

Evaluation of cytotoxicity of accessories used for traction of impacted teeth

Rogério Lacerda dos Santos¹, Matheus Melo Pithon¹, Fernanda Otaviano Martins²,
Maria Teresa Villela Romanos³, Antônio Carlos de Oliveira Ruellas⁴

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

² BS, Student in Microbiology, Department of Microbiology, Orthodontics Federal University of Rio de Janeiro, Brazil

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

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

Abstract

Aim: To test the hypothesis that gold-coated orthodontic accessories used for canine traction are less cytotoxic than those made of stainless steel. **Methods:** Six different orthodontic accessories were evaluated, three of them made from stainless steel (1 – bracket, 2 – button, 3 – mesh pad) and three made from a gold-coated alloy (4 – small mesh pad, 5 button, 6 – big mesh pad). Three control groups were also analyzed: Positive control (C+), consisting of Tween 80 cell detergent; Negative control (C-), consisting of PBS; and Cell control (CC), consisting of cells not exposed to any material. Dye-uptake technique, in which neutral red dye is incorporated into viable cells, was used to assess the cytotoxicity of the accessories. Viable cell counting was performed using a spectrophotometer. Data were analyzed statistically by ANOVA and Tukey's test. **Results:** Statistically significant differences ($P < 0.05$) were found between Groups 1-3 and Groups 4-6. However, no differences were found between Groups 1-3 and Groups C- and CC, and neither between Groups 4-6 and Group C+. **Conclusions:** The tested hypothesis was not confirmed since gold-coated orthodontic accessories were found to be more cytotoxic than those made of stainless steel.

Keywords: tooth, impacted, orthodontic appliance, orthodontic appliance design.

Introduction

Impacted teeth are a problem of increasing frequency. Several contributing factors for their occurrence can be mentioned, such as the normal growth of the braincase at the expense of the jaws; the diet, which are less demanding of the stomatognathic system; and the advances in preventive dentistry, which have allowed for children and adolescents suffering less dental mutilation and entering adulthood with all or most of their teeth in the arch¹⁻².

The most commonly impacted teeth are molars in adults and adolescents, and maxillary canines in children. The presence of impacted teeth can cause the development of important pathological conditions that can compromise the integrity of the maxillomandibular complex, and the patient's health¹⁻².

Diagnosis of impaction should be performed both clinically and radiographically³. Once impaction is diagnosed, one should opt for the following: no intervention, if the patient so desires; self-transplantation; extraction; occlusal reestablishment by means of prosthesis; surgical exposure and orthodontic traction to move tooth towards the line of occlusion⁴⁻⁶. This latter option has been proven efficient, mainly when dental impaction is adequately diagnosed. There exists in

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Correspondence to:

Rogério Lacerda dos Santos
Rua Ipatinga, 170, Planalto
Divinópolis- MG- Brasil - CEP: 35501-191
E-mail: lacerdaorto@hotmail.com

the literature a multitude of techniques for this purpose^{5,7-11}. All techniques available require trans-surgical bonding of accessories to the teeth, which will offer anchorage for application of orthodontic force. Basically, the orthodontic accessories are manufactured from stainless steel and gold. Gold-coated accessories are described in the literature as being less irritating to oral tissues because of their noble alloy¹². In spite of its biocompatibility, a disadvantage of gold is its high cost¹³. The aim of the present study was to test the hypothesis that gold-coated accessories are less cytotoxicity than the stainless steel ones.

Material and methods

Cell Culture

The cell line used for this study was mouse L929 fibroblasts obtained from the American Type Culture Collection (TCC, Rockville, MD, USA) and cultivated in Eagle's minimum essential medium (MEM) (Cultilab, Campinas, SP, Brazil). The cell culture was supplemented with 2 mM of L-glutamine (Sigma, St. Louis, MO, USA), 50 µg/mL of gentamicin (Schering Plough, Kenilworth, NJ, USA), 2.5 µg/mL of fungizone (Bristol-Myers-Squibb, New York, USA), 0.25 mM of sodium bicarbonate solution (Merck, Darmstadt, Germany), 10 mM of HEPES (Sigma), and 10% of fetal bovine serum (FBS) (Cultilab), and kept at 37°C in a 5% CO₂ environment.

Accessories

The sample consisted of 6 different types of orthodontic accessories (Figure 1), which were divided into 6 groups according to their characteristics as shown in Table 1. To verify the cell response to extreme situations, three other control 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 (Polioxietileno-20-Sorbitan); and Group C- (negative control), consisting of PBS solution (phosphate-buffered saline) in contact with the cells.

Cytotoxicity assay

The materials were previously sterilized by exposure

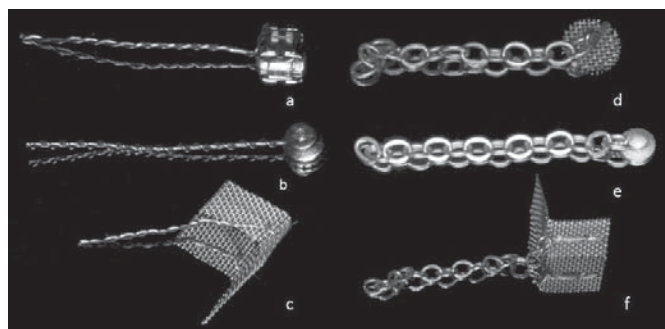


Fig. 1. Orthodontic accessories.

Table 1. Groups and materials with their respective characteristics.

Groups	Accessories	Attachment	Material	Composition
1	Bracket	Braided wire	Steel	Cr: 17.0 – 20.0%, Ni: 8.0 – 10.5%, Fe 65%-69.5%
2	Button	Braided wire	Steel	
3	Big mesh pad	Braided wire	Steel	
4	Small mesh pad	Chain	Gold	88.0 Sn, 1.5 Sb, 9.0 Pb and 1.5 Cd
5	Button	Chain	Gold	
6	Big mesh pad	Chain	Steel	

to ultra-violet light (Labconco, Kansas, Missouri, USA) for 1 h. Next, 30 samples of each material were placed in 24-well plates containing Eagles' MEM (Cultilab, Campinas, São Paulo, Brazil). The culture medium was replaced with fresh medium every 24 h, and the supernatants were collected after 1 and 7 days for toxicity analysis to L929 cells. The supernatants were placed in a 96-well plate containing a single layer of L929 cells and then incubated at 37°C for 24 h in a 5% CO₂ environment. After the incubation period, cell viability was determined using the "dye-uptake" technique described by Neyndorff et al.¹⁴ (1990), which was slightly modified. After the 24-h incubation period, 100 µL of 0.01% neutral-red staining solution (Sigma) was added to the medium in each well of the plates, and these were incubated for 3 h at 37°C to allow the dye to penetrate into the living cells. After this period, the cells were fixed using 100 µL of 4% formaldehyde solution (Reagen, Rio de Janeiro, Brazil) in PBS (130 mM NaCl; 2 mM KCl; 6 mM Na₂HPO₄ 2H₂O; 1 mM K₂HPO₄, pH = 7.2) for 5 min. Next, 100 µL of 1% acetic acid solution (Vetec, Rio de Janeiro, RJ, Brazil) with 50% methanol (Reagen, Rio de Janeiro, RJ, Brazil) was added to the medium to remove the dye. Absorption was measured after 20 min by using a spectrophotometer (BioTek, Winooski, Vermont, USA) at 492 nm wavelength.

X-Ray Dispersion Analysis

The metallic alloy of the accessories was characterized by X-ray dispersion using a JEOL scanning electron microscope (SEM) (2000 FX, Tokyo, Japan). The accessories were cut with a precision sectioning machine (Isomet, Buehler, IL, USA) and the samples were ultrasonicated (Ultramet 2002, Buehler, Illinois, USA), dried and subjected to SEM analysis.

Statistical Analysis

Statistical analyses were performed by using Statistical Package for the Social Sciences version 13.0 program (SPSS Inc, Chicago, IL, USA). and means and standard deviations were calculated for descriptive statistical analysis. The mean numbers of viable cells were calculated and analyzed statistically by ANOVA and Tukey's test at 5% significance level.

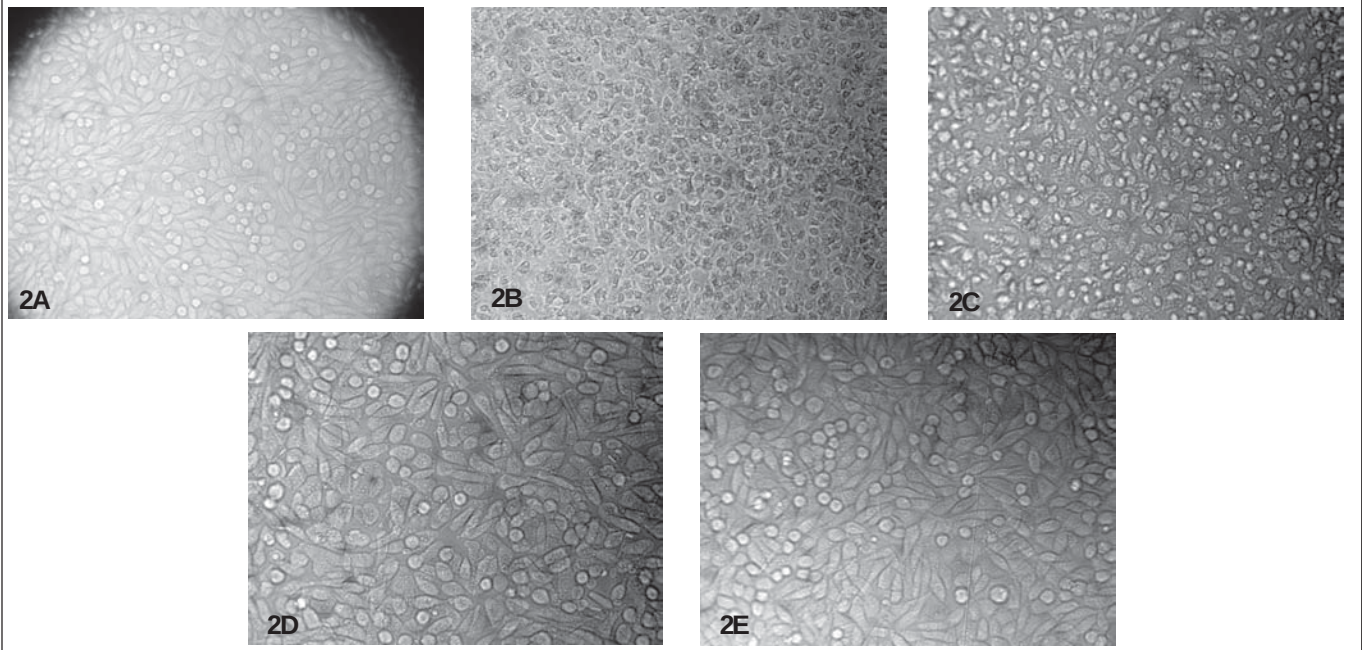


Fig. 2. Cell aspect a- groups 1, 2 e 3; b- groups 3,4 e 5; c- Positive control; d- Negative control; e- cell control.

Results

The results obtained in the cytotoxicity assay revealed that gold-coated accessories were more cytotoxic than the stainless steel accessories and the controls C- and CC ($P < 0.05$), as shown in Table 2 and Figure 2.

Table 2. Mean number of viable cells (and standard deviations) in the studied groups.

Group	1 day		7 days	
	Mean (SD)	Stat	Mean (SD)	Stat
1	248 (21.77)	A	773.22(46.11)	A
2	255.55(73.05)	A	776.33(58.80)	A
3	289 (33.11)	A	782.33(54.90)	A
4	100.11(30.08)	B	149.33(39.15)	B
5	111.44(37.97)	B	161.88(49.93)	B
6	92.66(28.27)	B	148.22(41.30)	B
C+	76.33(8.38)	B	138.33(53.74)	B
C-	272.22(17.22)	A	767.44(126.94)	A
CC	259.22(14.77)	A	783.22(141.89)	A

Mean = mean number of viable cells; SD = Standard Deviation;

St* = Same indicate no statistically significant difference ($p > 0.05$).

Discussion

The success in clinical orthodontics involves not only having an accurate the technique, but also following biosecurity norms and preventing local and systemic reactions of the materials used¹⁵⁻¹⁶. Based on this and considering the lack of studies on the biocompatibility of orthodontic accessories employed during traction of impacted canines, the present study was designed to assess the

cytotoxicity of these materials by performing an *in vitro* study with L929 fibroblast culture.

Investigations of the cytotoxicity of metals have taken different approaches in relation to cell characteristics and functions. Studies have evaluated the adhesion, proliferation, and metabolism of cells such as 3T3, Balb/c, mice fibroblasts, W138, and fibroblasts and osteoblasts¹⁷⁻¹⁸. In the present study, the behavior of L929 mouse fibroblast was assessed by using the dye-uptake technique.

The vital dye neutral red method was employed to evaluate cell viability. Analysis of neutral red is a cell survival/viability assay based on the capacity of viable cells to incorporate and process the neutral red within their lysosomes. This is normally performed by adherent cells. The neutral red is a weak cationic dye that penetrates the cell membrane and accumulates intracellularly within the lysosomes (lysosomal pH < cytoplasmic pH), where combines with the anionic part of the lysosomal matrix¹⁹. The changes in either cell surface or lysosomal membrane result in lysosomal membrane fragility and other changes that become gradually irreversible. Such alterations resulting from the action of xenobiotics decrease the absorption and process of neutral red dye. Therefore, it is possible to distinguish viable, damaged or dead cells, which is the basis of this essay. The amount of dye incorporated into the cells is measured spectrometrically, being directly proportional to the number of cells with intact membrane.

This method was first used by Pithon et al.²⁰, who compared it to the agar diffusion method for evaluating the cytotoxicity of orthodontic materials and found that both methods provide adequate cytotoxic evaluation.

In order to assess the cell response to extreme situations, a positive control group (C+) was included into this study to cause cell damage. The material used in the positive control

group was a non-ionic surfactant (Tween), a toxic agent to biological membranes²¹. This non-ionic surfactant consists of polyethylene sorbitol fatty acid esters, which is characterized by stimulating protein secretion in microorganisms²², for altering cell morphology and wall surface²³. On the other hand, a recognizably non-toxic agent (PBS solution) was used as a negative control in order to assess only the physical effect on the cells, as no other substance was put in contact with the cells. Other studies²⁴ have used silver amalgam and stainless steel orthodontic wire as positive and negative controls, respectively. The use of such controls was aimed at assessing the cytotoxicity of accessories made of stainless alloy similar to that of conventional orthodontic wires. However, this methodology would not allow us having a parameter to quantify the toxicity of the materials we have studied.

During the experimental period, gold-coated accessories were as cytotoxic as the stainless steel ones. The groups of stainless steel accessories were shown to be biocompatible, since no differences in cytotoxicity were found ($P > 0.05$) from cell and negative control groups. Microscopic evaluation of these groups showed morphologically normal cells adhered to the bottom of the plates. These findings are important since such characteristics are strictly related to the capacity of cell proliferation and differentiation as reported by Serrano et al.²⁵.

On the other hand, the cells in the groups of gold-coated accessories were non-adherent, rounded, small, and dark-colored, which are all characteristics of dead cells. These groups showed cytotoxicity since no statistically significant differences were found between them and positive control group, the latter using Tween 80 cell detergent, which is a very cytotoxic agent that causes irreversible lesion to cell membrane ($P > 0.05$).

X-ray dispersion analysis was performed to evaluate the alloy components of the orthodontic accessories and identify and quantify chemical substances. The results of such analysis corroborated the cytotoxicity assay. The components of stainless steel accessories were the same ones found in the orthodontic wires used by Freitas²⁴ as negative control, with both studies exhibiting no cytotoxicity. On the other hand, the gold-coated accessories showed a very small amount of gold (1%) compared to other elements such as 87.7% tin (Sn), 1.5% antimony (Sb), 9.0% lead (Pb), and 1.5% cadmium (Cd), all known toxic substances, which explains our findings. The concentration of gold informed by the manufacturer was very low, perhaps being incorporated only to give the accessories a noble aspect. According to Syrjanen et al.²⁶, low-gold content alloys are more likely to be toxic, a fact observed in the present study.

The analysis of each component of this alloy individually showed that although tin was most predominant, the cytotoxicity found here cannot be attributed to this element in particular since previous studies reported no cytotoxicity for it²⁷. Another component found was antimony, which is toxic depending on its chemical state. The metallic antimony detected is relatively innocuous, but stibnite is highly toxic²⁸.

Other elements forming the gold-coated accessories, lead and cadmium have been shown to be cytotoxic as well. The former is a non-essential element having heavy metal

characteristics that accumulates inside the organism, interacting with living cells and affecting virtually all organ systems, for its toxicity mechanisms involve important biochemical processes, such as inhibiting or mimicking calcium action and interacting with proteins. The lead toxicity is mainly due to its interference with the functioning of cell membranes and enzymes, forming stable complexes with sulfur, phosphorus, nitrogen, or oxygen ligands²⁹. Likewise, cadmium is also toxic as this chemical substance may alter mitochondrial permeability, representing a progressive process until cell death. Li et al.³⁰ demonstrated that this ion may cause mitochondrial dysfunction, including inhibition of respiration, loss of potential of membrane transportation, and release of c-cytochromes. Çelik et al.³¹ observed that cadmium might induce alterations in the DNA of intact cells as well as hinder their repair potential.

Size of the accessories caused only litter influence on cytotoxicity, with larger accessories presenting slight reduction in cell viability but with no statistical significance.

The hypothesis that gold-coated orthodontic accessories are less cytotoxic could not be proved, as they had only a golden coating. On the other hand, orthodontic accessories made of stainless steel were non-toxic to fibroblast cells.

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