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In vitro biological response to a light-cured composite when used for cementation of composite inlays

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ABSTRACT

Objective. To define the cytotoxicity of a photo-cured composite when used as a bonding system under a composite inlay.
Methods. Composite specimens were photo-cured with or without a 2 mm composite inlay interposed between them and the light source. Samples were extracted in complete cell culture medium and the obtained eluates applied to primary cultures of human pulp and gingival fibroblasts. After 72 h of incubation, cell viability was evaluated by MTT assay. Survival rates were calculated with respect to negative controls.
Results. Both shielded and unshielded composite samples were cytotoxic to pulp and gingival cells. The inlay shielded composite samples reached a significantly higher level of cytotoxicity compared to the unshielded ones.
Significance. The results suggested that the cytotoxicity of a light-cured composite resin used as a bonding system for indirect composite restorations may be significantly increased as a result of an inlay light-shielding effect.

1. Introduction

Resin-based indirect restorations have been extensively used during the last decade as an esthetic alternative in posterior teeth, especially when large or multiple cavities are restored [1]. Indirect composite restorations offer some benefits with respect to the direct ones, such as a higher mechanical performance together with a significant reduction in polymerization shrinkage, limited to the dual-cured luting cement [2,3]. The main drawback remains the existence of a mechanical gradient between the cement layer and the inlay material. For instance, currently used luting cements are less wear resistant (due to their lower filler content), so that the formation of a marginal groove between tooth and restoration, the staining of the cement layer, and finally the failure, primarily for esthetic reasons, can occur [2–5].

The utilization, as a bonding system, of the same light-cured composite used for the fabrication of the inlay itself, has been recently proposed as an alternative to the conventional and more diffused dual-cured cements [6,7]. The rationale underlying this approach is that by using the same material for both the inlay and the bonding system, the mechanical gradient at the tooth-inlay interface can be reduced, improving the endurance of the cement layer over time. Furthermore, cement could be photo-cured at convenience after the inlay sitting, without the disadvantage of an early hardening which can occur using traditional dual-cured systems. The reduced mechanical properties of dual-cured cements with respect

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to composite resins has been shown in a recent paper [7], where Park et al. measured the microhardness of two types of restorative composites cured under a pre-polymerized resin composite overlay, compared to a conventional dual-cured luting cement. In that study, composite resins showed higher microhardness values than dual-cured cement, under any tested condition.

However, as known, the polymerization rate of a photo-cured material can be significantly affected by the intensity of the curing light. Several studies evidenced the dramatic effect of the thickness of composite increments on the their cure level and how poor polymerization can occur at depths greater than 2 mm [8–11]. A decreased degree of cure is known to consort not only with a decay of composite mechanical properties [11–14], but also with an alteration of the biological ones [15–18], with an increase of leached free monomers [19,20], that are demonstrated to be cytotoxic [21] and potentially harmful for the pulp [22] and the oral mucosa [23].

In this study, the authors wanted to investigate whether the biological properties of a photo-cured composite, in relation to primary cultures of human pulp and gingival fibroblasts could be affected when it is used as a bonding system under a pre-polymerized inlay.

2. Materials and methods

2.1. Materials and chemicals

The inlay and all the samples were fabricated using the composite Filtek Supreme (3M ESPE, St. Paul, MN, USA), whose characteristics are listed in Table 1.

All tissue culture biologics were purchased from Gibco Laboratories (Grand Island, NY, USA). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Inlay fabrication

To simulate an indirect restoration (inlay), a disc of composite (2 mm thickness × 7 mm diameter) was placed in a polyethylene mold and fully cured for 120 s on both sides by a conventional halogen light unit (Demetron Optilux, Kerr Company, USA; light intensity: 550 mW/cm^2). The light intensity was measured using a calibrated dental radiometer commonly available to clinicians. Light intensity was measured through the pre-polymerized inlay with the digital dental radiometer built into the Optilux curing light (Kerr, USA); the value was: 70 mW/cm^2.

Table 1 – Characteristics and composition (according with the manufacturer) of the material used to fabricate inlay and test samples

Product	Filtek Supreme
General description	Resin-based composite
Manufacturer	3M ESPE, St. Paul, MN, USA
Shade	A4
Composition	BIS-GMA, BIS-EMA, UDMA, TEGDMA, Nanosilica filler, zirconia/silica nanocluster and particles

2.3. Sample fabrication

Composite specimens (discs 1 mm thickness × 7 mm diameter) were placed on polyethylene strips and polymerized, applying the light tip close to the bottom of the strips. Some discs were cured with the inlay interposed between them and the light tip (shielded samples – SS) and some without the inlay (unshielded samples – US). All specimens were irradiated for 20 s with the above mentioned curing light unit and immediately processed for the eluate preparation.

2.4. Eluate preparation

The shielded and unshielded specimens were extracted in glass vials following standardized procedures [23]. High density polyethylene discs were used as negative control (Ctr) in parallel with the resin composite specimens. Eluates were prepared in cDMEM-F12, that is DMEM-F12 supplemented with 10% FBS, 1% L-glutamine, 1% pen-strep, and 0.25 μg/mL fungizone. The ratio between the specimen surface area and the extraction volume was 150 mm^2/mL.

After a 24 h incubation period at 37 °C under static conditions, the eluates were filtered through 0.22 μm cellulose acetate filters and immediately used for the cytotoxic assay.

2.5. Cell cultures

Primary human pulp cells (HPC) and gingival fibroblasts (HGF) were cultured by using an explant technique. Human deciduous molars were extracted just before their spontaneous exfoliation and immediately washed with sterile saline solution. The access to the pulp chamber was obtained by carefully removing the residual thin dentin floor with a sterile excavator and the pulp tissue aseptically removed. Gingival tissue was obtained from surgical operations (e.g. frenulectomies, flap operations); only the connective layer was dissected from the gingival samples by means of a surgical blade. Both pulp and gingival explants were washed two times with phosphate buffered saline (PBS) supplemented with antibiotics (1% pen-strep, 0.25 μg/mL fungizone) and cut into small pieces with a sterile surgical blade. Tissue fragments were digested in 1 mL of cDMEM-F12 containing 1 mg/mL collagenase at 37 °C for 3 h. After the incubation, released cells and tissue fragments were pelletted at 300 × *g* for 10 min, resuspended in cDMEM-F12, and plated in 35 mm Petri dishes at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air. First cell islets were visible after 3–4 days, while confluence was reached in about 1–2 weeks. Cell cultures between the second and fifth passages were used in this study.

2.6. Cytotoxicity assay

Effect of control (Ctr) and test eluates (US and SS) on cell viability was measured by MTT assay. Both HGF and HPC were seeded in 24-well plates (4×10^4 cells/well) in cDMEM-F12. After overnight attachment, cells were exposed to the eluates (1 mL/well) for 72 h. Then, the cells were rinsed with PBS and