

## Short Communication

# Intraoral Elastics Used in Orthodontic Practice: An *In vitro* Study on Cytotoxicity and Behavior Cellular

Freitas MPM<sup>1\*</sup>, Gerzson, DRS<sup>1</sup>, Closs LQ<sup>1</sup>, Vargas IA<sup>1</sup> and Machado D<sup>2</sup><sup>1</sup>Department of Orthodontics, University in Canoas, Brazil<sup>2</sup>Department of Immunology, Pontifical Catholic University of Rio Grande do Sul, Brazil

## \*Corresponding author

Maria Perpétua Mota Freitas, ULBRA- Lutheran University of Brazil- Orthodontic and Pediatric Dentistry Department. Av. Farroupilha, 8001. Canoas, RS. Brazil, Tel: 55 51 3477 4000; Email: perpetuamf@hotmail.com

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## Abstract

Biocompatibility should be a basic requirement prior to the use of dental materials in clinical practice. **Objective:** To test the null hypothesis that the elastics employed in orthodontics is not cytotoxic for fibroblasts.

**Materials and methods:** This *in vitro* study was performed using a culture of mice fibroblasts (lineage NIH/3T3), divided into four groups (n=10 each): control, negative control (stainless steel archwire), positive control (amalgam disks), and test group (elastics). After cell culture in complete Dulbecco modified eagle medium and achievement of confluence in 80%, the suspension was added to the plates of 24 wells containing the specimens and incubated in an oven at 37°C for 24 hours. The plates were analyzed on an inverted light microscope, photomicrographs were obtained, and the results were recorded as response rates based on modifications of the parameters of Stanford according to the size of the diffusion halo of the toxic substance and quantity of cell lysis.

**Results:** The results revealed a maximum response rate for the elastics, as well as severe inhibition of cell proliferation and growth, more round cells with mostly darkened and granular aspects, suggesting lysis with cell death. A similar response was seen in the positive control group.

**Conclusion:** The hypothesis is rejected. The elastics used in orthodontics represent a highly cytotoxic material for the cells analyzed.

## INTRODUCTION

Biocompatibility of dental materials has been the subject of widespread speculation and uncertainty. Particularly in orthodontics, various materials remain in direct contact with oral tissues for long periods of time. The potential adverse reactions to these materials have been widely investigated [1].

The latex has been used in orthodontics since this specialty was first introduced in the form of extraoral and intraoral elastics for occlusion detailing and maxillomandibular fixation after orthognathic surgery [1].

The advent of vulcanization (Charles Goodyear, 1839) led to latex being used in over 40,000 items, such as: Dental products (gloves, masks, rubber dams and orthodontic accessories), medical equipment (gloves, catheters, tubes, implants and prostheses), household gloves, balloons and balls, condoms, stickers, pacifiers, carpets, footwear, mats and sporting goods [2]. As a result, a high prevalence of hypersensitivity to latex has

emerged due to its widespread use in manufactured products containing rubber [3].

Many reports on different reactions to latex are described in the literature. Allergic reactions caused by latex range from swelling and stomatitis to erythematous oral lesions and respiratory reactions culminate in anaphylactic shock [4]. These reactions are present in 3% to 17% of cases and are caused by component proteins [5].

Most of the cases are prevalent in women [6], these reactions usually appear at a young age and are very frequent in adults working in at risk professions such as dentists and other healthcare professionals. Among healthcare professionals (physicians, nurses and dentists), 7% show a delayed allergic reaction (contact dermatitis), while 3% show an immediate reaction. In 1993, after numerous reports had been published on this issue, the labels of medical products containing latex were required to inform such content [7].

In a study conducted over a period of 10 years (from 1978 to 1988), 7000 patients were evaluated. Of these, 4860 were tested and a total of 14.7% had one or more positive reactions to rubber additives [8]. Specifically in the dental field, a questionnaire-based survey was conducted with dentists in England and Wales. At Least 135 Professionals as well as one or more patients reported adverse effects to the use of gloves containing latex [9]. The authors of this study are aware of the potential toxic effects of latex on human tissues, as reported in the literature. This investigation therefore aimed to test the null hypothesis that the elastics employed in orthodontics is not cytotoxic for fibroblasts.

## MATERIAL AND METHODS

The cytotoxicity study was revised and approved by the Institutional Review Board of PUCRS (protocol n. 1148/05-CEP) and was conducted on cell culture, using the lineage NIH/3T3 (mice fibroblasts) to evaluate the response rate, determined from modifications in the parameters of Stanford [10].

### Specimens

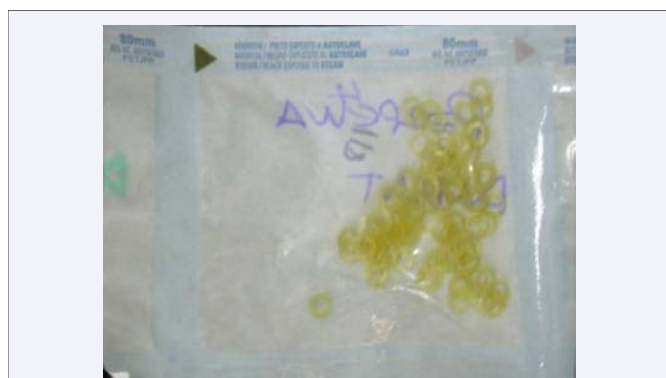
Four study groups (Figure 1) were established, as presented in Table 1. Group 1 was included only for the control of cell growth.

### Fabrication of specimens

The sequence for fabrication of specimens for amalgam discs is described in Table 2.

### Cell culture

Mice fibroblasts lineage NIH/3T3, purchased from ATCC® (USA) and manipulated at the Laboratory of Cell Biology and Respiratory Diseases of the Institute of Biomedical Research of the São Lucas Hospital at PUCRS, were unfrozen and cultured in D-MEM culture medium (Dulbecco's Modified Eagle Media, Invitrogen, USA), supplemented with 10% of bovine fetal serum, 100 U/mL of penicillin, 100 µg/mL of streptomycin and 50 µg/mL of gentamicin (complete D-MEM), in culture bottles (TPP, Switzerland) and incubated at a temperature of 37 °C in a humidified oven containing 5% of CO<sub>2</sub>, which was changed twice a week until the cells reached confluence in 80%. After confluence, the cells were detaching by trypsinization using 0.1% Trypsin-EDTA (Gibco, Grand Island, NY) and counted in a Neubauer chamber (Optik Labor, Germany). The suspension was added on



**Figure 1** Intraoral elastics after sterilization.

Procedure	
1	Prepare the amalgam DS80 in amalgamator SDI® for 7s.
2	Place of the mixture on a rectangular acrylic plate.
3	Press a second plate on the mixture until a 1.5-mm space is established between the two plates (final thickness of the amalgam disc).
4	Use an amalgam carrier with 2-mm diameter opening to determine the disc width.
5	Polishing with abrasive rubber point.
6	Rinsing and drying.
7	Sterilization in autoclave.

Index of halo size	Index of quantity of cell lysis
0 = no detection of halo around or under the specimen	0 = no lysis
1 = halo limited to the area under the specimen	1 = up to 20% of the halo with lysis
2 = halo not greater than 25% the extent of the specimen	2 = 20 to 40% of the halo with lysis
3 = halo not greater than 50% the extent of the specimen	3 = 40 to 60% of the halo with lysis
4 = halo greater than 50% the extent of the specimen, yet not involving the entire plate	4 = 60 to 80% of the halo with lysis
5 = halo involving the entire plate	5 = more than 80% of cell with lysis in the halo

plates of 24 wells (10 wells for the control of cell growth of the control group, 10 containing amalgam discs, 10 with orthodontic archwires and 10 with silver soldering), in 500 µL, with density of 4.5 x 10<sup>5</sup> cells per well. Finally, the cultures were once again incubated for 24 hours.

### Analysis of cytotoxicity

After the 24-hour period, the plates were analyzed on an inverted light microscope Axiovert 25® (Carl Zeiss SMT, Inc. Thornwood, New York, USA) with 10X objective and photomicrographs were obtained. The results were recorded as response rates, according to the modified parameters of Stanford [10], are presented in Table 3.

The rates were calculated in relation to the halos observed, being two numbers separated by a bar, in which the first represented the size of the diffusion halo of the toxic substance and the second indicated the quantity of cell lysis. Qualitative cell analysis was also performed based on the characteristics of cell proliferation, growth, morphology and adhesion.

## RESULTS AND DISCUSSION

The results showing the response rates are depicted in Table 4. As can be observed, there was a maximum response rate of 100% of the samples in the Group 4, unlike the Group 1 (cell control group) and Group 2 (negative control), which exhibited a minimal rate, as expected.

Qualitative cellular analysis disclosed an increase in the

**Table 3:** Response rates, according to modification of the parameters of Stanford.

	Group 1 (n=10) Control group	Group 2 (n=10) Negative control	Group 3 (n=10) Test group	Group 4 (n=10) Positive control
<b>Material</b>	-	Archwire segments	Intraoral elastics	Amalgamdiscs (GS80)
<b>Brand</b>	-	Morelli®, São Paulo, Brazil	GAC®®, New York, USA	SDI Ultramat®, Victoria, Australia
<b>Dimension</b>	-	0.018"	3/16	2mmx1.5mm
<b>Cleaning</b>	-	Rinsing/Drying	Rinsing/Drying	Rinsing/Drying
<b>Sterilization</b>	-	Autoclave	Autoclave	Autoclave

**Table 4:** Response rates obtained in the groups evaluated.

Group 1 Control group		Group 2 Negative control		Group 3 Test group		Group 4 Positive control	
n	Response rate	n	Response rate	n	Response rate	n	Response rate
1	0/0	1	0/0	1	5/5	1	5/5
2	0/0	2	0/0	2	5/5	2	5/5
3	0/0	3	0/0	3	5/5	3	5/5
4	0/0	4	0/0	4	5/5	4	5/5
5	0/0	5	0/0	5	5/5	5	5/5
6	0/0	6	0/0	6	5/5	6	5/5
7	0/0	7	0/0	7	5/5	7	5/5
8	0/0	8	0/0	8	5/5	8	5/5
9	0/0	9	0/0	9	5/5	9	5/5
10	0/0	10	0/0	10	5/5	10	5/5

number of cells in the Group 1 and 2, as well as confluent growth and fusiform cells, typical of normal fibroblast development (Figure 2a, b). Moreover, in the Group 3 (Figure 2c), a severe inhibition of cellular proliferation and growth occurred along with an increased number of circular cells and, in large part, with a blackened appearance similar to Group 4 (positive control) (Figure 2d), suggestive of lysis and cellular death (Figure 3).

Biocompatibility should be a basic requirement prior to the use of dental materials in clinical practice. Given that no dental material is absolutely safe; their selection should be based on the assurance that benefits outweigh biological risks [8].

Founded on this assumption and considering the scant studies available on the biocompatibility of materials in orthodontics, the authors of this study aimed to assess specifically the cytotoxicity of intraoral elastics, considering the time that they remain in the oral cavity of patients in direct contact with their tissues.

Based on these reports, several methods have been proposed to assess the cytotoxicity of materials, including experiments with NIH/3T3 cell line mouse fibroblasts using modified Stanford parameters (1980) [10-12] and investigations on proliferation by means of microscopic analysis of cellular growth and division [13-15]. By the same token, other authors have proposed the use of other cell types such as Balb/c, L929, W138, human fibroblasts or osteoblasts [16,17]. This research focused on the use of NIH/3T3 cell line given their availability in the authors' laboratories, and their validation in cytotoxicity studies.

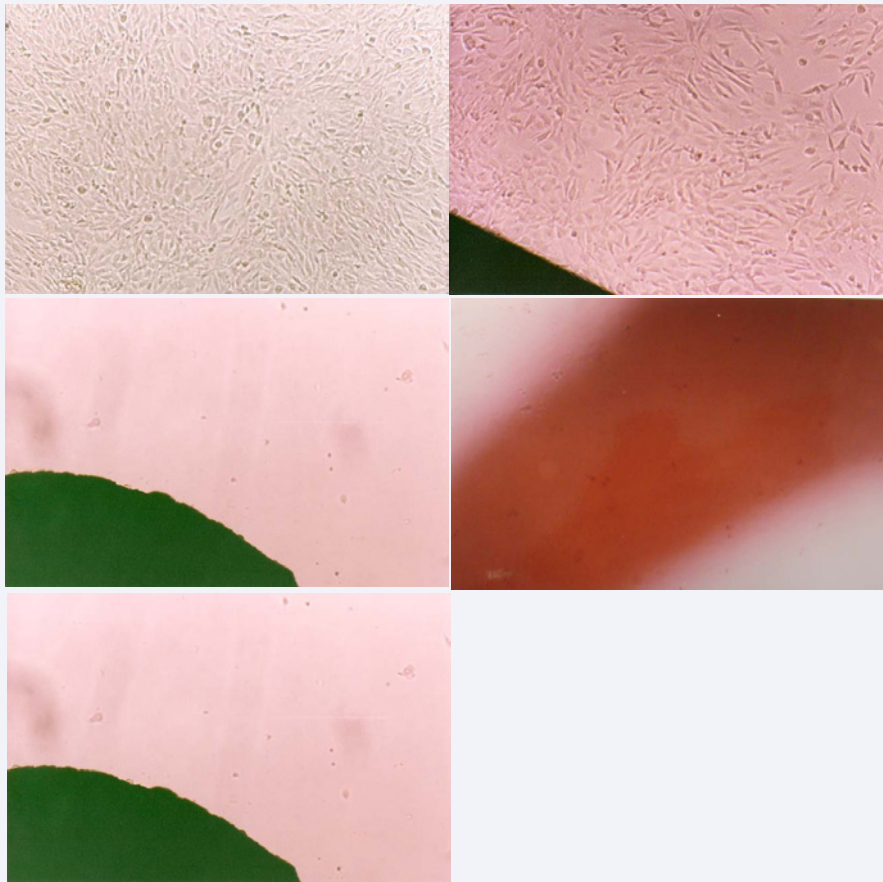
Cells in the control group exhibited a normal pattern with significant proliferation and confluent growth (Figure 2a). The

test group, in turn, showed a halo of growth inhibition in 100% of the samples as well as a high degree of cellular lysis, indicative of cytotoxicity (Figures 2c, 3). Whereas in the normal life cycle of fibroblasts these cells enter into contact with a surface, then attract one another, adhere to one another, eventually spreading in the medium; and considering that adhesion quality influences cell morphology, ability to proliferate and differentiate, it is likely that the components in the elastics initially prevented cell adhesion and, as a result, subsequent events [14].

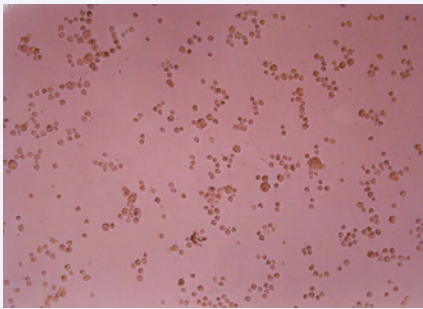
Ideally, by calculating the percentage of cells in the "S" phase one could substantiate this process, be it by immunocytochemistry or by thymidine incorporation, based on the fact that cell adhesion to the substrate may be critical for cell proliferation. This phenomenon occurs because the cells become adherent when they begin to divide early in the "S" phase, determining a peculiar array of fibronectines [15].

Regarding cell morphology, the response of the fibroblasts to the presence of elastic specimens led to the emergence of more rounded, isolated and notably dark cells, suggesting inhibition of cellular activities or cell death, unlike the control group, in which the fibroblasts exhibited an elongated shape, confluent and dense growth, typical of a normal pattern. Similar conclusions were reached in other studies [18-20].

In seeking to explain which component could be associated with such changes, it was observed that latex is primarily composed of large molecules which do not appear to cause allergies. Furthermore, polyisoprene is the main constituent of the rubber, in addition to proteins, which comprise a total of



**Figure 2** Cell aspect in (a) the control group, (b) negative control, (c) test group, and (d) positive control.



**Figure 3** Response rates, according to modification of the parameters of Stanford.

2% to 3% of the end product. Moreover, low molecular weight substances such as additives, antioxidants and vulcanizers are commonly used in the manufacture of rubber and responsible for producing the desired properties which may cause adverse reactions. These additives are often released from rubber gloves and boots especially in warm and humid environments [9]. According to Hanson, Lobner (2004) [1], zinc - a component present in the latex pre-vulcanization phase- is considered as the causative factor of cytotoxicity of the material in question. As an alternative for clinical use and in line with previous evidence-based examples, these authors - aware of the documented cytotoxicity of urinary catheters - suggested that their use be

suspended and replaced by silicone catheters [1]. Considering the above evidence, this may also constitute a viable alternative for use in orthodontic practice. Nevertheless, further laboratory and clinical research is warranted to clarify the actual risks involved in using these materials.

## CONCLUSIONS

The orthodontic intraoral elastics used in orthodontics exhibit severe cell toxicity resulting in inhibition of proliferation, growth, and development of the cells analyzed.

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