Cytotoxicity of temporary resins for orthodontic miniscrew covering

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Abstract

Introduction: The covering of orthodontic miniscrews (MS) with temporary resins can relieve the patient’s discomfort and prevent soft tissue injuries. The direct contact with these tissues for prolonged periods justifies the need of biocompatibility tests. The aim of this study was to evaluate the cytotoxicity of light-polymerized temporary fillings used for MS covering.

Methods: Two types of light curing temporary resins, Bioplic (Biodinâmica, Ibiporã, Paraná, Brazil) and Top Comfort (FGM, Joinville, Santa Catarina, Brazil) were assessed for their cytotoxicity in L929 fibroblastic cells. Four groups were analyzed: two experimental groups and two control groups (cell control and positive control). After the incubation period, the cells were examined in an inverted microscope (E600 Nikon Eclipse, Japan) and cell viability was determined using the dye uptake method. The optical density was measured with a spectrophotometer (BioTekTM, Winooski, Vermont, USA). Intergroup comparisons were performed with ANOVA and Tukey tests.

Results: Top comfort groups revealed to be more toxic at 48 h (0.488 ± 0.068), 72 (0.519 ± 0.101) and 7d (0.248 ± 0.102) (P<0.05).

Conclusions: The resins evaluated presented differences in the toxic activity. Top comfort presented an extended period of cytotoxicity until one week of the study.

Introduction

The use of orthodontic miniscrews (MS) is increasingly common in the contemporary treatment planning due to the need of an efficient and controlled movement of the teeth, especially in cases of patients with periodontal disease, deficient dentition and those who are not compliant with treatment demands [1-3]. The risks concerning the use of miniscrews includes complications during the insertion and removal procedures, problems in the loading process and soft tissue injury, such as aphthous ulceration, inflammation and infection [4].

The simple use of a wax pellet, separating elastic or a healing abutment can prevent the discomfort caused by traumatic lesions [4]. Recently, Marquezan et al. presented the covering of the miniscrew head with a temporary resin as an alternative to prevent these injuries (Figure 1) [5]. The direct contact with organic tissues for prolonged periods justifies the need of biocompatibility studies to investigate de safety of using the orthodontic appliances, [6,7] including metal alloys, [7-10] elastomeric ligatures [11,12] and resin-based materials [13,14].

The biocompatibility of resin composites is directly influenced by the amount and nature of organic substances released [15], derived from incomplete polymerization or degradation over the time [16,17]. Despite several studies have been carried out on orthodontic adhesives [18-20], there was little emphasis regarding the biocompatibility of temporary composites used for covering miniscrews. The aim of this study was to evaluate the cytotoxicity of light-polymerized temporary fillings used for orthodontic miniscrew covering.

Material and methods

Sample

Two types of light curing temporary resins, used for covering of orthodontic MS - Bioplic (Biodinâmica, Ibiporã, Paraná, Brazil) and Top Comfort (FGM, Joinville, Santa Catarina, Brazil) were assessed for their cytotoxicity in L929 fibroblastic cells. Four groups were analyzed: two experimental groups and two control groups (cell control and positive control). After the incubation period, the cells were examined in an inverted microscope (E600 Nikon Eclipse, Japan) and cell viability was determined using the dye uptake method. The optical density was measured with a spectrophotometer (BioTekTM, Winooski, Vermont, USA). Intergroup comparisons were performed with ANOVA and Tukey tests.

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Figure 1. Clinical use of temporary resins for miniscrew coverage. A: Bioplic; B: Top Comfort.
Top Comfort (FGM, Joinville, Santa Catarina, Brazil) (Table 1) - were selected for the assessment of their cytotoxicity.

The experimental groups, with three samples each, were divided based on the type of resin evaluated: group A – Bioplic and group B – Top comfort. As the test was carried out in triplicate, there were, in total, 9 values for each group. All the resins used in the study were in sealed packages and obtained from the same product lot.

**Sample preparation**

For the sample preparation, the materials used were previously sterilized in autoclave and with ultra-violet light exposure for 1 hour. The higher viscosity and consistency of the Bioplic resin enabled the removal of the material with a tooth sculpture tool. However, due to the fluid nature of the Top comfort resin, the material was transferred from the tip of the material tube to a tooth probe. This technique enabled the removal of similar quantities of both materials.

According to the manufacturer’s instructions, the photopolymerization (DB 685 – Dabi Atlante, Ribeirão Preto, SP, Brazil) of Bioplic lasted 40 seconds and Top Comfort 30 seconds. After the preparation, all the samples were transferred to a 24-well plate containing MEM for cytotoxic evaluation.

**Cell culture**

Mouse L929 fibroblasts lineage cells (American Type Culture Collection - ATCC, Rockville, MD, USA) were selected and cultivated in Eagle’s minimum essential medium (MEM) (Cultilab, Campinas, Brazil) with 2 mM of L-glutamine (Sigma, St.Louis, Missouri, USA), 50 µg/mL of gentamicin (Schering Plough, Kenilworth, New Jersey, USA), 2.5 µg/mL of fungizone (Bristol-Myers-Squib, New York, USA), 0.25 mM of sodium bicarbonate solution (Merck, Darmstadt, Germany), 10 mM of HEPES (Sigma, St. Louis, Missouri, USA), and 10% of foetal bovine serum (FBS) (Cultilab, Campinas, SP, Brazil). Then, the cell culture medium was incubated for 24 hours at 37°C in a 5% CO₂ atmosphere.

**Cytotoxicity assessment**

The 24-well plate MEM was replaced with fresh medium for every 24 hours and after 0, 24, 48, 72 hours, 7, 14 and 21 days the supernatants were collected in triplicate for the toxicity analysis to L929 cells. For each evaluation, the supernatants were transferred to 96-well plates with a single layer of L929 cells and maintained at 37°C for 24 hours in 5% CO₂ environment. In order to examine the cells reaction to extreme conditions, two extra groups were included: Group C (positive control) constituted of cells in contact with Tween and sodium fluoride.

Depending on the cytotoxicity level, the other groups presented the removal of similar quantities of both materials.

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**Statistical analysis**

Data were evaluated with the software SPSS (version 17, SPSS Inc., USA). The normality and homogeneity of variables were verified by Kolmogorov-Smirnov and Levene’s tests. Intergroup comparisons were performed by ANOVA/Tukey post-hoc tests. The level of significance was 5%.

**Results**

From the morphological evaluation of the extreme groups, group D showed the majority of spindle-shaped cells consistent with fibroblasts in normal development. However, in group C, the presence of rounded and granular cells indicated an environment of cellular apoptosis. Depending on the cytotoxicity level, the other groups presented the same tendency of the groups mentioned above.

**Table 1.** Composition of the resins tested with their respective manufacturers and manufacturing lot.

| Resin       | Manufacturer          | Composition                                                                 | Lot      |
|-------------|-----------------------|-----------------------------------------------------------------------------|----------|
| Bioplic     | Biodinâmica, Ibiporã, Parana, Brazil | bis-GMA [21], dimethacrylate groups (40%); organic filler (25.18%); silicium dioxide, catalysts and sodium fluoride. | 706/14   |
| Top comfort | FGM, Joinville, Santa Catarina, Brazil | Methacrylic monomers (as bis-EMA, TEGDMA and UDMA), stabilizer, camphorquimine, co-initiator, pigments and inorganic fillers (40%) of boro-aluminium-silicate and silica nanoparticulate. | 270813   |

Figure 2. Samples of Bioplic (A) and Top Comfort (B), obtained through different techniques, but presenting similar dimensions.
The results of quantitative intergroup comparisons for the experimental groups are given in Table 2. Significant differences were found between the experimental and control groups, for all of the times assessed (P<0.05). At 0 h, no group presented cytotoxic behavior, with the only exception for group C (positive control).

At 24h, group A (0.082 ± 0.007) exhibited severe toxicity and group B (0.356 ± 0.135) a moderate toxic activity (cell control: 0.703 ± 0.053).

The Bioplic group (A) presented a satisfactory percentage of cell viability from 48 h to 21d period. However, Top comfort group (B) revealed to be more toxic at 48 h (0.480 ± 0.068), 72 h (0.519 ± 0.101) and 7d (0.248 ± 0.102) (P<0.05). At 14 and 21 days, both of the groups presented moderate percentage of cell viability with the exception of the group C (positive control).

**Discussion**

The biocompatibility assessment of temporary resins for orthodontic MS covering is justified by the proximity of these materials to the periodontal and soft tissues, such as gingiva and oral mucosa. The potential releasing of substances may induce to an inflammation process by the presence of water-soluble components into the oral cavity and the direct interaction with surrounding tissues [23]. Despite the fact that and in vitro toxic activity cannot imply the same for an in vivo application, the lack of cytotoxicity can support the clinical safety as it is used clinically. Considering that the levels of biocompatibility of both of resins were acceptable, but not ideal, further studies using as it is prolonged cytotoxic effect at 48 h, 72 h and 7 days, may be explained by the presence of TEGDMA in its matrix. It should be highlighted that as the details regarding the quantity of monomers, initiators and size of inorganic fillers were not supplied by both manufacturers, we were limited to establish any cause and effect relationship.

At 0 h (Zero) the levels of cell viability above the cell control were observed. These findings were related to the exposure of cells with a high number of passages to non-toxic concentrations of some metals such as titanium, aluminum and vanadium, which are the main components of orthodontic miniscrews [31,32]. All of the groups presented lower levels of cytotoxicity from the 14th day of the study, which were considered to be acceptable, despite the significant differences from the cell control. The present study identified potential toxic activity for Top comfort resin that remained until one week of experiment suggesting further evaluation of the long-term effects of this material at low quantities, as it is used clinically. Considering that the levels of biocompatibility of both of resins were acceptable, but not ideal, further studies using different methodological assays for both of resins are needed.

**Conclusion**

- A severe toxic activity was observed by the Bioplic group and a moderate one by the Top comfort group at 24 hours of the study.
- Top comfort group presented an extended period of cytotoxicity for 48 h, 72 h and 7 days.
- From the 14th day of the study both of the resins presented reasonable values of cell viability.

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Table 2. Statistical analysis with mean, standard deviation and cell viability values of the experimental groups (*SD indicates standard deviation **Different letters indicate statistical differences at p<0.05).

| Groups     | Mean   | SD      | Cell viability (%) | Mean   | SD      | Cell viability (%) | Mean   | SD      | Cell viability (%) | Mean   | SD      | Cell viability (%) |
|------------|--------|---------|--------------------|--------|---------|--------------------|--------|---------|--------------------|--------|---------|--------------------|
|            | 0 h    | 24 h    | 48 h               | 72 h   |
| A          | 0.393  | 0.031   | 110.01             | 0.082  | 0.007   | 11.66              | 0.589  | 0.035   | 87.91              | 0.659  | 0.122   | 80.66              |
| B          | 0.425  | 0.039   | 120.05             | 0.356  | 0.135   | 50.64              | 0.488  | 0.068   | 72.83              | 0.519  | 0.101   | 63.52              |
| C          | 0.072  | 0.010   | 20.33              | 0.132  | 0.002   | 18.77              | 0.073  | 0.008   | 10.89              | 0.111  | 0.002   | 13.5               |
| D          | 0.354  | 0.028   | 100.00             | 0.703  | 0.053   | 100.00             | 0.670  | 0.090   | 100.00             | 0.817  | 0.030   | 100.00             |

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anchorage during orthodontic brace treatment with implants or other surgical methods. 

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