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Cytokine release from human leukocytes exposed to silorane- and methacrylate-based dental materials

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ABSTRACT

Objectives. Silorane-based dental monomers contain an epoxy functional group. Less is known about the toxicological and inflammatory potential of silorane-based composites. Therefore we compared the release of 24 cytokines from human leukocytes after incubation with silorane-based Filtek™ Silorane (Silo) and methacrylate-based TetricEvo Flow® (TC).

Methods. Leukocytes from nine healthy test persons (P) were incubated with Silo or TC for up to 72 h. All 24 h cytokines were quantified with a magnetic bead assay.

Results. Silo stimulates the leukocytes to higher release of cytokines when compared to TC. 72 h after beginning the experiment, leukocytes from P6 incubated with Silo secreted more than an 18-fold amount of interleukin (IL)-6 when compared with leukocytes incubated with TC (771.8 vs 42.1 pg/ml).

Only leukocytes from P8 incubated with Silo release up to 14.4 pg/ml IL-2 after 72 h.

Significance. The significantly higher induction of cytokines with Silo in comparison to TC is test person independent. This indicates a higher sensitization potential for Silo. Because of the cytokine release pattern (especially the release of T-cell dependent IL-2) from leukocytes from P8 after incubation with Silo it is likely that P8 can develop an allergic Type IV sensitization to Silo. Therefore the cytokine release assay is a helpful tool for providing information about possible immunological reactions to dental resins in individual cases as well as for a general risk assessment and comparison between different dental materials.

1. Introduction

The inflammatory mediators are a large family of molecules which play an important role in numerous physiological and pathological processes in our organism [1]. Within this cross-serial regulatory network they can have additive/synergistic as well as antagonistic effects. Inflammatory mediators can be released from different type of cells, e.g. from peripheral (blood) leukocytes after their stimulation. To start an immune response, human leukocytes can produce different inflammatory mediators like polypeptide hormones, complement factors, coagulation factors, enzymes like proteases and lipases [2]. Cytokines, a subgroup of the polypeptide

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hormones, interact with specific receptors and can trigger autocrine, paracrine and endocrine reactions by the release of proinflammatory substances, e.g. interleukin (IL)-1, IL-6, IL-10 and tumor necrosis factor (TNF)-α [2].

The release of inflammatory mediators from cells like human leukocytes can be caused for different reasons, e.g. defence against microorganisms and parasites. In our modern times this release can also be stimulated by xenobiotic substances like 2-Hydroxyethyl methacrylate (HEMA) or Triethylene glycol dimethacrylate (TEGDMA) [3] or erosion particles from methacrylate based polymer networks [4]. In relation to methacrylate based materials it is known that they can cause allergic reactions in patient and dental personal as well as cytotoxic and genotoxic effects in vitro [5–7]. One reason for these effects is the release of dental monomers and additives after polymerization of the dental material because of the low conversion rate of about 55–65% [8,9].

A new class of monomers, named silorane, an acronym for siloaxane and oxirane, was presented a few years ago [10]. Filtek™ Silorane has lower polymerization shrinkage and a higher depth of cure compared with a lot of methacrylate based dental composites [11]. However less data is available regarding possible cytotoxic effects and the release of substances from the polymerized silorane.

The release of components from dental materials has a marked influence on the structural stability and especially on the biocompatibility of the material. The induction or alterations in the regulation of inflammatory markers can be helpful in the assessment of the biocompatibility of the dental composite [12]. Particularly in the early stages, after exposition to dental composites as well as by exposure to sublethal concentrations the secretion and expression of inflammatory markers is an important indicator for latent cellular damage.

Therefore the aim of our study was to compare the amount and the releasing kinetic of 24 inflammatory markers after exposure of human leukocytes to the dental methacrylate based restorative material TetricEvo Flow® and the silorane based restorative material Filtek™ Silorane to the 72 h after beginning the experiment.

2. Materials and methods

2.1. Preparation of specimen

From the light-curable tooth restorative materials TetricEvo Flow® (TC) (Ivoclar Vivadent, Ellwangen, Germany; LOT M61775) and Filtek™ Silorane (Silo) (3M ESPE, Seefeld, Germany; LOT 0FM) specimens of approximately 100 mg (thickness of 1.8 mm, diameter of 6 mm; color A2, with a resulting surface of the cylinder of 90.4 mm^2) were prepared under photolaboratory conditions. The specimens were covered with plastic matrix strips (Frasaco, Tettnang, Germany) and polymerized according to the instructions of the manufacturer by using an Astralis 10® light source (Ivoclar Vivadent).

2.2. Cell culture

9 ml blood was taken from 9 healthy test persons (P) (8 female, 1 male) in a Li-heparin tube (Becton Dickinson Vacutainer®, Heidelberg, Germany). 500 μl Roswell Park Memorial Institute medium (RPMI)-1640 medium (Sigma–Aldrich, Steinheim, Germany) were added to 500 μl whole-blood sample in 5 ml tubes (Sarstedt, Nuermbracht, Germany; LOT 1071001) under cell culture conditions. From the blood from each test person 4 tubes were prepared. 100 μl lectin from *Phytolacca americana* (Sigma–Aldrich; LOT 110M77102V) were added to tube 1 (positive control). Silo or TC were added to tubes 2 and 3, respectively. No further substances were added to tube 4 (negative control). The cells were stored at 37 °C in 5% (v/v) CO_2 atmosphere. 24, 48 and 72 h after the beginning of the experiment 50 μl from the cell free supernatant were taken and stored at −20 °C.

2.3. Quantification of cytokines

Inflammatory mediators were quantified with the magnetic bead assay Bio-Plex Pro™ (Bio Rad Laboratories, Munich, Germany; LOT 310009465) test kit using the Bio-Plex® detection system and software (Bio Rad Laboratories). Following mediators were quantified: IL-1β, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12(p70), IL-13, IL15, IL-17, granulocyte colony-stimulating factor (G-CSF), interferon-gamma (IFN-γ), monocyte chemoattractant protein-1 (MCP-1); major Cdk9-interacting elongation factor (MCAF)), macrophage inflammatory proteins (MIP)-1β, MIP-1α, TNF-α, vascular endothelial growth factor (VGEF), Eotaxin, fibroblast growth factor (FGF) basic, granulocyte-macrophage colony-stimulating factor (GM-CSF). The treatment of the samples as well as standard dilution and calibration followed the manufacturer's instructions. Briefly, all standards and samples were equilibrated to 25 °C. The test was performed on 96-well filter plates. After wetting the filter plates with assay buffer and removing it, 50 μl of the coupled bead solution was added to each well. Then 50 μl of the standard or sample were added to the wells, respectively, and incubated for 30 min at 25 °C with constant shaking (300 rpm). Afterwards, each well was washed three times with wash buffer and 25 μl of the solution with detection antibodies was added to each well and incubated for 30 min at 25 °C with constant shaking (300 rpm). Next, each well was washed three times with wash buffer and 50 μl streptavidin–phycoerythrin (streptavidin–pe) solution was added to each well and incubated for 10 min at 25 °C with constant shaking (300 rpm). Hereafter, each well was washed three times with wash buffer and 125 μl assay buffer were added to each well and shaken for 30 s at 1100 rpm. Then the 96-well filter plates were analyzed in the Bio-Plex® detection system.

2.4. Calculations and statistics

The data were analyzed with the Bio-Plex Manager™ software. From the measured amount of inflammatory mediators in the leukocyte cultures incubated with Silo or TC, the amount of inflammatory mediators measured in the native culture was subtracted. P8 was excluded from the statistics and separately discussed. The data are presented as mean ± CV. The statistical significance ($p < 0.05$) of the differences between the release of inflammatory mediators caused