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ORIGINAL ARTICLE

Genotoxicity and cytotoxicity induced by eluates from orthodontic glass ionomer cements in vitro

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Abstract The aim of this study was to investigate genotoxicity and cytotoxicity of some orthodontic glass ionomer cements commercially available by means of the single cell gel (comet) assay. For this purpose, five commercial orthodontic glass ionomer cements (Vidrion C®, Meron®, Optiband®, Multicure® and Ultra Band Lok®) were tested in murine fibroblasts in vitro. For this purpose, eluates from each cement were prepared according manufactures instructions at 0, 2, 4, 8, 18, 32 and 64 days of immersion in artificial saliva at 37 °C. All orthodontic glass ionomer cements failed to induce cytotoxicity to murine fibroblasts for all periods evaluated in this study. However, Vidrion C® was able to induce genotoxicity after 64 days of exposure to eluates. Meron® also demonstrated genotoxicity as depicted by increasing DNA damage on 2nd day. Multicure® demonstrated genotoxicity on 32nd day and Ultra band Lok on 18th, 32nd days of exposure. Taken together, our results demonstrated that orthodontic cements derived from resin-modified glass ionomer composite (Multicure®) and compomer (Ultra Band Lok®) cause genetic damage in mammalian cells in vitro.

1. Introduction

Nowadays, orthodontic therapy is increasing around the world as a result of establishment masticatory function and/or esthetic purposes. Herein, orthodontists have routinely used glass ionomer cements with success for bonding brackets. In order to facilitate its manipulation as far as to increase its immediate tensile strength, resin-modified glass ionomer cements and compomers have been incorporated to clinical usage, by incorporation of 2-hydroxyethyl methacrylate (HEMA), triethylene glycol dimethacrylate (TEGMA), urethane dimethacrylate (UDMA) and bisphenol A glicidyl

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methacrylate (BisGMA) (Reichl et al., 2006a,b). Taking into consideration that these dental materials are in close contact with oral tissues over long periods of time, it is mandatory to evaluate the behavior on biological test systems to ensure protection either to patients or to clinicians (Ribeiro et al., 2006a,b).

Biocompatibility is the ability of some material to perform with an appropriate host response when applied to biological tissues. To date, there are many experimental models able to evaluate the biocompatibility of materials used in dental and/or medical practices using different methodologies and end-points so far. To evaluate genotoxicity and cytotoxicity are particularly relevant biologically, because they are closely related to the initiation phase of carcinogenesis process, due to cell cycle proliferation stimulus, or an error of mitotic phase secondary to cell deoxyribonucleic acid (DNA) damage with subsequent repair capacity impaired (Bull et al., 2006). For this reason, it would important to investigate if, and to what extent, some glass ionomer cements used in orthodontics induce genetic damage and cellular death in mammalian cells.

The single-cell gel (comet) assay in alkaline version was designed as a rapid, simple and reliable biochemical technique for evaluating DNA strand breaks in mammalian cells (McKelvey-Martin et al., 1993). Some advantages of the single cell gel (comet) assay have been elected when compared to other genotoxicity assays because it is cheap and with reproducible results (Brendler-Schwaab et al., 2006). The basic principle of the single-cell gel (comet) assay resides on the migration of DNA fragments as a result of double strand breaks, single strand breaks, adducts and incomplete repair sites through agarose matrix under electrophoresis conditions. Nucleoids look like comets with a head (the nuclear region) and a tail containing DNA fragments by conventional light microscopy (Tice et al., 2000). Previous studies conducted by our research group have performed the single cell gel (comet) assay under different protocols and paradigms (Angelieri et al., 2011; Da Silva et al., 2007; Braz et al., 2006; Guilheiro et al., 2014). Therefore, the methodology is useful for assessing DNA damage in mammalian cells.

Accumulating evidence suggests that cell membrane integrity is a suitable biological phenomenon for distinguishing dead from live cells (Kroemer et al., 2009). The trypan blue method is a suitable assay for evaluating cytotoxicity in experimental investigations (Zanatta et al., 2012) including biocompatibility tests. The rationale of this methodology resides on the fact that dead cells incorporates trypan blue dye into the cytoplasm as a result of loss of membrane selectivity, whereas live cells remain unstained (Tennant, 1964).

The aim of this study was to evaluate genotoxicity and cytotoxicity induced by orthodontic glass ionomer cements by single cell gel (comet) assay and trypan blue exclusion test in vitro. Certainly, such data will contribute for a better understanding the biological behavior of these compounds on eukaryotic cells.

2. Materials and methods

2.1. Cell Culture

Murine fibroblast cells (lineage 3 T3-L1) were obtained from the American Type Culture Collection and cultured at 37 °C in a humidified atmosphere of 5% CO_2/95% air. The cells were maintained in a growth medium containing the following constituents: Dulbecco's modified Eagle's medium (Invitrogen Corp., Grand Island, NY, USA) with 25 mmol/L glucose, 1 mmol/L pyruvate, 4.02 mmol/L L-alanyl-glutamine and 10% fetal calf serum (Sigma Aldrich, St. Louis, MO, USA). Confluent cells were detached with 0.15% trypsin (Invitrogen Corp.) for 5 min. After that, 2 mL of complete medium was added and the cells were centrifuged at 1000 rpm (180*g*) for 5 min. Cell suspension was counted using a Neubauer chamber and seeded in 96-well microtitre plates (Corning Glass, Corning, NY, USA) at a density of 1×10^4 cells per well (at a concentration of 1×106/mL).

2.2. Cell treatment

Two specimens (6 mm × 2 mm) of each cement were made according to manufactures' instructions. The following orthodontic cements were used in this study: two conventional glass ionomer cements (Vidrion C®, SS White, Brazil and Meron®, VOCO, Germany), two resin-modified glass ionomer cements (Optiband®, Ormco, USA and Multicure®, 3 M Unitek, USA) and a compomer (Ultra Band Lok®, Reliance Orthodontics, USA). The resin-modified glass ionomer cement and cement were light cured for 40 s.

Each specimen was individually immersed in 7 ml of artificial saliva at 37 °C for a period up to 64 days. One milliliter of each eluate was extracted at 0, 2, 4, 8, 18, 32 and 64 days. After that, each eluate obtained from experimental periods established in this setting were exposed to murine fibroblasts for 1 h at 37 °C in triplicate as described elsewhere (Angelieri et al., 2011).

The negative control group was treated with vehicle control (artificial saliva), during 1 h at 37 °C. For the positive control group, both cells were exposed to methyl methanesulfonate (MMS, Sigma Aldrich) at 1 μmol/mL during 3 min at 37 °C. Each treatment was performed consecutively three times to ensure reproducibility. The protocol was established in a previous study conducted by our research group (Angelieri et al., 2011; Matsumoto et al., 2014).

2.3. Trypan blue exclusion test

After completing the experimental periods, murine fibroblasts cells from all groups (1×105 mL cells) were incubated to 90 μL 0.4% trypan blue solution diluted in PBS for 5 min. Neubauer chamber was used to measure the total number of death cells.

2.4. Single cell gel (comet) assay

For genotoxicity assay (Tice et al., 2000), a volume of 10 μl of murine fibroblasts cells ($\sim 1 \times 10^4$ cells) for all experimental groups was added to 120 mL of 0.5% low-melting-point agarose at 37 °C, layered onto a pre-coated slide with 1.5% regular agarose, and covered with a coverslip (slides were prepared in duplicate per treatment). After brief agarose solidification in a refrigerator, the coverslip was removed and the slides were immersed in the lysis solution (2.5 M NaCl, 100 mM ethylenediaminetetraacetic acid [EDTA], 10 mM Tris-HCl buffer pH = 10, 1% sodium sarcosinate with 1% Triton X-100 and 10% DMSO) for 1 h. Prior to electrophoresis, the slides were

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