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Original Research Article

Nuclear anomalies in the buccal cells of children under dental treatment

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Abstract

Introduction: Since some dental materials may be aggressive to a person's body, studies involving such materials seem to be necessary. **Objective:** This study was conducted to evaluate the genotoxicity of dental materials through micronucleus (MN) test. **Material and methods:** Exfoliated buccal cells of 4-to-12 year-old children, who were on some type of dental treatment, were collected either before or after the treatment ending. Each sample was composed of 1,000 cells per patient. Student's t test was used for comparison. **Results:** The dental materials were divided into 3 groups, as follows: cement, monomers, and their combination. Treatments using monomer + cement-based materials were found to increase significantly the number of binucleated (BN) cells, ($p < 0.05$) which indicate several degenerative nuclear changes. **Conclusion:** The combination of cement-based dental material with monomers increases the cytotoxic action of dental materials.

Introduction

Dental materials may produce aggressive effects caused by monomers release and/or other organic and inorganic components [31]. As most dental materials release small portions of several substances on both the pulp and oral cavity, it is essential to prove their biocompatibility and toxicological profile. Appropriate regulation should ensure that the genotoxicity be either abolished or decreased [17].

The main dental materials used in pediatric dental treatment are: glass ionomer cements (GIC), zinc oxide - eugenol (ZOE), and resin composites (RC). GIC maintains the surrounding environment suitable for remineralization as well as interferes with the bacteria metabolism by fluoride releasing [2, 26]. They are employed as pulp capping, occlusal sealant, and restorative material. Also, hydrophilic monomers can be incorporated to GIC, so-called resin-modified glass ionomers (RMGICs). The polymerization reactions make monomers into polymers. Consequently, the residual monomers released may cause intense cytotoxic effects [9, 34]. ZOE is often used in low-cost, easy-dealing temporary restoration [7]. Its effects can vary, as follows: point mutation inducer [27]; cytotoxicity [18, 27]; antimicrobial [6, 31]. RC is commonly used in permanent restoration. It presents less abrasion, offering easy handling. It also can be found in different colors to match to the tooth to be restored [3]. These materials may have some genotoxic potential. An increasing number of dental restorations have been performed over the past decade with significantly increase in local and systemic adverse effects such as cytogenetic changes [29].

Chromosome damages are widely used as biomarkers in monitoring human exposure to carcinogenic agents [8, 20, 36]. Binucleated (BN) cells may indicate several kinds of degenerative nuclear changes [10]. BN are cells which have two similar-sized nuclei (almost the same size). Such nuclei are not overplaced, but may be side by side. They have the same color, an intact nuclear membrane, and are within the cytoplasm [14]. Micronuclei (MN) are free round or egg-shaped corpuscles, about 1/3 to 1/16 of the nucleus size [4, 5, 25]. They are usually found beside the main nucleus and are similar in shape, color, and chromatinic body distribution [14]. Such structures are a result of chromosome fragments or entire acentric chromosomes which are lost during a cell division. For this reason they are not included in the daughter cells' nuclei, thus remaining in the cytoplasm of interphase cells [5, 10, 16, 35]. MN takes 7-16 or 30 days to be formed [6, 28, 33, 37, 38]. Such period is related to the amount of time that the basal cells take to reach the surface and exfoliate.

It is extremely important to know how the materials act in human tissues because they are in close contact with oral mucosa [30]. This study aims to evaluate the genotoxicity of dental materials used in pediatric treatment through micronucleus (MN) test of exfoliated buccal cells.

Material and methods

Study Design

The study was approved by the Ethics Committee of Unilavras (CAAE, 0036/06). The sample comprised 72 children divided into control group (n = 40) and test group (n = 32). Inclusion criteria during this study were as follows: children who had never undergone any dental treatment; children aged 2-12 years (mean age of five years). There were no children with neurological diseases and related genetic alterations. Their participation in the research was previously authorized by their parents or children's legal guardians.

Exclusion criteria comprised children with damages in oral mucosa that preclude the collection of cells and whose parents had not signed the clarified consent form or did not want to participate in the study.

Test group patients were divided into 3 experimental subgroups according to the dental material used, as follows: Group I - Cement-type materials: glass ionomer cement and zinc oxide cement + eugenol (n = 8); Group II - Monomer-based materials: composite resin, dental sealant, and adhesives (n = 13); Group III: combination of both (n = 11). The genetic material used was obtained by collecting oral mucosa cells by scrubbing the surface with a wood stick. The first collection was done before the treatment in order to find out the basal index of both micronucleus and binucleus cells likely to be found in the mucosa.

One month after the treatment, a new sample collection was performed in the treated patients when they returned for either a follow-up or new treatment appointment This time period was chosen because is related to the mitoses that the altered cells would suffer, thus showing clearly their micronucleus formation. The control group consisted of patients' oral mucosa cells before the insertion of any type of dental material.

Genotoxicity evaluation (micronucleus test)

After the inner part of the cheek was scrubbed by using a wood stick [10, 38], the epithelial cells collected from buccal mucosa were smeared onto clean microscope glass slides fixed in 70% alcohol, air dried, and stained with 2% acetic orcein [15], for 30' [23]. A light microscope at 100 X magnification on coded slides was used for MN analysis and then microphotographed (Samsung SDC-312). Two thousand cells per patient were analyzed, as follows: 1,000 before and 1,000 after the treatment [19, 24]. After both the micronucleus and the binucleated cells were quantified, the frequency analysis of both alterations was performed only in the non fragmented, overplaced or overcrowded cells with untouched nuclei, according to the acceptance criteria, as described by Fenech *et al.* (2000). Data statistic analysis was performed by Student's t test [19] for non-parametric data.

Results and discussion

The epithelial tissue of the oral cavity was collected for the test, because it is in close contact with dental materials and is constantly renewed [1]. In addition to this, it can act as a tool for biomonitoring human populations exposed to genotoxic agents [12, 22].

The MN average frequency in a healthy population is about 1 to 3 out of 1,000 cells [12]. However, in our study, nearly 1% of the control patients exhibited MN score of 7 to 8 before treatment (figure 1). This suggests that those children had probably been influenced by genotoxic agents [1, 22, 31].

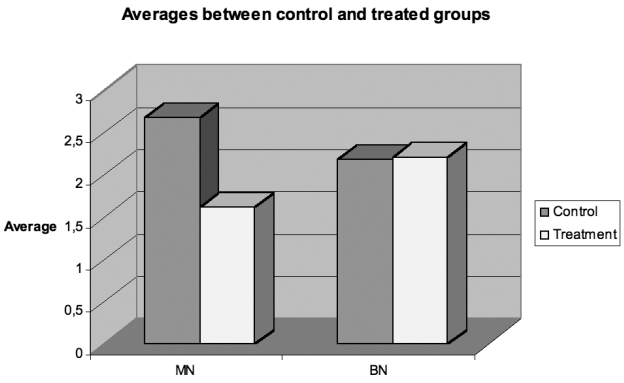

Figure 1 – Mean values of BN and MN average in control and experimental groups

Normal, binucleated, and micronucleated cells were found in all studied groups. The binucleated cells (figure 2) presented two nuclei, nearly the same size instead of overplaced. They showed the same color, their nuclear membrane was intact and within the same cytoplasm, as described by Fenech *et al.* (2003). On the other hand, the nucleus of the micronucleated cells (figure 3) was shorter than the main nucleus diameter, round or oval in shape, separated from the main nucleus [14]. Diler and Ergene [11] analyzed nuclear anomalies in buccal cells of calcite factory workers and found micronucleus and binucleated cells suggesting significant cytogenetic damage.

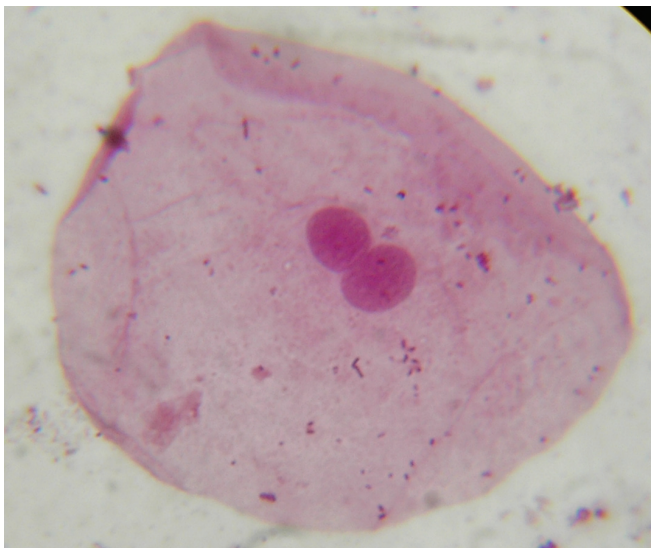

Figure 2 – Binucleated cell

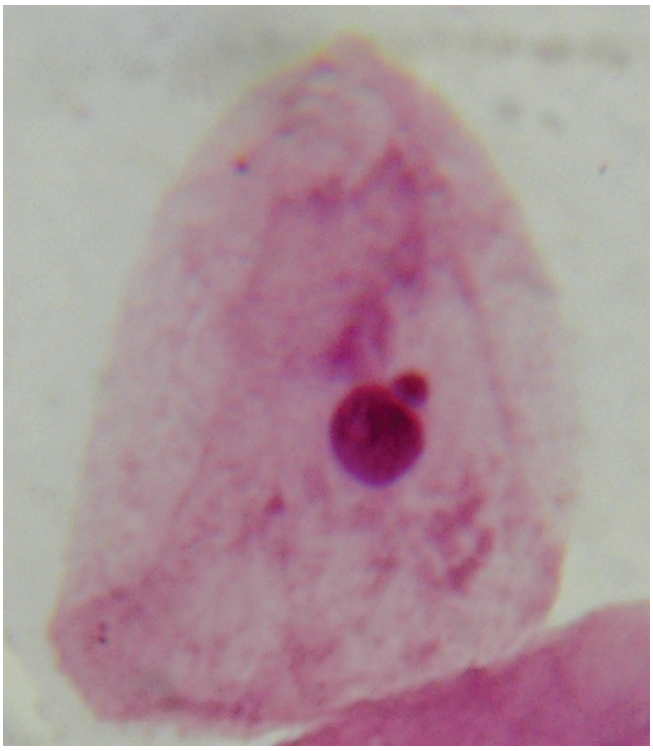

Figure 3 – Micronucleated cell

The cement materials did not caused decrease and/or increase of BN or MN cells in Group I patients (table I). The treatment with monomers materials (Group II) did not significantly alter the frequency of MN, because pre- and post-treatment MN frequency score was similar (table I).

It is important to consider that both MNs and BNs have a time-determined formation. The dental material constantly releases small portions of its own substances in the oral cavity [17], especially during the first 30 days after its insertion [2]. Therefore, the time period of this study was enough for all the possible variations appear.

The increase of the frequency of BN for Group III was significantly influenced by the cement + monomer combination (table I). Similarly results were found in people working in oil and oil-products stations [11] and in between smoking and exposure to benzene workers [12].

Many materials, particularly root canal sealers, remain in contact with vital tissues for a long period, when cellular aggression by chemical, physical and mechanical elements may occur, being the respiratory system one of the first cells to be affected. Some aggressor agents, particularly chemical substances, block important enzyme systems of the protein synthesis and/or the generation of ATP; others lead to the generation of harmful intracellular products or, still, act directly destroying vital structural components of the cell [30]. This may justify the appearance of binucleated cells (Group III). Senne *et al*. (2009) analyzed zinc oxide-eugenol cements and resin, finding that all tested sealers were cytotoxic. Reis *et al*. [24] studied the genotoxic effect of ethanol on oral mucosa cells and observed that the frequency means of micronucleated cells and micronuclei were significantly higher in the group of exposed individuals, when compared to the control group.

Table I – Evaluation of BN and MN in Groups I, II and III

Group	BN		MN	
	Mean ± SE	t Test 5%	Mean ± SE	t Test 5%
I – Treatment	2.180 ± 0.714	0.0922 *(NS)*	1.330 ± 0.122	0.8928 *(NS)*
I – Control	1.355 ± 0.760		1.299 ± 0.356	
II – Treatment	2.019 ± 0.375	0.0531 *(NS)*	1.696 ± 0.674	0.7913 *(NS)*
II – Control	1.441 ± 0.541		1.790 ± 0.486	
III – Treatment	2.413 ± 0.644	0.0069 *(S)*	1.821 ± 0.504	0.9371 *(NS)*
III – Control	1.588 ± 0.177		1.791 ± 0.722	

(NS) Not significant
(S) Significant. The treatment values were significantly different from the control at $p < 0.05$
(SE) Standard Error

Celik *et al*. (2003) and Fenech *et al*. (1999) state that binucleated cells indicate cytotoxicity. The resin-modified glass ionomer cements (RMGIC) have hydrophilic monomers incorporated to the glass ionomer cement. When RMGICs are subpolymerized, they convert monomers into polymers. In such case

the released residual monomers may produce an intense cytotoxic effect [34]. The RMGIC cytotoxic effect was noticed in MDPC-23 odontoblastic cells culture [9]. When combined, the chemical composition of cements + monomers becomes similar to that of RMGIC. For this reason, group III materials were found to be related to cytotoxic damages. Such results were reached by comparing the number of BNs found either before or after the exposure to dental material. Although BNs are not directly involved with DNA, they interfere with the late events occurring in cell division [11, 39]. Because their consequences are still unknown, further cytogenetic studies regarding to the perpetuation of such cells in the oral mucosa are necessary.

In order to analyze genotoxicity or cytotoxicity in lymphocyte culture, the use of cytochalasin B is needed. This substance paralyses the cytokinesis, which promotes the nucleus division, resulting in a two-nucleus cell [13, 41]. The cement + monomer combination used in this study showed significant result for BN, suggesting a direct influence on cytokinesis similarly to cytochalasin B. Its action can be explained by the inhibition of telophase, consequently the cell reaches the epithelial surfaces with two nuclei. Because the action of the evaluated substances interferes directly into the cell instead of the gene, a cytogenetic damage occurs [11].

Biological markers may express the dose amount of exposure to carcinogens and their interaction with macromolecules, as DNA [24]. Consequently, a greater emphasis should be given to methods that detect the genotoxic human activity. The biomarkers may be used for the prevention of serious diseases, and detection of high-risk patients.

Conclusion

It can be concluded that a significant BN increase was noticed when Group III materials were used, suggesting that the combination of a cement-based dental material with monomers intensify the cytotoxic action. The combination of MN analysis with other nuclear anomalies, such as BN, was found to enhance the sensitiveness as well as the evaluation of both cytotoxicity and genotoxicity in scrubbed cells of the exfoliated buccal mucosa.

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