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Biological evaluation of enamel sealants in an organotypic model of the human gingiva

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ABSTRACT

Objectives. Various sealant materials have been suggested to decrease decalcification during orthodontic treatment. However, only a few in vitro studies on the cytotoxicity of resinous pit and fissure sealants have been published, and to the best of our knowledge no similar studies are available for the enamel sealants used in orthodontics. Therefore, we aimed to characterize the possible adverse effects of enamel sealants, especially on the gingival epithelium.

Methods. Organotypic cultures of the human gingival mucosa were used to assess the possible impact of six enamel sealants. Differentiation and apoptosis were determined by immunofluorescent staining. The pro-inflammatory cytokines IL-1β and IL-6 were quantified by ELISA. Cytotoxicity was measured using MTS assays in monolayer cultures of human gingival fibroblasts. Leaching of monomers from enamel sealants was quantified using HPLC.

Results. The differentiation of the organotypic gingival mucosa remained unaffected. All under-cured and several standard-cured sealants (Light Bond™ Sealant, Light Bond™ Filled Sealant, and L.E.D. Pro Seal®) significantly induced apoptosis in the organotypic model. Light Bond™ Sealant, Light Bond™ Filled Sealant, and L.E.D. Pro Seal® caused a significant induction of pro-inflammatory cytokines. Reducing curing time had an influence on cytotoxicity in monolayer cultures of primary human oral cells. All resin-based sealants leached monomers.

Significance. Enamel sealants might exert adverse effects on the gingival epithelium. Due to the vicinity of the enamel sealant to the gingival epithelium, and the large surface area of applied sealants, these materials should be carefully applied and sufficiently cured.

Abbreviations: HPV, human papilloma virus; IL-1, interleukin-1; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; DAPI, (4′,6-diamidino-2-phenylindole); MTS, (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium); FCS, fetal calf serum; HBS, Hanks' buffered salt solution; DMEM, Dulbecco's modified Eagle medium; HPLC, high performance liquid chromatography; Bis-GMA, bisphenol A diglycidyl dimethacrylate; UDMA, urethane dimethacrylate; TEGDMA, triethylene glycol dimethacrylate; HEMA, hydroxyethyl methacrylate.

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1. Introduction

Decalcification of the enamel surface is one of the most common unwanted side effects of orthodontic treatment with fixed appliances [1–3]. The use of various sealant materials has been suggested to decrease decalcification during orthodontic treatment. In particular, filled and unfilled photo-activated resin-based smooth enamel surface sealants and caries infiltrants, as well as different types of varnishes and liners, have all been recommended as non-compliant caries preventive measures [4–7].

Dental materials used in the oral cavity are not inert, and almost all materials may release substances. These substances could cause adverse local and/or even systemic effects [8,9].

Regarding resin-based dental materials, previous studies have clearly demonstrated that numerous substances such as (co)monomers [e.g. bisphenol-A-diglycidyl dimethacrylate (bis-GMA), urethane dimethacrylate (UDMA), triethylene glycol dimethacrylate (TEGDMA), hydroxyethyl methacrylate (HEMA)] or additives [e.g. camphorquinone (CQ), butylated hydroxytoluene (BHT)] can be eluted from these materials into an adjacent liquid phase and may exert cytotoxic effects on human oral cells [10,11]. In addition, more recent studies have identified toxic metabolic intermediates of composite (co)monomers that might significantly contribute to cytotoxicity [12,13]. Light-induced resin-based dental materials are cytotoxic before polymerization and directly thereafter, whereas set specimens cause less severe effects [14]. Therefore, due to the amount of possible residual monomer, light-curing time and conversion rates are considered important factors related to cytotoxicity [15–18]. Also, the filler formulation might affect the degree of cytotoxicity [19]. In addition to light-curing time, conversion rate, and filler formulation, a recent review has highlighted that the release of components from polymerized resin-based dental materials is dependent on the surface area that is exposed to the solvent [20].

Enamel sealants, applied to the total labial surface of tooth crowns, will expose large surface areas to potential solvents. During application, sealants might even come into direct contact with gingival epithelia and excess material might deposit in the gingival sulcus. However, no comprehensive data are available so far regarding the cytotoxicity of these materials. Hence, the aim of the present study was to evaluate the cytotoxic effects of different commercially available (and commonly used) sealant materials. We used an organotypic co-culture model of the gingival epithelium challenged with sealant-treated enamel slices to evaluate their impact on epithelial differentiation and apoptosis. The levels of the pro-inflammatory cytokines IL1-β and IL-6 were measured in culture supernatants to assess local inflammatory and immune responses. Cell viability assays were performed on human gingival fibroblasts exposed to leachings of sealants in artificial saliva. Finally, leachable monomers from sealants were quantified by high performance liquid chromatography (HPLC).

2. Materials and methods

2.1. Sealants

The sealants used in this study are presented in Table 1.

2.2. Cell culture and organotypic co-culture of gingival cells

Gingival tissue and alveolar bone were obtained from patients following extraction of third molars. Informed consent was obtained from volunteers who were undergoing removal of wisdom teeth for medical reasons. The local ethics committee (Medical Faculty, University of Heidelberg; 80/94S147/2010) approved harvest of the tissues.

The isolation of gingival cells was performed as described previously [21]. Briefly, gingival tissues were separated into epithelial and connective tissue elements. Epithelial tissue was dissociated using dispase (2.4 U/ml, 37 °C, 30 min). Cells were seeded and subcultured in keratinocyte growth medium (Promo Cell, Heidelberg, Germany) at 37 °C in a humidified 5% CO_2 incubator. Keratinocytes were immortalized using the E6 and E7 genes of human papilloma virus 16 (HPV-16) using recombinant retroviruses [22].

Gingival fibroblasts were obtained from the remaining connective tissue of explant cultures as described by [21].

Gingival fibroblasts were cultured in Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Karlsruhe, Germany) supplemented with 10% fetal calf serum (FCS), 2 mM L-glutamine, antibiotics, and antimycotics at 37 °C in a humidified 5% CO_2 incubator. Immortalized gingival keratinocytes were used between passages 14 and 16. Gingival fibroblasts were used between passages 4 and 6 both for the organotypic cultures and for the monolayer cultures used for viability assays.

Organotypic cultures of human gingival cells were established as described by [21]: bovine collagen type I (Life Technologies, Darmstadt, Germany) was polymerized at a concentration of 3 mg/ml in Hanks' buffered solution (HBS, 10×), gingival fibroblasts were added in FCS at a concentration of 1×10^5 cells/ml collagen, and collagen gels were polymerized by neutralization with NaOH (1 N). Collagen gels containing gingival fibroblasts were allowed to polymerize and were immersed in organotypic culture medium (DMEM/Ham's F-12 1:1 mixture, Life Technologies, Darmstadt, Germany) supplemented with bovine pituitary extract [0.004 mg/ml], epidermal growth factor (EGF; recombinant human) [0.125 ng/ml], insulin (recombinant human) [5 μg/ml], hydrocortisone [0.33 μg/ml], epinephrine [0.39 μg/ml], transferrin, holo (human) [10 μg/ml], and $CaCl_2$ [0.06 mM]. After 24 h, gingival keratinocytes (7×10^5 cells per collagen gel) were seeded on the surface of the collagen gels and allowed to adhere for 24 h. At this stage organotypic cultures were lifted onto metal grids allowing air contact of the upper keratinocyte layer. Organotypic culture medium was changed every second day and the cultures were matured for 21 days.