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Genotoxicity of Orthodontic Elastomerics: An in Vitro Study

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Abstract

Introduction: The biocompatibility of intraoral elastomerics used in Orthodontics continues to give cause for concern, especially with relation to genotoxicity, since there are not yet any published studies that have investigated these methods. In response to this, the objective of this study was to conduct “in vitro” tests of these materials' genotoxicity to the fibroblasts of rats (L929 lineage), analyzing possible relationships with presence or absence of latex in a range of commercially-available brands. **Materials and Methods:** A total of 36 elastometric test specimens, produced by 3M Unitek®, American Orthodontics®, GAC®, Morelli®, RMO® and TP Orthodontics®, were divided into 9 experimental groups. Genotoxicity was assessed using the Comet assay. Cell growth was used as a negative control and 1% sodium hypochlorite as a positive control. Data were tested statistically using one-way ANOVA and Dunnett's post-hoc test to a significance level of 5%. **Results:** The results showed that all groups of elastomerics from all of the different commercial brands exhibited high percentages of damage to cell DNA, irrespective of whether or not they contained latex, and were significantly different from the negative control ($p<0.05$). Means for groups of elastomerics containing latex were higher than means for products without latex, but the difference was not statistically significant ($p>0.05$). **Conclusions:** It was concluded that all of the elastomerics evaluated were genotoxic to the cells assessed, irrespective of latex content or commercial brand, and there were no differences between elastics from each brand with or without latex, suggesting that genotoxicity is not exclusively related to this component.

Keywords: Orthodontics, toxicity, elastomerics, latex, genotoxicity, biocompatibility.

Introduction

The biocompatibility of dental materials has become a subject of great interest, whether because of increased rates of the clinical manifestations of the allergic reactions they cause in patients or because of increased awareness and knowledge of the adverse effects that are possibly caused by using these materials. [1] Specifically with regard to Orthodontics, many materials remain in direct contact with organic tissues for long periods of time, as is the case of elastomerics. [2,3]

Biocompatibility can be defined as the capacity for tissues to be brought into contact with a given material without manifesting any type of toxic, irritant, inflammatory, allergic, mutagenic or carcinogenic reaction, while the occurrence of any type of adverse reaction is defined as toxicity. [2, 4, 5]

Genotoxicity includes mutagenic and carcinogenic processes and is therefore of great importance when selecting materials that are safe for patients. [6]

Elastomerics began to be used in Orthodontics at the end of the nineteenth century and their use has increased as their properties have been improved. They are widely used to substitute metallic ligatures for moving teeth, whether for retracting teeth, closing gaps or correcting inter-arch relationships, and also as auxiliaries for extraoral devices, and are important instruments for achieving favorable results during treatment. [7,8,9,10]

In terms of composition, orthodontic elastomerics can be made from latex or synthetic materials. [11] In general, latex-based materials are obtained from natural rubber, which is sourced from the rubber tree (Hevea brasiliensis) and has the chemical formula cis-1,4-polyisoprene and is widely utilized because it has better properties such as flexibility, low cost and a greater capacity to return to its original dimensions after suffering deformations. [7,8,12] Synthetic materials, produced from polyurethane, and also called plastics, are obtained by chemically transforming coal, petroleum and certain vegetable alcohols. [13]

Although they have a wide range of clinical applications, materials obtained from latex have a large potential for causing allergies. Allergic reactions reported by patients range from swelling and stomatitis to erythematous lesions, respiratory reactions and anaphylactic shock. [5, 14, 15]

In response to these reported effects of using elastomerics, manufacturers have proposed alternatives that do not have latex in their composition. However, although there are published studies investigating these materials' cytotoxicity, there is scant literature on their genotoxicity or on the true relationship of these effects with latex.

Based on these findings, and with the objective of elucidating issues related to the biocompatibility of intraoral elastomerics used in orthodontic treatment, the authors of this study decided to assess the genotoxicity of these materials, analyzing possible relationships with presence or absence of latex in a range of commercially-available brands.

Materials and Methods

Sample

The sample comprised 36 intraoral elastomerics (3/16”), which were allocated to nine experimental groups, each with n=4, as shown in Table 1. The following commercial brands were tested: American Orthodontic® (St. Louis-Illinois, USA), RMO® (Denver-Colorado, USA), Morelli® (Sorocaba-São Paulo, Brazil), TP Orthodontic® (La Porte-Indiana, USA), GAC® (Islandia-

New York, USA), and 3M Unitek® (Saint Paul-Minessota, USA).

A Negative Control C(-) was included using cell growth and a Positive Control C(+) was set up with 1% sodium hypochlorite.

Cell Cultures

Fibroblasts from L929 lineage rats were cultured in single layers in 75 cm² culture flasks (TPP) containing DMEM (Gibco) medium supplemented with 10% of fetal bovine serum (Cultilab) and antibiotics (a mixture of streptomycin, 1% penicillin and 0.1% gentamycin, obtained from Gibco) at 37°C in an incubator (ThermoScientific) with 5% CO2.

Comet Assay

In order to determine genotoxicity of the elastomerics, 1X105 cells per well were seeded on 24-well plates (TPP) (and incubated for 24 hours in full DMEM medium, then the cells were washed in DPBS before being subjected to the treatments with the various different elastics. At the end of treatments, cells were washed in DPBS at 37ºC and trypsinized with 350 µL of trypsin. After 5 minutes, the same cells were re-suspended in complete medium and the cell suspension volume was immediately used for the assay.

	TYPE	COMMERCIAL BRAND	n	Latex	Color
Control (-)	Cell growth	-	4	-	
GROUP 1 (G1)	Intraoral elastic (3/16)	American Orthodontics®	4	Yes	Natural
GROUP 2 (G2)	Intraoral elastic (3/16)	American Orthodontics®	4	No	Transparent
GROUP 3 (G3)	Intraoral elastic (3/16)	RMO®	4	Yes	Natural
GROUP 4(G4)	Intraoral elastic (3/16)	RMO®	4	No	Colored
GROUP 5 (G5)	Intraoral elastic (3/16)	Morelli®	4	Yes	Natural
GROUP 6 (G6)	Intraoral elastic (3/16)	Morelli®	4	No	Transparent
GROUP 7 (G7)	Intraoral elastic (3/16)	TP Orthodontics®*	4	Yes	Natural
GROUP 8 (G8)	Intraoral elastic (3/16)	GAC®*	4	Yes	Natural
GROUP 9 (G9)	Intraoral elastic (3/16)	3M UNITEK®*	4	Yes	Natural
Control (+)	1% Sodium hypochlorite	1% Sodium hypochlorite	4	-	

* Brands that do not sell latex-free elastics.

Table 1: Experimental groups and characteristics of the elastomerics

Before the cells were analyzed, they were mixed with low gelling point agarose gel and distributed to glass slides that had been previously prepared with a normal gelling point agarose gel coating. These slides were then submerged in a lysing solution and subjected to an electric field to induce migration of free DNA fragments out of the nuclei. After electrophoresis in alkaline conditions (pH>13), the slides were stained with ethidium bromide and nuclei of cells with broken DNA were observed (Figure 1).

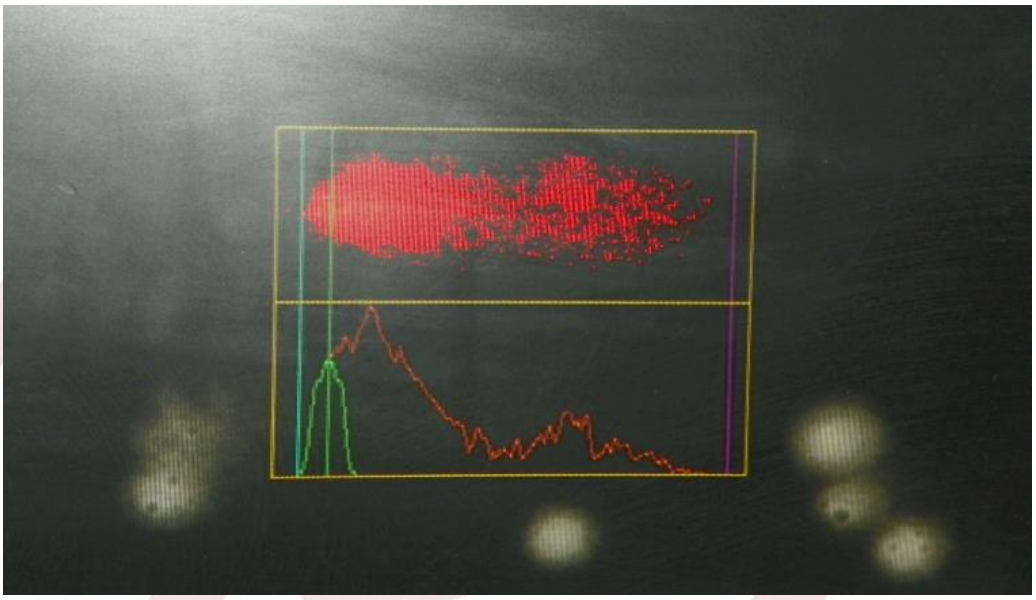


Figure 1: Cell with comet

After staining, slides were viewed under a fluorescence microscope (Olympus BX51). A total of 100 cells were analyzed per sample, i.e. 25 cells per repetition were counted. Intact nuclei have a round appearance, whereas free DNA from damaged cells migrate in the direction of the anode, creating a tale of fragments with an appearance reminiscent of a comet. These fragments can be of varying sizes and may still be connected to the nucleus by a single tail. The comets were classified using Comet Assay IV software (Perceptive).

Statistical Analysis

One-way analysis of variance (ANOVA) and Dunnett's post hoc test were used for statistical analysis of the genotoxic activity of the samples, with significance set at $p<0.05$.

Results

As can be observed from the data contained in Table 2, all groups of elastomerics, with and without latex, from all of the different commercial brands tested proved to be genotoxic, and results were significantly different from the negative control ($p<0.05$). It should also be pointed out that groups G5 (Morelli®, containing latex) and G9 (3M Unitek®, containing latex) were so genotoxic that they could not be analyzed using the Comet test.

Controls	Comet Test (% of DNA in Tail) Mean ± SD	Coefficient of Variance (%)
Negative	5.5±2.2	**40%**
Positive	35.1±12.2[a]	**34.75%**
Elastics		
G1 – AMO® (containing latex)	62.2±6.6 [a.c]	**10.61%**
G2 – AMO® (without latex)	37.6±23.8 [a]	**63.30%**
G3 – RMO® (containing latex)	30.7±10.3 [a]	**35.55%**
G4 - RMO® (without latex) **G5 - Morelli® (containing latex)**	30.6±18.8 [a] *	**61.44%**
G6 - Morelli® (without latex)	16.3±7.0 [b]	**42.94%**
G7 – TP® (containing latex)	31.3±7.2 [a]	**23 %**
G8 – GAC® (containing latex)	16.2±6.1 [b]	**37.65%**
G9 - Unitek® (containing latex)	*	

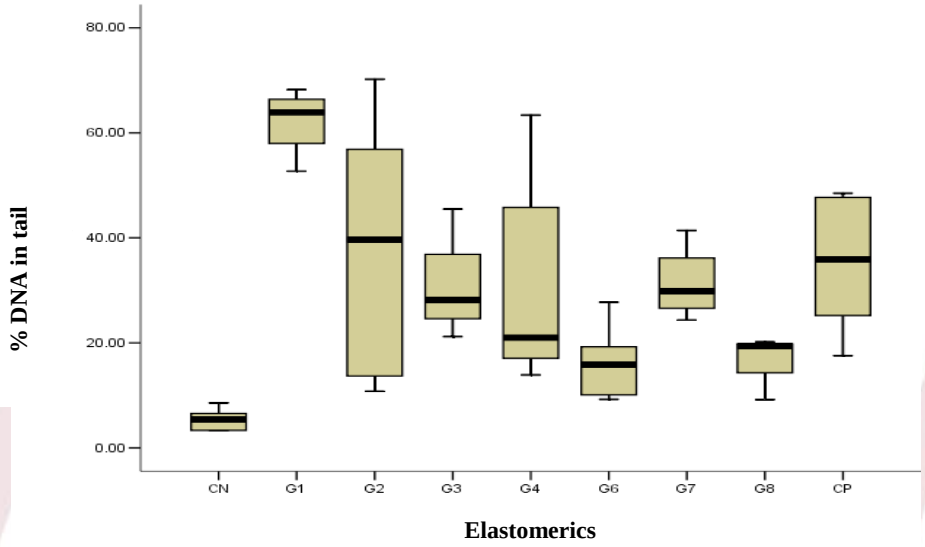
a Significantly different from the negative control, P<0.001; b Significantly different from the negative control, P<0.05; c Significantly different from G6 and G8, P<0.01. One-way ANOVA and Dunnett's post-hoc test.

Table 2: DNA damage after exposure (24 hours) of L929 cells, for the groups investigated

Means for groups of elastomerics containing latex were similar to means for latex-free elastomerics, except for groups G1 (American Orthodontics® containing latex) and G6 (Morelli® without latex).

Among the groups containing latex, the most genotoxic groups, in ascending order, were as follows: GAC®, RMO® TP Orthodontic®, American Orthodontics®, Morelli® / 3M Unitek ®. Among the groups without latex, Morelli®, RMO® and American Orthodontics® were the most genotoxic.

Graph 1 shows the minimum and maximum values observed for each group, showing that variations were considerable, particularly in the groups of latex-free elastomerics G2 (American Orthodontics®) and G4 (RMO®).



Graph 1: Box plots - Group oportions of DNA damage.

Discussion

According to the results shown in Table 2, all of the elastomerics tested, with and without latex, from all brands, proved to be genotoxic, and groups G5 (Morelli®) and G9 (3M Unitek ®), both containing latex, stood out as extremely genotoxic, to the extent that it was not possible to quantify the comet size. According to Marins et al. (2012), this finding is not uncommon and can be explained by the high cytotoxic percentage of the material during the cell culture, exceeding 30% and meaning it is not feasible to use the Comet test. [16] Similar results were observed in earlier studies, such as one published by Santos et al. (2012), who demonstrated the cytotoxicity of Morelli® brand elastomerics for the cells tested, although they used tests of cell viability. [17]

Six of the nine groups of elastomerics analyzed contained latex in their composition, and caused a high percentage of DNA damage and there was only a statistically significant difference between G1 (American Orthodontics®) with 62.2% and G8(GAC®) with 16.2%. This difference may be related to different compositions and/or manufacturing processes. The primary cause of allergic reactions of any type are proteins contained in the natural latex. [16,5] Allergy to natural latex is primarily due to the presence of Hevein and prohevein, which account for 70% of the proteins contained in latex. [17]

Analyzing presence or absence of latex, there were no statistically significant differences between elastomerics from the same manufacturer, and differences were only detected between groups G1, with latex (American Orthodontics®), and G6, without latex (Morelli®).

These findings shine new light on the etiology of these reactions, which had hitherto been associated with the latex content of the materials and, if the allergy is to be understood, it is extremely important to analyze the elastomerics' production processes, which involve collection, centrifugation, coagulation, vulcanization and

application of a powder coating. [17] The different manufacturing processes of these elastomerics may hold the key to explaining these differences in genotoxicity, since, in general, stabilizing agents may be added in order to improve mechanical properties and these may include zinc, an ion considered to be neurotoxic, antioxidants and antiozonant. [18]

Another finding supporting the conclusion that differences in genotoxicity are not exclusively linked with latex is the observation that groups G6 and G8 exhibited similar behavior, with the lowest genotoxicity values, of 16.3% and 16.2% of DNA in the tail respectively, even though G6 does not contain latex.

Trevisan (2014) [19] studied the cytotoxicity of the same elastomerics listed in Table 1, finding that over the first 24h the majority of the brands that did contain latex in their composition (TP®, Morelli®, 3M®, GAC® and American Orthodontics®) caused low mean cell viability, and consequently higher cell toxicity, even exhibiting lower values than the positive control (C+), in contrast with the RMO® brand, which had a similar result to the negative control (C-), and exhibited the highest cell viability score. These results support the hypothesis that there are differences in the production processes5 or basic composition of the elastomerics, especially in the case of the RMO® brand, and also differences in release of the components involved.

Aware of these differences, and hoping to investigate their characteristics and possible consequences on genotoxicity values, the authors of this study contacted the manufacturers, but they were unanimous in claiming that the exact compositions of their elastomerics are trade secrets.

Another factor that could contribute to differences in genotoxicity is the presence of coloring agents in the elastomerics, according to a study by Holmes et al. (1993), [20] who tested elastomerics in a range of colors and found that cell lysis was greater during the first 24 hours with colored elastomerics, although the difference was not significant. This may explain, for example, the similarity in genotoxicity between groups G3 and G4, both from the RMO® brand, which, despite respectively containing and being free from latex, had percentages of DNA in the tail of 30.7% and 30.6%. Since G4 was the only colored elastomeric without latex in all of the experimental groups, its greater toxicity, despite being free from latex, may be related to coloring agents in its composition. Along the same lines, Trevisan (2014) [19] reported identical results for the same brand after studying cytotoxicity. In contrast, a study by Santos et al. (2009) [2] that assessed cell viability with elastomerics of a variety of colors found that pigmentation did not interfere with greater or lesser cell lysis.

One important detail that should be analyzed is the high standard deviations in the results for the different groups assessed (Table 2, Graph 1), showing that the data are highly variable, and in common with many studies of genotoxicity available in the literature, such as: Angieleri et al. (2011) [3], Zhilong et al. (2011), [21] Haffez et al. (2011), [22] Angieleri et al. (2012), [23] and Gonçalves et al. (2014). [24]

Theoretically there is no plausible explanation for these observations, but, working from the principle that the Comet assay requires reading of a relatively large number of comets (50 on average) for analysis with an optical microscope, and that, to avoid repeating the analysis of the same comet, the image process involves a complete scan of the slides, from top to bottom (Brianezi et al. (2009), [25] this would allow insertion of readings at minimum and maximum values. Added to this, according to Burlinson et al. (2007) [26, 27] when doses are cytotoxic a reduction in DNA migration can be detected, caused by loss of cells damaged or killed during processing of the sample and/or electrophoresis.

Finally, it is clear that the results reported here corroborate the idea that although latex is a proven allergen, it is not the only agent that causes adverse effects. Further research must be undertaken with the approval of the manufacturers to allow the composition of the elastomerics to be divulged, if the true etiology of the toxic effects

caused by these products is to be elucidated.

Conclusions

All of the groups and all different brands were genotoxic, irrespective of whether they contained latex or not.

Among the groups containing latex, the most genotoxic groups, in ascending order, were as follows: GAC®, RMO® TP Orthodontic®, American Orthodontics®, Morelli® / 3M Unitek®. Among the groups without latex, Morelli®, RMO® and American Orthodontics® were the most genotoxic.

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