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Modulation of gingival cell response towards dental composites

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ABSTRACT

Objective. This aim of this study was to investigate the cell behavioural response on clinically applied dental composites in exposition-relevant target cells, i.e. human gingival fibroblasts (HGF) and epithelial keratinocytes (HGK).

Methods. HGF and HGK were exposed to eluates of Ceram X™, Filtek™ Silorane, Filtek™ Supreme XTE, Fusio™ Liquid Dentin and Vertise™ Flow. Eluates were created by storing material samples in respective cell culture medium, for 24 h and 72 h (n = 17), according to ISO 10993-12:2012. Cell response was evaluated at eluate exposure periods of 24 h and 72 h by (i) impedance analysis-based real-time monitoring of adhesion and proliferation, (ii) semi-quantitative indirect immunofluorescence (sq-IIF) detection of tissue-specific biomarkers, and (iii) ELISA-detection of pro-inflammatory interleukin (IL)-6.

Results. Generally, cell behavioural response towards the eluates was gradual in HGK and HGF, the latter exhibiting a less pronounced modulation *per se*. In HGK, ERK 1/2 was mainly activated after 24 h by Fusio™ Liquid Dentin and Vertise™ Flow, while an increase in biomarker expression occurred time-delayed. A 72 h exposure of HGK to eluates of Filtek™ Supreme XTE, Fusio™ Liquid Dentin and Vertise™ Flow significantly decreased secreted IL-6 amounts. In HGK, the impedance analysis revealed less proliferation and/or adhesion in case of Fusio™ Liquid Dentin and Vertise™ Flow with matched other composites.

Significance. In detail, protein expression and secretion is modulated particularly in terms of signal transduction, differentiation and inflammation. On cell biological level, all tested materials modulated the analysed features of cell behaviour with emphasis on the self-adhering composites.

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1. Introduction

Composite materials are nowadays widely used in modern dentistry as direct filling materials due to their improved aesthetic and mechanical properties. Increased requirements concerning their physicochemical characteristics and their handling led to their further development. Composites mainly consist of a resin-based organic matrix, inorganic reinforcing fillers and a silane-coupling agent [1]. In order to improve the mechanical performance of the composite materials, modifications were performed first on fillers and later on the organic matrix. Methacrylates such as BisGMA, UDMA and TEGDMA were supplemented or replaced by polysiloxane and silorane monomers. Furthermore, self-adhering composites were designed by integrating amphiphilic monomers like 4-MET, GPDM and HEMA into the organic matrix [2], thereby presenting a new category of flowable composites.

The clinical success of composite materials is determined, amongst other parameters, by their biocompatibility, including a good performance towards cells and tissues. Several studies have shown that polymerized dental composites can release substances, mainly elements of the organic matrix, in considerably high amounts [3–9]. Due to cytotoxic, genotoxic, immunological, allergic and estrogenic effects of the eluted substances, high concern exists about their possible negative impacts on human health [10–12]. Monomers like TEGDMA, HEMA, BisGMA and UDMA reduced cell survival in cell cultures of human oral tissues [13,14]. Additionally, eluted substances from composite materials decreased RNA levels in human gingival keratinocytes [15], and cell cycles of dental pulp cells and gingival epithelial cells were arrested when the cell cultures were treated with HEMA [16]. Moreover, TEGDMA generated a breakdown of the mitochondrial membrane potential in gingival fibroblasts and therefore led to cell death via apoptosis [17]. In addition, it has been shown that gingival fibroblast can metabolize methacrylate monomers to epoxymethacrylates, the latter considered being highly toxic and mutagenic [18–20]. The adverse effects of dental monomers are largely transmitted by a disturbance of the cells' redox balance through the generation of reactive oxygen species (ROS) [21,22]. ROS influence the cells' intracellular signal transduction by changing activities of signal transduction molecules such as the mitogen-activated protein-kinase (MAPK) pathway [23]. MAPK in turn regulate downstream transcription factors and thereby play a central role in regulating cell behaviour such as proliferation, differentiation, morphology and apoptosis resistance [24,25].

However, the majority of the studies focus on toxic effects of specific dental composite components whereas little knowledge exists about putative sub toxic effects of the composites on oral tissues [11,19]. A few studies have considered the effect of composite resins on the gene expression of human cells [15,26,27]. The total RNA-levels in human gingival keratinocytes were found to be reduced by eluates of composite materials [15] and TEGDMA induced alterations of gene expression in human dental pulp cells [26]. Moreover, released substances of composite materials modulated RNA-levels of human gingival cell systems by decreasing total RNA amounts and by increasing the expression of biomarkers of inflammation, apoptosis and differentiation [27]. Although the sensitive method of gene expression analysis may indicate variations of cell regulations, protein expression does not necessarily vary concomitantly. Therefore, it is of importance to monitor cell behaviour on the protein level by evaluating possible cell responses towards dental composites.

In the present study, human gingival keratinocytes (HGK) and human gingival fibroblasts (HGF) were exposed to eluates of five composite materials with the aim to evaluate the effects of the eluates on the gingival cells' protein expression. In order to gain basic information about time-dependent (i) proliferation, (ii) viability and (iii) morphology of the gingival cells, they were continuously analysed by a Real-Time Cell Analyzer. In detail, the protein analysis was performed by indirect immunofluorescence staining and ELISA in order to detect sub toxic effects. Biomarkers under study address features of cell behaviour, such as proliferation, differentiation, adhesion, inflammation and signal transduction to improve our understanding on biological sub toxic adverse effects, which emerge from dental composites on oral target tissue cells. The null hypotheses tested were that: (i) composite eluates affect the protein expression of HGK and HGF, (ii) composition of the materials plays an important role on the observed effects, (iii) the monitored effects are time-dependent with respect to both the elution time and incubation period.

2. Materials and methods

2.1. Composite materials

In the present study five different composite materials were used: an Ormocer [Ceram X^{TM} (Dentsply DeTrey, Konstanz, Germany)], a Silorane [Filtek^{TM} Silorane (3M ESPE, Seefeld, Germany)], a nanohybrid [Filtek^{TM} Supreme XTE (3M ESPE, Seefeld, Germany)] and two self-adhering composites [Fusio^{TM} Liquid Dentin (Petron Clinical, Orange, California, USA)] and Vertise^{TM} Flow (Kerr Corporation, Orange, California, USA)]. Detailed information about the chemical composition of the materials is given in Table 1.

2.2. Sample preparation and generation of composite eluates

Sample preparation and storage were performed according to ISO 10993-12:2012 [28]. For the preparation of standardized cylindrical specimens (diameter 6 mm and thickness 2 mm) of each material (n = 34), molds were used and placed on a transparent polyester matrix strip (Kerr Hawe, Switzerland). The composite material was inserted into the moulds in one step, flattened and then covered by a second matrix strip. The polymerization and polishing of the samples were carried out by using a LED light-curing unit (Elipar® Freelight 2, 3M ESPE, Seefeld, Germany) with constant light intensity (>1000 mW/cm^2) according to the manufacturers' instructions.

In order to achieve an appropriate disinfection, the composite samples were washed for 60 s with ethanol 70% (Sigma Aldrich, St. Louis, MO, USA), left to dry and then stored in 800 μl of cell culture media per sample. Half of the samples

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