td>
<td>0.108</td>
<td>0.005</td>
<td>15.9</td>
</tr>
<tr>
<td>P</td>
<td>0.329g</td>
<td>0.346</td>
<td>0.008</td>
<td>35.7</td>
<td>0.100g</td>
<td>0.114</td>
<td>0.007</td>
<td>16.4</td>
</tr>
</tbody>
</table>

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