ORTHODONTICS & CRANIOFACIAL RESEARCH

ORIGINAL ARTICLE

Evaluation of the cytotoxicity of latex and non-latex orthodontic separating elastics

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Structured Abstract

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Objective – To test the hypothesis that a difference in cytotoxicity exists between latex and non-latex orthodontic separating elastics.

Material and Methods – Five intra-oral separating elastics from different manufactures (four latex and one non-latex) were divided into five groups of 15 elastics each: Group MA (non-latex elastics, Masel), Group MO (natural latex, Morelli), Group DE (natural latex, Dentaurum), Group TP (natural latex, TP Orthodontics) and Group UN (natural latex, Unitek). The cytotoxicity assay was performed using cell cultures (epithelial HEp-2 cells originating from human laryngeal carcinoma) that were submitted to the cell viability test with neutral red (dye-uptake) at 24, 48, 72 and 168 h. Analysis of variance (ANOVA) with multiple comparisons and Tukey's test were employed ($p < 0.05$).

Results – The results showed no statistically significant differences between groups MA, DE, TP and UN in relation to Group CC (cell control) for experimental times of 24, 48 and 168 h ($p > 0.05$). Morelli, Dentaurum, TP Orthodontics and Unitek elastics induced a great amount of cell lyses at 72 h.

Conclusion – One can demonstrate that the Masel elastic induced less cell lysis compared with other elastics, but all trademarks were found to be clinically biocompatible.

Clinical relevance – Separating orthodontic elastics are used in the interdental subgingival region with the aim to separate the teeth for placement of orthodontic bands. However, latex has been known to cause allergy. As these materials are widely used in clinical orthodontics, care regarding the cytotoxicity of orthodontic elastics should be taken. Thus, clinically proven biocompatible materials should be acquired whenever possible.

Key words: biocompatibility; cytotoxicity; elastics; orthodontics

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Introduction

Recent studies concerned the biocompatibility of different types of orthodontic materials (1, 2). Latex separating elastics are commonly used in orthodontic treatment, however, the protein content of latex is a known allergen. Allergy caused by latex proteins has been well documented (3), including immediate hypersensibility reactions (4). Amongst the allergic

reactions caused by orthodontic elastics, swelling and stomatitits, erythematous oral lesions, respiratory reactions and even anaphylactic shock, the most severe form of allergy (5, 6), can be cited. Latex allergy occurs in 3–17% of the cases (7). Because latex allergy is prevalent of among occupationally exposed groups and patients, the need for non-latex alternatives is increasing.

Pre-vulcanized latex is produced by mixing pure natural latex, which has the highest molecular weight (8), with stabilizers such as zinc oxide and chemically vulcanized materials. The resulting mixture is then heated until 70°C (9). Although zinc is known to be neurotoxic (10), the amount released by orthodontic elastics can be ingested as research studies show no evidence of harm (11). Anti-ozone and anti-oxidant agents are also added to latex during the manufacture of orthodontic elastics (8). This process has the advantage of producing latex with higher mechanical properties, thus increasing its strength and elasticity (9, 11). However, natural latex is not in the category of materials known to be entirely inoffensive (12, 13).

The use of cell culture (CC) medium for testing the toxicity of dental products is a valid way of understanding the biological behaviour of such materials (12). The objective of the present *in vitro* study was to test the hypothesis that difference in cytotoxicity exists between latex and non-latex orthodontic separating elastics.

Material and methods

Blue-coloured separating intra-oral elastics (4.4 mm) from five different manufacturers were selected for cytotoxicity study, being four of natural latex and one containing no latex at all (Table 1). The samples were divided into five groups of 15 elastics each: Group MA (Natural silicone elastics, modular, Masel, Bristol, PA, USA), Group MO (Natural latex, modular, Morelli, Sorocaba, São Paulo, Brazil), Group DE (Natural latex, modular, Dentaurum, Ispringen, Germany), Group TP (Natural latex, bulk pack, TP Orthodontics, La Porte, IN, USA) and Group UN (Natural latex, bulk pack, 3 M Unitek, Monrovia, CA, USA).

The elastics used in this study belonged to the same production line for each trademark. Copper amalgam (Pratic NG 2, Vigodent, Rio de Janeiro, Brazil) was used as positive control, whereas a glass cylinder served as a negative control, both in standardized sizes (Table 1).

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

Groups	Trademark	Main composition	External diameter (mm)	Reference no
MA	Masel	Natural silicone	4.4	4108–720
MO	Morelli	Natural latex	4.4	60–04–201
DE	Dentaurun	Natural latex	4.4	774–200–01
TP	TP orthodontics	Natural latex	4.4	352–000C
UN	Unitek	Natural latex	4.4	406–084
C+	Dental copper amalgam. Pratic NG 2. Vigodent (control positive)			
C–	Cylinder glass (control negative)			

The CC model used was the monolayer containing Hep-2 line cells (human laryngeal carcinoma) was maintained in Eagles' minimum essential medium (Cultilab, Campinas, Brazil) by adding 0.03 mg/ml of glutamine (Sigma, St. Louis, MO, USA), 50 μg/ml of garamicine (Schering Plough, Kenilworth, NJ, USA), 2.5 mg/ml of fungizone (Bristol-Myers-Squibb, New York City, NY, USA), 0.25% sodium bicarbonate solution (Merck, Darmstadt, Germany), 10 mM of HEPES (Sigma) and 10% bovine foetal serum (Cultilab, Campinas, Brazil) for growth medium or no bovine foetal serum for maintenance medium only. Next, the CC medium was incubated at 37°C for 48 h.

The elastics were previously sterilized with ultraviolet radiation (Labconco, Kansas, MO, USA) for 30 min for each surface (14). The cytotoxicity of these orthodontic elastics was determined through the dye-uptake technique (15), which is based on the neutral red absorption by living cells. Because these elastics are usually maintained in the oral cavity for up to 168 h (7 days) to allow separation between teeth for the placement of orthodontic bands, different periods of time were considered: 24, 48, 72 and 168 h. These experimental periods represent the time maintenance under CC conditions before removal of the elastics.

Dye-uptake

Volumes of 100 μl of Hep-2 cells were distributed into 96-well microplates. After 48 h, the growth medium was replaced with 100 μl of Eagles' minimum essential

medium (MEM) obtained following incubation in the different types of elastics at 24, 48 72 and 168 h. Eagles' MEM was employed because it is the same type of material used for cell maintenance, thus not influencing the results. Positive and control groups consisted of culture medium put in contact with amalgam and cylinder glass respectively. The experiment was performed four times.

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, Brazil) in phosphate buffered solution (130 mM of NaCl; 2 mM of KCl; 6 mM of Na_2HPO_4 2 H_2O; 1 mM of K_2HPO_4 1 mM; pH 7.2) were added to promote cell attachment to the plate. After 5 min, 100 μl of 1% acetic acid (Vetec) and 50% methanol (Vetec) were added to remove the dye. After 20 min, a spectrophotometer (Bio Tek, Winooski, VT, USA; Fig. 1) at 492 nm wavelength (λ = 492 nm) was used for data reading. This test was repeated three times and each test was used 15 new elastics samples for each group.

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

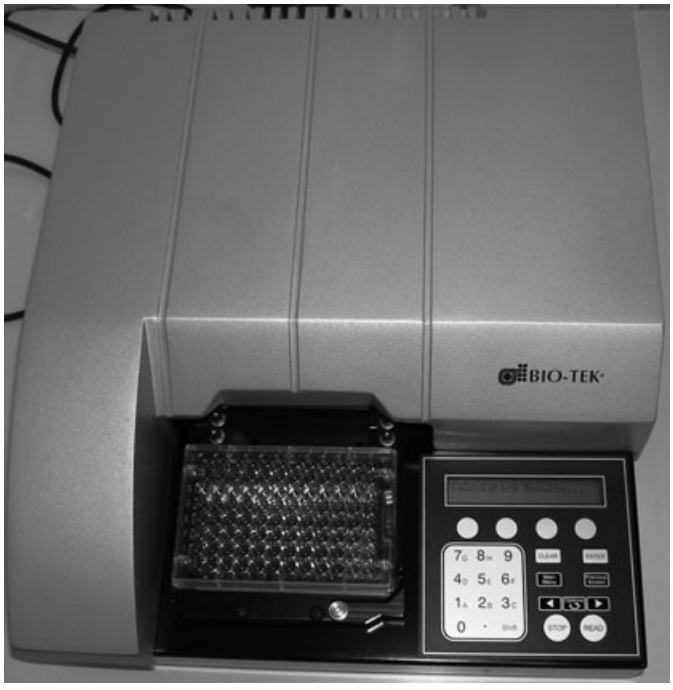

Fig. 1. Spectrophotometer used for reading of the optical density (Bio Tek®).

Results

The results showed no statistically significant differences between elastics from groups MA (natural silicone, Masel), DE (natural latex, Dentaurum), TP (natural latex, TP Orthodontics) and UN (natural latex, 3M Unitek) in relation to group CC (cell control) for experimental times of 24, 48 and 168 h ($p > 0.05$) (Tables 2 and 3).

Morelli, Dentaurum, TP Orthodontics and Unitek elastics trademarks induced a greater amount of cell lysis at 72 h compared with the other experimental times, a significant difference ($p < 0.05$) was noted between the groups MO, DE, TP, UN and the group CC at 72 h (Tables 2 and 3).

The Masel elastics induced less cell lysis in all experimental times compared with the other groups, but also no statistically significant difference was observed ($p > 0.05$; Tables 2 and 3).

Discussion

The CC model used in the present study was the monolayer (16, 17). This model was used together with the dye-uptake technique because the cytotoxicity of the materials can be determined by defining viable, damaged and dead cells. The amount of dye incorporated into the cells is directly proportional to the number of cells with intact membrane, which allows distinguishing the cytotoxicity of each elastic.

The per cent of viable cells was obtained by comparing the mean optical density (OD) in the control group (cells with no contact with elastics) to that obtained from supernatants of CC that had been in contacted with elastics. A 50% toxicity was calculated for CC (Tables 2 and 3).

In this study, we opted to use copper amalgam as a positive control (14) and glass cylinder as a negative control, since the CC were placed into glass bottles because such a material is adequate for this cytotoxicity assay. The cytotoxicity potential of dental amalgam comes from the presence of mercury, although other potentially neurotoxic substances are

Table 2. **Descriptive statistics for optical density of elastics at 24 and 48 h**

		Time (24 h)				Time (48 h)			
Groups	n	Mean	Median	SD	Viable cells (%)	Mean	Median	SD	Viable cells (%)
CC	15	0.784[a]	0.799	0.121	100.0	0.688[a]	0.706	0.119	100.0
C–	15	0.763	0.789	0.112	97.4	0.679	0.698	0.125	98.8
C+	15	0.275[b]	0.295	0.110	35.1	0.195[b]	0.218	0.119	28.4
MA	15	0.747[a]	0.762	0.111	95.4	0.668[a]	0.690	0.112	97.1
MO	15	0.704[b]	0.738	0.119	89.8	0.638[a]	0.669	0.111	92.8
DE	15	0.731[a]	0.752	0.112	93.3	0.653[a]	0.680	0.119	95.0
TP	15	0.733[a]	0.750	0.121	93.6	0.650[a]	0.671	0.119	94.6
UN	15	0.736[a]	0.766	0.119	94.0	0.659[a]	0.689	0.117	95.9

n = 15. Values followed by same letters are not significantly different ($p > 0.05$) for the same time.

Table 3. **Descriptive statistics for optical density of elastics at 72 and 168 h**

		Time (72 h)				Time (168 h)			
Groups	n	Mean	Median	SD	Viable cells (%)	Mean	Median	SD	Viable cells (%)
CC	15	0.742[a]	0.764	0.129	100.0	0.820[a]	0.838	0.124	100.0
C–	15	0.734	0.769	0.115	99.0	0.804	0.829	0.110	98.1
C+	15	0.253[b]	0.282	0.121	34.2	0.277[b]	0.302	0.114	33.9
MA	15	0.714[a]	0.740	0.119	96.3	0.792[a]	0.819	0.117	96.6
MO	15	0.607[b]	0.631	0.117	81.9	0.762[a]	0.798	0.127	93.0
DE	15	0.647[b]	0.679	0.119	87.2	0.788[a]	0.817	0.119	96.1
TP	15	0.652[b]	0.686	0.127	87.9	0.786[a]	0.806	0.120	95.9
UN	15	0.655[b]	0.680	0.119	88.3	0.792[a]	0.825	0.129	96.7

n = 15. Values followed by same letters are not significantly different ($p > 0.05$) for the same time.

also found depending on its composition and manufacturer (10).

As sterilizations is a prerequisite for cytotoxicity assays, ultraviolet radiation (14) was used in this study for 30 min for each elastic surface. It was observed that all elastics exhibited the same colour aspect and malleability following sterilization with UV light.

Because natural latex rubber has been increasingly used as dental material, many cytotoxicity issues have been reported as well (18). Conservants such as sulphur and zinc oxide as well as antioxidants such as di-thiocarbohydrates, *N*-nitrosodibutylamine and *N*-nitrosopiperidine are all known to be cytotoxic substances (19). Holmes et al. (13) have verified whether the colourants used in the fabrication of coloured latex could have some toxic effect. Their results showed that these colourants exhibited low toxicity. However, such an effect is clinically inoffensive.

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 reactions (20) have been related to the use of orthodontic elastics (21), which is characterized by presence of small vesicles or acute oedema and complaints of itching and burning.

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 the Masel non-latex separating elastics and it was observed that this material induced a lesser amount of cell lysis compared with latex elastics. As the powder covering the 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 to standardize the samples as composition and quantity of powder present in the elastics could interfere with the results.

According to Schmalz (12), 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, 22) and medications (23).

Evidence of this cytotoxic feature was shown following exposition of the elastics to CC medium. Natural latex separating elastics from Morelli, Dentaurum, TP Orthodontics and Unitek trademarks induced a greater amount of cell lysis at 72 h compared with the other experimental times of 24, 48 and 168 h, suggesting a greater release of toxic ingredients at 72 h, because of a possible latex degradation and release of allergenic proteins, which was not shown on days 1 and 2, and not persisted on day 7. This fact shows that release of allergenic proteins from latex is neither constant nor continuing. However, all natural latex elastics were found to be biocompatible after the 3rd experimental day. Holmes et al. (13), who showed evidence of cytotoxicity in latex separating elastics, corroborate these findings.

Further studies using elastics without the removal of surface powder, and use of saliva for maintenance of the elastics, as well as assessment of the mechanism of delayed cell lysis, can contribute to better describe in detail the cytotoxicity behaviour of these materials. As these materials are widely used in clinical orthodontics, care regarding the cytotoxicity of orthodontic elastics should be taken, mainly with regard to intra-oral elastics as they have a very close contact with gingiva. Thus, clinically proven biocompatible materials should be acquired whenever possible.

Conclusion

The non-latex separating elastic from Masel trademark induced a lesser amount of cell lysis compared with natural latex elastics. Elastics of all manufacturers are clinically biocompatible.

Clinical relevance

Separating orthodontic elastics are used in the interdental subgingival region with the aim to separate the teeth for placement of orthodontic bands. However, latex has been known to cause allergy. As these materials are widely used in clinical orthodontics, care regarding the cytotoxicity of orthodontic elastics should be taken. Thus, clinically proven biocompatible materials should be acquired whenever possible.

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