Original Article

Braz J Oral Sci.
April/June 2009 - Volume 8, Number 2

Cytotoxicity of polycarbonate orthodontic brackets

Matheus Melo Pithon[1], Rogério Lacerda dos Santos[1], Fernanda Otaviano Martins[2], Antônio Carlos de Oliveira Ruellas[3], Lincoln Issamu Nojima[3], Matilde Gonçalves Nojima[3], Maria Teresa Villela Romanos[4]

[1] Specialist in Orthodontics and Master in Orthodontics, Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro (RJ), Brazil; Doctorate Student in Orthodontics, UFRJ, Rio de Janeiro (RJ), Brazil

[2] Graduate Student in Microbiology and Immunology, UFRJ, Rio de Janeiro (RJ), Brazil; Trainee at the Professor Paulo de Goés Institute of Microbiology, UFRJ, Rio de Janeiro (RJ), Brazil

[3] PhD in Orthodontics, UFRJ, Rio de Janeiro (RJ), Brazil; Adjunct Professor, UFRJ, Rio de Janeiro (RJ), Brazil

[4] PhD in Microbiology and Immunology, UFRJ, Rio de Janeiro (RJ), Brazil; Adjunct Professor, UFRJ, Rio de Janeiro (RJ), Brazil

Abstract

Aim: To assess the cytotoxicity of polycarbonate orthodontic brackets. **Methods**: Polycarbonate brackets from two different manufacturers, namely, Composite bracket (Morelli™) and Silkon Plus bracket (American Orthodontics™), were assessed. In addition to these two experimental groups, other three control groups were included: Positive Control Group (C+) consisting of amalgam cylinders, Negative Control Group (C-) consisting of glass rods, and Cell Control Group (CC) consisting of cells not exposed to any material. All brackets were previously sterilized under ultra-violet light (UV) and, then, immersed in Eagle's minimum essential media (MEM) for 24 hours, after which the supernatants were removed and placed into contact with L929 fibroblast cells. Cytotoxicity was evaluated at 24, 48, 72 and 168 hours. After contact with MEM, the cells were further incubated at 37°C for 24 hours and 100 mL of 0.01% neutral red dye were added. The cells were incubated again at 37°C for three hours to incorporate the dye. After this period, the cells were fixed and viable cell counting was performed by spectrophotometry at 492 nm wavelength. **Results**: No statistically significant difference was found between the experimental groups (1 and 2) and the negative and cell control groups ($p > 0.05$). The Positive Control Group exhibited high cytotoxicity throughout experimental period are differed significantly from the other groups ($p < 0.05$). **Conclusions**: Polycarbonate orthodontic brackets were found not to be cytotoxic within the evaluated experimental period.

Keywords: orthodontic brackets, cytotoxicity tests, immunologic, cell culture techniques.

Introduction

Dentistry has the main purpose of keeping or improving the patient's quality of life by preventing diseases, relieving pain, and improving the masticatory efficacy, phonetics and/or esthetics. Most of these objectives require replacement or alteration of the existing dental structure as well as changes in tooth positioning. Developing and selecting biocompatible materials have been one of the major challenges in Dentistry[1]. Metals, ceramics, polymers and composites are the four groups of materials that are currently employed[2].

Little scientific information on these materials was available until the middle of the last century. Toxic, inflammatory, allergic or mutagenic reactions are the possible biological responses to these materials[3-6]. Toxicity is one of the main parameters for evaluating the biological response and the potential damage to cells and tissues related to the use of such materials[7,8].

Received for publication: April 3, 2009
Accepted: July 24, 2009

Correspondence to:
Matheus Melo Pithon
Centro Odontomédico Doutor Altamirando da Costa Lima
Avenida Otávio Santos, 395, sala 705 – Recreio
CEP 45020-750 – Vitória da Conquista (BA), Brasil
E-mail: matheuspithon@bol.com.br

In Dentistry, various kinds of materials are used for transitory restoration (wires, bands, brackets and resins) during medium and long periods of time, and the orthodontic appliance consists of a series of these materials. In attempt to make the orthodontic fixed devices esthetically more acceptable, the manufacturers are producing lingual brackets and accessories mimicking tooth colors.

Polycarbonate is a material for application in a wide variety of areas. This material is formed with small molecules called monomers or giant molecules called polymers. They are produced through chemical reactions that may be reversible or not, spontaneous or stimulated (by heat or reagents), in which the monomers combine chemically to form long ramified molecules with the same centesimal composition[9].

The polymers usually employed in the orthodontic materials may be divided into three groups with distinct characteristics: 1) finished material to be used in its original shape; 2) cast polymers to be used for structuring a variety of removable or functional artifacts, and 3) polymeric materials for impression, adhesion and sealing.

Esthetic plastic orthodontic brackets are comprised in the first group. These accessories are not chemically resistant when in contact with solvents and, under high temperatures, allow migration of monomers away from the original products[6].

It is important to point out that, once inside the mouth, any of these materials creates a dynamic interface whose interactions may cause changes, thus leading to either an active biological reaction to the material (i.e. biocompatibility) or degradation or corrosion of the material itself[10,11]. Therefore, the biocompatibility depends on the release of elements from these materials. In addition, composition, pretreatment, and manipulation of these apparatuses influence on the release of such elements[3,4].

Under these circumstances, it is of crucial importance to evaluate the cytotoxicity of polycarbonate brackets to be clinically used in Orthodontics in order to detect any possible harmful effect of the materials to the oral cavity.

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, Missouri, USA), 50 μg/mL of gentamicin (Schering Plough, Kenilworth, NJ, USA), 2.5 μg/mL of fungizone (Bristol-Myers-Squibb New York, NY, USA), 0.25 mm of sodium bicarbonate solution (Merck™, Darmstadt, Germany), 10 mm of HEPES (Sigma), and 10% of fetal bovine serum (FBS) (Cultilab), then being kept at 37°C in a 5%-CO_2 environment.

Orthodontic brackets

The sample consisted of polycarbonate brackets from two different manufacturers, which were divided into two groups: Group 1, composite bracket (Morelli, Sorocaba, São Paulo, Brazil) and Group 2, Silkon Plus bracket (American Orthodontics, Sheboygan, WI, USA).

Controls

To verify the cell response to extreme situations, other three groups were included in the study: Group C+ (positive control), consisting of amalgam cylinders; Group C- (negative control), consisting of glass rods in contact with the cells; Group CC (cell control), consisting of cells not exposed to any material.

Cytotoxicity assays

The materials were previously sterilized by exposing them to ultra-violet light (Labconco Corporation, Kansas City, MO, USA) for one hour. Next, three samples of each material were placed in 24-well plates containing Eagles' MEM (Cultilab). The culture medium was replaced with fresh medium every 24 hours, and the supernatants were collected after 24, 48, 72, and 168 hours (7 days) for analysis of the toxicity 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 hours a in 5%-CO_2 environment. After the incubation period, cell viability was determined using the "dye-uptake" technique described by Neyndorff et al.[12], but slightly modified. After the 24-hour incubation period, 100 μL of 0.01% neutral-red staining solution (Sigma) were added to the medium within each well of the plates, and these were incubated for 3 hours at 37°C to allow the dye to penetrate the living cells. After this period, the cells were fixed using 100 μL of 4% formaldehyde solution (Reagen, Rio de Janeiro, RJ, Brazil) in PBS (130 mM NaCl; 2 mM KCl; 6 mM Na_2HPO_4 $2H_2O$; 1 mM K_2HPO_4, pH = 7.2) for 5 minutes. Next, 100 μL of 1% acetic acid solution (Vetec, Rio de Janeiro, RJ, Brazil) with 50% methanol (Reagen) were added to the medium to remove the dye. Absorption was measured after 20 minutes by using a spectrophotometer (Biotek Instruments Inc., Winooski, VT, USA) at 492 nm wavelengths.

Statistical analysis

Statistical analyses were performed by using a SPSS version 13.0 software (SPSS Inc., Chicago, IL, USA), and means and standard deviations were calculated for descriptive statistical analysis. The values for the amount of viable cells were submitted to ANOVA and Tukey's test to determine whether statistically significant differences existed between the groups. Significance level was set at 5% for all analyses.

Results

The results of the cytotoxicity of polycarbonate orthodontic brackets are listed in **Table 1**.

Table 1. Statistical analysis with means and standard deviations for the studied groups

Groups	1st day		2nd day		3rd day		7th day	
	M. Cel./sd	Statistics	M. Cel./sd	Statistics	M. Cel./sd	Statistics	M. Cel./sd	Statistics
1	503.5 (50.05)	A	502.6 (113.7)	AC	328.4 (25.23)	A	958.7 (70.29)	A
2	496.4 (112.0)	A	457.4 (66.0)	A	284.7 (27.33)	B	922.3 (62.53)	A
c+	274.6 (62.21)	B	251.6 (50.23)	B	212.6 (37.8)	C	581.6 (62.74)	B
c-	539 (6.06)	A	513.3 (35.6)	AC	318 (26.84)	AB	951.6 (55.53)	A
cc	566 (21.3)	A	579 (126.2)	C	319.6 (18.86)	AB	1041.3 (81.03)	A

M. Cel.: mean values for the amount of viable cells; sd: standard deviation; Same letters indicate no statistically significant differences.

In the first day, no statistically significant differences were found between the experimental groups in relation to Positive Control and Cell Control Groups. On the second day, however, a statistically significant difference was observed between Group 2 and Cell Control Group. On the third day, Groups 1 and 2 differed significantly from each other, but there were no statistically significant differences between the Positive Control and Cell Control Groups. At the end of the experiment (seventh day), the brackets exhibited low cytotoxicity, with no statistically significant differences between them and between the control groups. Group C+ showed high level of cytotoxicity during the whole period of the study.

Discussion

The development and selection of biocompatible materials have been one of the greatest challenges in the area of health care. Toxic, inflammatory, allergic or mutagenic reactions are the possible biological responses to these materials, and cytotoxicity is one of the main parameters for biological evaluation. The goal of orthodontic treatment is to promote tooth movements by using a series of materials, including the brackets. These accessories, available in metallic, plastic, and ceramic compositions, are attached to tooth surface and, consequently, are in direct contact with oral tissues and saliva. Changes in the properties of these materials may cause harmful effects on the surrounding tissues, leading to the development of inflammatory processes. Based on these premises, the present study assessed the cytotoxicity of polycarbonate brackets, as they represent a great demand due to their esthetic appearance and low cost in comparison to ceramic brackets.

The method in which vital dye neutral red is used was employed to evaluate cell viability. Analysis of neutral red is a cell survival/viability essay 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 within the lysosomes (lysosomic pH < cytoplasmatic pH), where it combines with the anionic part of the lysosomic matrix[13]. The changes in either cell surface or lysosomic 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 in the cells is measured by spectrometry, being directly proportional to the number of cells with intact membrane.

This method was firstly used by Pithon et al.[14], who compared it to the agar diffusion method for evaluating the cytotoxicity of orthodontic materials and found that both methods provide adequate cytotoxic evaluation. The results obtained in the first, second, third days and at the end of the experiment are in accordance with the findings of Costa et al.[15], who assessed the cytotoxicity of stainless steel brackets with nickel in the composition.

Under these conditions, the polycarbonate brackets showed no toxic effects on cells for all study periods. This may have occurred due to the fact that only new brackets were evaluated, so none of them were exposed to chemical, thermal or mechanical agents, which, in turn, would release residual monomers, as previously suggested[6].

Although four experimental periods were used (24, 48, 72, and 168 hours), these periods are very short in comparison to the length of time in which the brackets remain within the oral cavity, 30 months on average. In face of the initial results herein obtained, further researches with longer experimental periods are necessary.

The evaluation of cytotoxicity is important to clarify the biological mechanism by which the cytotoxic effect is produced[16], as well as the action mechanism of different materials during the material-tissue interaction[17,18]. The presence of *in vitro* cytotoxic effect does not mean that the material is toxic for *in vivo* application. On the other hand, the absence of cytotoxic effect is guarantee of good clinical response[18]. Therefore, the results from *in vitro* studies should be judiciously compared to clinical outcomes as there is still controversy on this issue[19].

Based on the findings of the present study, it may be concluded that polycarbonate brackets were not cytotoxic within the experimental period of zero to seven days.

References

1. Jorge JH, Giampaolo ET, Pavarina AC. Cytotoxicity of the dental materials. A literature review. Rev Odontol UNESP 2004;33:65-8.
2. Phillips RW. Materiais Dentários. São Paulo: Elsevier; 2005.
3. Morais LS, Serra GS, Muller CA, Palermo EFA, Andrade LR, Meyers MA, et al. In vivo metal ion release from Ti-6AL-4V orthodontic mini-implants. Revista Matéria 2007;12:290-7.
4. Gioka C, Bourauel C, Hiskia A, Kletsas D, Eliades T, Eliades G. Light-cured or chemically cured orthodontic adhesive resins? A selection based on the degree of cure, monomer leaching, and cytotoxicity. Am J Orthod Dentofacial Orthop 2005;127:413-9; quiz 516.

5. Hanson M, Lobner D. In vitro neuronal cytotoxicity of latex and nonlatex orthodontic elastics. Am J Orthod Dentofacial Orthop 2004;126:65-70.
6. Howdeshell KL, Peterman PH, Judy BM, Taylor JA, Orazio CE, Ruhlen RL, et al. Bisphenol A is released from used polycarbonate animal cages into water at room temperature. Environ Health Perspect 2003;111:1180-7.
7. Siqueira Goncalves T, Minghelli Schmitt V, Thomas M, Lopes de Souza MA, Macedo de Menezes L. Cytotoxicity of two autopolymerized acrylic resins used in orthodontics. Angle Orthod 2008;78:926-30.
8. Kao CT, Ding SJ, Min Y, Hsu TC, Chou MY, Huang TH. The cytotoxicity of orthodontic metal bracket immersion media. Eur J Orthod 2007;29:198-203.
9. Anusavice KJ. Phillips, materiais dentários. 11ª ed. Rio de Janeiro: Elsevier; 2005.
10. Oh KT, Kim KN. Ion release and cytotoxicity of stainless steel wires. Eur J Orthod 2005;27:533-40.
11. Jonke E, Franz A, Freudenthaler J, Konig F, Bantleon HP, Schedle A. Cytotoxicity and shear bond strength of four orthodontic adhesive systems. Eur J Orthod 2008;30:495-502.
12. Neyndorff HC, Bartel DL, Tufaro F, Levy JG. Development of a model to demonstrate photosensitizer-mediated viral inactivation in blood. Transfusion 1990;30:485-90.
13. Griffon G, Marchal C, Merlin JL, Marchal S, Parache RM, Bey P. Radiosensitivity of multicellular tumour spheroids obtained from human ovarian cancers. Eur J Cancer 1995;31A:85-91.
14. Pithon MM, Santos RL, Ruellas ACO, Fidalgo TK, Romanos MTV, Mendes GS. Citotoxicidade in vitro de elásticos ortodônticos: comparação entre duas metodologias. Rev Saúde Com 2008;4:19-26.
15. Costa MT, Lenza MA, Gosch CS, Costa I, Ribeiro-Dias F. In vitro evaluation of corrosion and cytotoxicity of orthodontic brackets. J Dent Res 2007;86:441-5.
16. Schmalz G. Use of cell cultures for toxicity testing of dental materials-advantages and limitations. J Dentistry 1994;22:6-11.
17. Santos RL, Pithon MM, Oliveira MV, Mendes GS, Romanos MTV, Ruellas ACO. Cytotoxicity of introral orthodontic elastics. Braz J Oral Sci 2008;7:1520-5.
18. Pizzoferrato A, Ciapetti G, Stea S, Cenni E, Arciola CR, Granchi D, et al. Cell culture methods for testing biocompatibility. Clin Mater 1994;15:175-90.
19. Wennberg A, Mjor IA, Hersten-Pettersen A. Biological evaluation of dental restorative materials - a comparison of different test methods. J Biomed Mater Res 1983;11:23-36.