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Gene expression analysis of conventional and interactive human gingival cell systems exposed to dental composites

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ABSTRACT

Objectives. The aim of this study was the detection of putative gene expression-related effects of dental composites in conventional and interactive gingival cell systems.

Methods. Conventional monoculture (MC) and interactive cell systems (ICS) comprising human gingival fibroblast (HGF) and immortalized human gingival keratinocytes (IHGK) were exposed for 24 h and 7 days according to ISO10993-12:2012 manufactured eluates of different composites (Ceram X®, Filtek™ Supreme XT, Filtek™ Silorane, Fusio™ Liquid Dentin, and Vertise™ Flow). qRT-PCR-based mRNA analysis for biomarkers indicating cell proliferation, differentiation, apoptosis, inflammation, and adhesion was performed. Apoptotic cells were quantified by annexin-V labeling.

Results. Due to low RNA amounts, qPCR could not be performed for Vertise™ Flow and Fusio™ Liquid Dentin at day 7. At 24 h, flowables yielded increased transcription for biomarkers of inflammation and apoptosis in IHGK, irrespective of the cell system. HGF cultures displayed lower transcription for cell adhesion markers in both cell systems. Filtek™ Supreme XT showed increased differentiation by elevated filaggrin gene expression in both cell systems for IHGK at day 7, while Filtek™ Silorane and Ceram X® yielded elevation of inflammation biomarkers in both cell types. Annexin-V labeling revealed high apoptosis rates for both flowables and Filtek™ Supreme XT for IHGK, while low rates were detected for Filtek™ Silorane and Ceram X®.

Significance. Among the composites evaluated, exposition of IHGK and HGF in conventional and interactive cell systems demonstrated most pronounced gene expression alterations in response to flowables, coinciding with elevated levels of apoptosis.

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1. Introduction

The use of composite materials have gained popularity in dental treatment through the past decades because of their rapid polymerization, their ability to bond with tooth surfaces, their excellent mechanical properties, and their esthetic appearance. The organic resin matrix of these dental restorative materials is usually based on methacrylate monomers, such as BisGMA (bisphenol A glycol dimethacrylate), UDMA (urethane dimethacrylate), TEGDMA (triethylene glycol dimethacrylate), and BisEMA (bisphenol A ethoxylated dimethacrylate) [1]. Recently, some new resin systems such as ormocers and silorane have been introduced as alternative to the methacrylate-based composites [2]. Composite characteristics including reactivity, viscosity, and the polymerization shrinkage as well as the mechanical properties are determined by the chemistry of the monomer [3]. Despite the achievements made in recent composite technology, composite polymerization is still incomplete, thereby affecting their physical properties and clinical performance [4,5].

With respect to the clinical success of dental composite materials, a critical issue is their biocompatibility. In this regard, differential release of monomers from dental composite materials after storage in water, ethanol 75% or methanol has been described for BisGMA, TEGDMA, HEMA (2-hydroxethyl methacrylate) and UDMA [6,7]. Of note, induction of cytotoxicity and apoptosis in human dental pulp cells has been observed for TEGDMA, BisGMA, and HEMA [8–10]. TEGDMA and HEMA have been shown to induce apoptosis in several cell types through the generation of redox balance-disturbing reactive oxygen species (ROS) [11–14], which lead to DNA damage [15]. Substances like BisGMA, BisDMA and Bisphenol A are assumed to indirectly induce estrogen-like reactions [16,17]. A further critical issue is that harmful effects on periodontal cells have been described in a study on human gingival fibroblasts (HGF) by BisGMA-induced DNA double-strand breaks [18], while other studies on fibroblasts revealed HEMA-associated alterations in morphology and extracellular type-I collagen protein abundance [19].

Until now, studies elucidating putative hazardous effects of composite-eluted substances share two major handicaps. First, frequently pure monomers in various concentrations have been used for cell exposition, while studies recruiting authentically eluted substances from the polymerized composites are rare. Several parameters might influence the elution of the substances of the composite materials. Testing the original substances that are used for the manufacturing of the composite materials does not really represent the clinical situation. Although the release of the same substances has been certified by several authors, these substances are not the only ones that are eluted from the composite materials as further degradation products might be additionally eluted. Additionally, an interaction between the eluted substances and the production of other molecules might take place. Second, the majority of the toxicity tests have been carried out on rodent cell systems, which may yield discriminative results to human target tissue cells, for instance, human gingival fibroblasts. So far available *in vitro* studies on periodontal cells usually assess substance-related effects on cell behavioral features in conventional monolayer cell systems [12,18,20–22]. However, although the use of such conventional monolayers gives important information of the direct effect of a tested composite or its monomer on cell behavior, the translation of the elaborated results to in the *in vivo* situation is limited. This limitation arises from the lack of cell–cell interactions provided by the physiological, *i.e. in vivo* situation. Hence, the use of interactive gingival cell systems, comprising fibroblasts and keratinocytes would be beneficial in order to evaluate the effects of composite materials, due to the ability of the cells to cooperate with each other and therefore to simulate the *in vivo* situation. The differential cell behavior by using human gingival keratinocytes and gingival fibroblasts in an interactive cell system compared with the conventional use of the same cell types in monolayers has been stated in the past [23–25].

Therefore, the aim of the present study was to evaluate the effects of eluates of five polymerized dental composite materials with different chemistry on the gene expression of human gingival keratinocytes and human gingival fibroblasts in conventional and interactive cell systems. We hypothesized that eluates of these dental composites have impact on the gene expression of the investigated cells and that the crosstalk between the cells in an interactive cell system putatively modulate these alterations. Based on our hypothesis, the present gene expression analysis focuses on genes of cell behavioral features (i) apoptosis, (ii) inflammation, (iii) adhesion, (iv) proliferation, and (v) differentiation. In order to detect possible direct substance-related effects on cell behavior on the phenomenological level, early apoptosis was quantified in conventional cell systems.

2. Materials and methods

2.1. Composite materials

In the present study, three different composite materials were tested: a nanohybrid resin composite, Filtek™ Supreme XT (3M ESPE Dental Products, Seefeld, Germany), an Ormocer, Ceram X® (Dentsply DeTrey GmbH, Konstanz, Germany), a composite material representing the Silorane technology, Filtek™ Silorane (3M ESPE Dental Products, Seefeld, Germany) and two self-adhering flowable composite materials, Vertise™ Flow (KERR, Orange/CA, USA) and Fusio™ Liquid Dentin (Pentron Clinical, Wallingford/CT, USA). Detailed information about the composition of the composite materials and the manufacturers are given in Table 1.

2.2. Preparation of composite samples and eluates

From each composite material, 26 specimens (shade A3) were prepared. The samples were prepared according to ISO 10993-12:2012 using molds, allowing the production of standardized cylindrical specimens (diameter 7 and 2 mm thickness). The forms were positioned on a transparent plastic matrix strip lying on a glass plate and were filled with the composite material. The samples were built up in one increment. After inserting the material into the discs, a transparent plastic matrix strip (Kerr Hawe, Bioggio, Switzerland) was placed on top of them in order to avoid an oxygen-inhibited superficial