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Effect of curing regime on the cytotoxicity of resin-modified glass-ionomer lining cements applied to an odontoblast-cell line

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ABSTRACT

Objective. The aim of this in vitro study was to evaluate the cytotoxicity of resin-modified glass-ionomer lining cements submitted to different curing regimes and applied to an immortalized odontoblast-cell line (MDPC-23).

Methods. Forty round-shaped specimens of each experimental material (Fuji Lining LC and Vitrebond) were prepared. They were light-cured for the manufacturers' recommended time (MRT = 30 s), under-cured (0.5 MRT = 15 s), over-cured (1.5 MRT = 45 s) or allowed to dark cure (0 MRT). Sterilized filter papers soaked with either 5 μL of PBS or HEMA were used as negative and positive control, respectively. After placing the specimens individually in wells of 24-well dishes, odontoblast-like cells MDPC-23 (30,000 cells/cm^2) were plated in each well and incubated for 72 h in a humidified incubator at 37 °C with 5% CO_2 and 95% air. The cytotoxicity was evaluated by the cell metabolism (MTT assay) and cell morphology (SEM).

Results. Fuji Lining LC was less cytotoxic than Vitrebond ($p < 0.05$) in all the experimental conditions. However, the cytotoxicity of Fuji Lining LC was noticeably increased in the absence of light-curing while the same was not observed for Vitrebond. The length of light-curing (15, 30 or 45 s) did not influence the toxicity of both lining materials when they were applied on the odontoblast-cell line MDPC-23.

Significance. The light-activation plays an important role in reducing the cytotoxicity of Fuji Lining LC. Following the manufacturer' recommendation regarding the light-curing regime may prevent toxic effect to the pulp cells.

1. Introduction

Glass-ionomer cements (GICs) represent a category of bioactive dental materials which were introduced in the early 70s. Further improvement in the field of GICs led to the development of a light-cured hybrid GIC version, so called resin-modified glass-ionomer cements (RMGICs) [1]. The improved mechanical properties of RMGICs when compared to the conventional GICs have been attributed to the dual-curing system in those cements [2]. The incorporation of polimerizable water-compatible monomers such as 2-hydroxyethyl methacrylate (HEMA) to the formulation of conventional GICs

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resulted in enhanced flexural strength, diametral tensile strength, elastic modulus and wear resistance [2]. The setting mechanism of RMGICs consists of two main reactions: (1) a free-radical polymerization of the monomeric components resulting in a polymeric backbone; and (2) the classic acid–base reaction that initiates upon mixing of the cement and continues even after light-activation resulting in a polysalt matrix [3].

The incorporation of HEMA to the formulation of conventional cements has been proven to increase their toxic effects [4] and as a consequence, RMGICs have been regarded as more cytotoxic than conventional GICs [5–8]. Although the degree of monomer to polymer conversion of the RMGICs has not been determined [9], several studies have demonstrated that measurable quantities of HEMA are released into the storage solutions used [4,9–11]. Leached residual HEMA can easily diffuse through the dentinal tubules due to its hydrophilicity and low molecular weight, and reach dental pulp cells [4,11]. The magnitude of the damage that may be caused by residual monomers to the pulp cells is inversely proportional to the remaining dentin thickness between the cavity floor and the pulp tissue [12].

Although a true RMGIC must be capable of setting without being light-activated [13], higher levels of released HEMA are found when these cements are only allowed to cure chemically [9]. In contrast to studies which have been focused on fluoride release, little data is available concerning the release of organic substances from RMGICs and the influence of the time of light-activation on the cytotoxicity of these materials [4,9–11].

According to the recommended methodology to evaluate the cytotoxic effects of dental materials, cell culture tests have frequently been used [4,5,8,14,15] although there is no consensus regarding the target cell type. It has been described that following application of resin-based materials on deep cavities, residual components may diffuse through the dentinal tubules to reach a monolayer of odontoblast-cells which underlie the dentin substrate [16]. Consequently, it seems reasonable to evaluate the cytotoxic effects of dental materials and their components on the culture of odontoblasts, since the primary toxic effects of the diffusate in vivo occurs on this specific type of cell. Therefore, the odontoblast-cell line MDPC-23 (Mouse Dental Papillae Cell) which was established a few years ago [17] has actually been used to perform in vitro cytotoxic tests on dental materials [5].

Regarding the importance of the resin monomer to polymer conversion in the RMGICs and the toxic effects of the uncured soluble components in a moist environment, the purpose of this in vitro study was to evaluate the cytotoxicity of these resin-based dental materials when submitted to different curing regimes. The null hypothesis advanced was that the cytotoxic effect of resin-modified glass-ionomer lining cements is not affected by variation in the curing regime.

2. Materials and methods

Forty round-shaped specimens (2 mm thick and 4 mm in diameter) were prepared for each of the following resin-modified glass-ionomer lining cements: Vitrebond (3M/ESPE, St. Paul, MN, USA) and Fuji Lining LC (GC Corporation, Tokyo, Japan). The dental cements were mixed according to the respective manufacturers' instructions using the recommended powder:liquid ratio by weight. To avoid the incorporation of air within the specimens, freshly hand-mixed cements were applied into stainless-steel molds with cylindrical apertures using a Centrix syringe. The specimens were divided into four groups ($n = 10$) according to the time of light-activation: 30 s (manufacturer's recommended time, MRT), 15 s (under polymerization, 0.5 MRT) or 45 s (over polymerization, 1.5 MRT). In addition, a group of 10 specimens was allowed to set in the dark for 15 min (0 MRT), in an incubator at 37 °C.

After the insertion in the stainless-steel molds, the material was covered by a plastic matrix, and a standardized weight (500 g) was applied to promote the overflow of material. The selected specimens were light-activated with a curing unit (Optilux 500, Demetron/Kerr, Danbury, CT, USA). The light intensity was monitored with a radiometer (Optilux 500, Demetron/Kerr, Danbury, CT, USA, 530 mW/cm^2).

Ten sterilized round filter papers (Matheson Scientific Inc., E&D 613, MI, USA) of 4 mm diameter were soaked with phosphate-buffered saline solution (PBS) (negative control group) or with pure 2-hydroxyethyl methacrylate (HEMA) (positive control group). The composition, powder:liquid ratio and batch number of the materials are given in Table 1.

Table 1 – Materials composition, powder:liquid ratio and batch number

Material	Composition	Powder:liquid ratio (wt)	Batch number
Vitrebond 3M ESPE, Dental Products, St. Paul, MN, USA	*Powder*: 95% glass powder (O, SrO, criolyte, NH_4F, MgO, P_sO), 2% diphenyl-iodoniumchloride. *Liquid*: 35–45% modified polyacrilic acid, 20–30% 2-hydroxyethyl-methacrylate (HEMA), 30–40% water	1.4:1	20030110
Fuji Lining LC GC, Tokyo, Japan	*Powder*: 100% aluminum-silicate. *Liquid*: 65–70% polyacrilic acid, 8–10% 2-hydroxyethyl-methacrylate (HEMA)	1.4: 1	0206041
HEMA SIGMA Chemical Co., St. Louis, USA	98%-2-hydroxyethyl-methacrylate (HEMA)	–	–
PBS	8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4, 0.24 g KH_2 PO_4 1L Ultra-pure water	–	–