Cytotoxic outcomes of orthodontic bands with and without silver solder in different cell lineages

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Introduction: The safety of orthodontic materials is a matter of high interest. In this study, we aimed to assess the in-vitro cytotoxicity of orthodontic band extracts, with and without silver solder, by comparing the viability outcomes of the HaCat keratinocytes, the fibroblastic cell lineages HGF and MRC-5, and the kidney epithelial Vero cells. **Methods:** Sterilized orthodontic bands with and without silver solder joints were added to culture media (6 cm^2/mL) and incubated for 24 hours at 37°C under continuous agitation. Subsequently, the cell cultures were exposed to the obtained extracts for 24 hours, and an assay was performed to evaluate the cell viability. Copper strip extracts were used as positive control devices. **Results:** The extracts from orthodontic bands with silver solder joints significantly reduced the viability of the HaCat, MRC-5, and Vero cell lines, whereas the viability of HGF was not altered by this material. Conversely, the extracts of orthodontic bands without silver solder did not significantly modify the viability index of all evaluated cell lines. **Conclusions:** Except for HGF fibroblasts, all tested cell lines showed decreased viability percentages after exposure to extracts of orthodontic bands containing silver solder joints. These data show the relevance of testing the toxicity of orthodontic devices in different cell lines. (Am J Orthod Dentofacial Orthop 2017;151:957-63)

Cytotoxicity is secondary to cellular function alterations, which are mainly related to changes in metabolic pathways or intracellular processes.[1] When choosing a dental material, it is important to have a substantial knowledge of its composition, its allergenic properties, and its toxic effects.[2] Nevertheless, there are great concerns regarding the biocompatibility of orthodontic materials and the ions related to their potential toxicity. The biocompatibility of orthodontic materials is a critical issue because of the long-term contact with the oral mucosa and the potential corrosion of different materials.

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The use of orthodontic appliances has increased over the past years. Bishara[3] published a case report that showed an association between an oral lesion and an orthodontic retainer containing silver solder. This led to much research and discussion over orthodontic material biocompatibility. A regular orthodontic treatment lasts for approximately 24 months, but appliances used in the mixed dentition can remain in place for many years. Orthodontic bands are largely used, especially when auxiliary appliances are required. Silver solder is frequently necessary to connect the orthodontic bands to wires in both fixed and removable appliances. Maxillary expanders are often used for as long as 6 months and may be used for longer periods when associated with facemasks. Lingual arches, used as space maintainers, can remain in the oral cavity for several years, especially during the development of the permanent dentition. Ideally, when considering the potential toxic effects, the biocompatibility of orthodontic materials should be evaluated before any clinical use.[4]

Host response can be influenced by corrosion parameters, ion release profiles, and metal ion toxicity.[5] Saliva can act as a corrosive agent of orthodontic appliances.[6] When compared with stainless steel, silver solder shows greater corrosion, which is the possible cause of cytotoxic effects.[2] Silver solder coupled with a

Table. Cell lines data[12,13]

Cell line	*HaCaT*	*HGF*	*MRC-5*	*Vero*
Organism	Homo sapiens, human	Homo sapiens, human	Homo sapiens, human	Cercopithecus aethiops, African green monkey
Tissue	Skin	Mouth	Lung	Kidney
Cell type	Keratinocyte	Fibroblast	Fibroblast	Epithelial
Properties	Adherent	Adherent	Adherent	Adherent
Disease	Normal	Normal	Normal	Normal
Age	62 years	Not specified	14 weeks	Adult
Sex	Male	Female	Male	Not specified
Ethnicity	White	Not specified	White	Not specified

high-resistance nickel-chromium alloy shows corrosion when exposed to a saline solution of 0.9% sodium chloride.[7] In a cell culture model, Pianigiani et al[8] evaluated the biocompatibility of metallic dental materials. Oxidation was observed on the surface of the soldering material, suggesting the release of metal ions.

In-vitro assays provide relevant data when assessing the biocompatibility of dental materials, and it is crucial to understand the variables affecting their outcomes. The chosen cell line, the cell density, and the passage number are critical variables for in-vitro studies.[9] Even under identical culture conditions, different cell lines can show different levels of cytotoxicity.

In this study, we aimed to investigate the in-vitro cytotoxicity of orthodontic bands with and without silver solder and to compare the viability outcomes of HaCat, HGF, MRC-5, and Vero cell lines exposed to orthodontic band extracts. Our data showed different profiles of cytotoxicity after exposure to orthodontic materials, depending on the tested cell line.

MATERIAL AND METHODS

This in-vitro cytotoxicity study was approved by the ethics committee of the Pontifical Catholic University of Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil. The experiments were performed following the standards of the International Organization for Standardization (10993-5 and 10993-12).[10,11] The chosen cell lines are shown in the Table.

All cell lines were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Grand Island, NY), supplemented with 1% penicillin/streptomycin (Gibco), 0.1% fungizone (Gibco), and 10% fetal bovine serum (Gibco). The cells were incubated in 75-cm^2 flasks at 37°C, with a minimum relative humidity of 95% and an atmosphere of 5% carbon dioxide. The culture medium was replaced every 48 hours, and trypsin (Sigma-Aldrich, St Louis, Mo) was used to detach the cells when 80% confluence was reached.

Tests were performed with extracts of stainless steel orthodontic bands (36 mm; Morelli, Sorocaba, São Paulo, Brazil) with and without silver solder joints. Pure copper strips were used as the positive control. The stainless steel orthodontic bands were composed, according to the manufacturer, of 8% to 12% nickel and 18% to 20% chromium and iron (balance). For the orthodontic bands with silver solder joints, a segment of a stainless steel 1.0-mm diameter wire composed of 8% to 10% nickel and 17% to 19% chromium and iron (Morelli) was used; this was soldered onto an orthodontic band using a 10-cm silver solder alloy composed of 55% to 57% silver, 21% to 23% copper, 15% to 19% zinc, and 4% to 6% tin (Morelli), together with 20 mg of silver solder flux composed of boric acid, potassium bifluoride, potassium hydroxide, and water (Morelli). Carborundum discs were used to remove the excess wire, and polishing was accomplished with polishing discs (Dhpro, Paranaguá, Paraná, Brazil).

The orthodontic bands and copper strips were autoclaved before the sample's preparation. DMEM supplemented with 10% fetal bovine serum was used as the extraction vehicle because of its ability to support cellular growth and to extract polar and nonpolar substances. A ratio (surface area of the bands × volume of culture medium) of 6 cm^2 per milliliter was used.

Extractions were carried out in 4 microtubes (each, 1.5 mL) filled with DMEM supplemented with 10% fetal bovine serum containing the stainless steel orthodontic bands, the stainless steel orthodontic bands with silver solder joints, or the copper strips. The microtubes were kept at 37°C under agitation in a shaker (180 rpm) for 24 hours.

To determine cell viability, 20 μL of cell suspension was mixed with 20 μL of Trypan Blue, and the cells were counted with a Neubauer Chamber on an inverted microscope (Olympus Model CH30RF100; Olympus Optical, Shinjuku-ku, Tokyo, Japan). Approximately 7000 cells per well were seeded in 96-well plates, and 100 μL of DMEM was added. After a 24-hour incubation period, the medium was removed, and the cells were exposed to the culture medium containing extracts of the orthodontic bands with and without silver solder or to the extracts of the pure copper strips for additional 24 hours. The negative control was carried out by