Clin Oral Invest (2012) 16:215–224
DOI 10.1007/s00784-010-0508-5

ORIGINAL ARTICLE

Oxidative stress and cytotoxicity generated by dental composites in human pulp cells

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Received: 2 December 2010 / Accepted: 30 December 2010 / Published online: 18 January 2011

Abstract Dental composites are a source of residual monomers that are released into the oral environment. Since monomers act on cultured cells through reactive oxygen species (ROS), we hypothesized that composites generate ROS associated with cytotoxicity. Human pulp-derived cells were exposed to extracts of methacrylate-based materials including triethylene glycol dimethacrylate and 2-hydroxyethyl methacrylate-free composites (Tetric Ceram, Tetric EvoCeram, els, els flow, Solitaire 2) and a silorane-based composite (Hermes III). The materials were polymerized in the presence and absence of a polyester film and then extracted in culture medium. The generation of ROS was measured by flow cytometry, and cytotoxicity was determined as well. Methacrylate-based composites reduced cell survival but varied in efficiency. Undiluted extracts of Solitaire 2 specimens prepared in the absence of a polyester film reduced cell survival to 26% compared with untreated cultures. Cytotoxicity was reduced when specimens were covered with a polyester film during preparation. Cytotoxicity of the composites was ranked as follows: Solitaire 2>>els flow>Tetric Ceram=Tetric EvoCeram=els>Hermes III. The generation of ROS followed the same pattern as detected with cytotoxic effects. A positive correlation was found between ROS production and cell survival caused by extracts made from materials not covered with a polyester film. These findings suggest that components released from composites affect cellular signaling networks through ROS formation. Regenerative and reparative capacities of the dentine–pulp complex may be impaired by biologically active resin monomers released from composite restorations.

Keywords TEGDMA and HEMA-free composites · Silorane · Dental pulp cells · Reactive oxygen species · Cytotoxicity

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Introduction

The dentine–pulp complex is formed by differentiated and undifferentiated cell populations including odontoblasts, odontoblast-like cells, and pulp stem cells [1]. Odontoblasts as a monolayer of post-mitotic cells at the periphery of the dental pulp function in dentine matrix formation which contains mostly various types of collagen, fibronectin, and proteoglycans. External stimuli such as attrition, erosion, caries, or restorative procedures lead to regenerative or reparative dentinogenesis as a result of the stimulation of odontoblast function [2]. Then, odontoblasts increase the secretion of extracellular matrix molecules followed by mineralization to form tertiary dentine at the site of initial mild tissue damage. The formation of reparative dentine, on the other hand, requires odontoblast-like cells which replace original odontoblasts because of severe cell damage. It has been repeatedly discussed that substances released from dental restorative materials can reach pulp tissue after diffusion across dentine, especially in deep cavities. Therefore, the analyses of the biocompatibility of a dental restorative and its performance with respect to adjacent oral tissues are of particular interest. Recent research has focused on the analysis of mechanisms behind phenomena such as cytotoxicity, the disturbance

of appropriate responses of the cellular immune system, or the inhibition of the mineralization processes in odontoblasts in vitro [3]. The understanding of these processes of material-specific damage to pulp tissues, or modifications of the regeneration of dental hard tissue, will lead the way to the development of new strategies in modern dental therapies.

Methacrylate-based adhesively bonded dental composites are frequently used in dental practice and fulfill patients' requirements for esthetic and tooth-colored restorations. However, incomplete monomer conversion during polymerization of light curing methacrylate-based composite resins leads to the release of residual monomers from composite materials which may be bioactive in adjacent oral tissues [4]. Moreover, a resin-rich inhibited layer is generated at the surface of resin composites in the presence of oxygen during light irradiation. Oxygen inhibits the polymerization process of resin monomers by the formation of unreactive peroxy radicals, which react with themselves or other propagating radicals resulting in inactive products [5]. The amount of free monomers increases in the case of lower polymerization rates because of an oxygen-inhibited surface layer [6].

It has been discussed that components released from methacrylate-based composite resins and dentine-bonding agents, for instance, the monofunctional monomer 2-hydroxyethyl methacrylate (HEMA) or the bifunctional comonomer triethylene glycol dimethacrylate (TEGDMA), can affect pulp tissues and cells in physiological concentrations [7, 8]. The immediate formation of reactive oxygen species (ROS) in resin-exposed cells is paralleled by the depletion of the cellular ROS scavenger glutathione (GSH) and plays a central role in the regulation of resin monomer-induced tissue responses [9–11]. Oxidative stress is a signal for the activation of pathways which control cell death and survival as well as proliferation and differentiation through mitogen-activated protein kinases. The biochemical execution of apoptosis or the support of cell survival is finally determined by crosstalk between the regulators of distinct signaling pathways and the cellular redox status [12]. The monomer-induced disturbance of the intracellular redox homeostasis has been associated with the onset of apoptosis [10–14]. Moreover, resin monomers down-regulated gene expression in pulp cells and inhibited mineralization indicating a decrease in regenerative potential of the dentine–pulp complex [15–17]. Hence, biocompatibility and the understanding of cellular mechanisms are vital to manage and support dentine formation and pulp regeneration in dental therapy. Yet, despite the reports on monomer activity, the generation of ROS by current dental composite materials has yet to be demonstrated experimentally. Therefore, HEMA and TEGDMA-free methacrylate-based adhesives and composite resins appear to be advantageous with respect to biocompatibility. Alternatively, siloranebased composites are another approach for the reduction of polymerization shrinkage and the prevention of biologically adverse effects caused by restorative resin materials, probably due to the low solubility of individual compounds in water [18].

We hypothesized that the generation of ROS by extracts of dental resin composites might exceed the cellular capacity to balance oxygen homeostasis and lead to cell death. Therefore, ROS formation in pulp-derived monolayer cell cultures as well as cytotoxicity of current dental composite resins was analyzed in the present study. To this end, the effects of methacrylate-based materials were compared with TEGDMA- and HEMA-free materials and a composite based on siloranes.

Materials and methods

Test materials and chemicals

The information provided by the manufacturers on the compounds of composite resins tested in the current study is summarized in Table 1. Minimal essential medium alpha (MEMα), fetal bovine serum, penicillin/streptomycin, antibiotic/antimycotic, geneticin, and crystal violet were obtained from Gibco Invitrogen (Karlsruhe, Germany), and Accutase came from PAA Laboratories GmbH (Cölbe, Germany). 2′7′-dichlorodihydrofluorescin diacetate (H_2DCF-DA) came from Invitrogen Molecular Probes (Karlsruhe, Germany). TEGDMA (CAS-No. 109-16-0) was purchased from Sigma-Aldrich (Taufkirchen, Germany). All other chemicals used here were analytical grade.

Sample preparation

Samples of the resin materials were prepared in Teflon rings (5 mm in diameter, 2 mm high). Specimens were light-cured for 40 s (780 mW/cm^2) from each side without or against a polyester film. The use of a polyester film may reduce the likelihood of the occurrence of an oxygen-inhibited free radical polymerization of dental polymers, which leads to a lower degree of monomer conversion [6]. Seven specimens per material were transferred immediately after mixing into one insert (Millipore, Billerica, MA, USA; 0.4 μm filter) of a six-well culture plate. The test specimens were covered with 3 ml cell culture medium (MEMα supplemented with 10% fetal bovine serum, geneticin, and penicillin/streptomycin) and incubated in the dark for 24 h at 37°C. Thus, extracts of the test specimens were prepared at a ratio of 91.6 mm^2 sample surface area per milliliter cell culture medium following the recommendations of ISO

Table 1 Test materials, compounds, and components

Test material	Lot number	Manufacturer	Components
Tetric Ceram	D51466	Ivoclar Vivadent Schaan, Liechtenstein	Bis-GMA, UDMA, TEGDMA; barium glass, Ba-Al-fluorosilicate glass, ytterbium trifluoride, highly dispersed silicon dioxide, spheroid mixed oxide, additives, catalysts, stabilizers and pigments
Tetric EvoCeram	G19701		Bis-GMA, UDMA, ethoxylated-*bis*-EMA; fillers contain barium glass, ytterbium trifluoride, mixed oxide, prepolymer, additives, catalysts, stabilizers and pigments
Solitaire 2	010253	Heraeus-Kulzer GmbH Hanau, Germany	Di- and tetra-functional monomers, Ba-Al-F-silicate glass, silicon dioxide
els extra low shrinkage	07.2011-38; 04.2013-68	SAREMCO Dental AG Rebstein, Switzerland	*Bis*-GMA, *Bis*EMA, catalysts, inhibitors, pigments; silanized barium glass
els flow	07.2011-06; 04-2013-13		
Hermes III (Filtek Silorane)	151174-2	3 M Espe AG Seefeld, Germany	3,4-Epoxycyclohexylethylcyclo-polymethylsiloxane, *bis*-3,4-epoxycyclohexylethyl-phenylmethylsilane, silanized silicia glass, yttrium flouride
Vitrebond resin modified glass ionomer material	20030902	3 M Espe AG Seefeld, Germany	Modified polyacrylic acid, HEMA, water, photoinitiator; silicon dioxide, aluminum fluoride, strontium oxide, zinc oxide, cryolite, ammonium fluoride, magnesium oxide

10993-12 [19]. These original extracts (1:1) were then serially diluted in cell culture medium prior to testing.

Measurement of ROS

ROS production in cell cultures was analyzed using the oxidation-sensitive fluorescent probe H_2DCF-DA following published procedures [10, 20]. Human-transformed pulp-derived cells (tHPC) from routine cultures (MEMα supplemented with 10% fetal bovine serum, geneticin, and penicillin/streptomycin) were cultivated in six-well plates (2×10^5/well) 24 h at 37°C as described [21]. Then, the cells were exposed to serial dilutions of composite resin extracts (2 mL per well) for 1 h at 37°C, and TEGDMA (1.5 mmol/L) was used as a control for ROS production. One well was used for each concentration. DCFH–DA (10 mmol/l) was added 30 min prior to the end of exposure. The cell cultures were then detached with accutase, resuspended in culture medium, collected by centrifugation, washed with phosphate-buffered saline free of calcium and magnesium (CMF–PBS), and finally resuspended in 200 µl CMF–PBS. Subsequently, DCF fluorescence was determined by flow cytometry (BD FACSCalibur) at an excitation wavelength of 495 nm and an emission wavelength of 530 nm (Fl-1). Mean fluorescence intensities were obtained by histogram statistics using the WinMDI program (Version 2.8). At least four independent experiments were performed with each material extract. Mean fluorescence intensities were normalized to untreated control cultures (=1.0), and differences between median values were statistically analyzed using the Mann–Whitney *U* test (α=0.05; SPSS, Version 15.0 SPSS; Chicago, IL, USA).

Cytotoxicity testing of dental composite materials

Transfected human pulp-derived cells were grown at a density of 7.5×10^3 per well in 96-well plates for 24 h at 37°C. Then, cell cultures were exposed to 200 µl of original extracts of the test materials and serial dilutions of these extracts for 24 h at 37°C. Next, cell survival was determined using a crystal violet assay [22]. The cell cultures were washed with PBS–EDTA, fixed with 1% glutaraldehyde and stained with crystal violet (0.02% in water) at room temperature. The amount of crystal violet bound to the cells was dissolved with 70% ethanol, and optical densities were measured at 600 nm in a multi-well spectrophotometer (EL312; Biotek Instruments, Winooski, VT, USA). Four replicate cell cultures were exposed to each dilution of material extract in at least four independent experiments. Optical density readings were normalized to untreated control cultures (=100%), and differences between median values were statistically analyzed using the Mann–Whitney *U* test (SPSS 15.0; SPSS, Chicago, IL, USA) for pairwise comparisons

among groups. Correlations between median values of ROS production and cell survival rates were analyzed using best fits and corresponding equations in TableCurve 2D V5.01 (Systat Software Inc, Chicago, IL, USA).

Results

Production of reactive oxygen species by composite resins

The generation of ROS in tHPC was determined after exposure to extracts of composite resins including HEMA- and TEGDMA-free materials. Diluted extracts (1:2) as well as the original extracts of Tetric Ceram increased ROS production 1.4-fold and 1.7-fold compared with untreated cell cultures (Fig. 1). Similar ROS levels were produced by extracts of the TEGDMA-free Tetric EvoCeram in the absence of a polyester film. The application of a polyester film, however, inhibited ROS production (Fig. 1). The highest amount of ROS was detected with extracts of Solitaire 2 (twofold) when material specimens were prepared in the absence of a polyester film, and even diluted extracts (1:4 and 1:2) were significantly effective (Fig. 1). ROS production in extracted Solitaire 2 specimens was reduced (1.6-fold) when the samples were prepared in the presence of a polyester film. However, the differences between median values of ROS production caused by extracts of Solitaire 2 specimens covered or not covered with a polyester film were not significant. The light curing glass ionomer cement Vitrebond, which was used here as a positive control material, induced the formation of ROS very similarly to Solitaire 2. A reduction in ROS production in extracts from specimens prepared in the presence of a polyester film was not statistically significant (Fig. 1).

The formation of low amounts of ROS was also observed with extracts of HEMA- and TEGDMA-free materials (Fig. 2). Extracts of the composite els enhanced ROS levels about 1.6-fold compared with controls in the

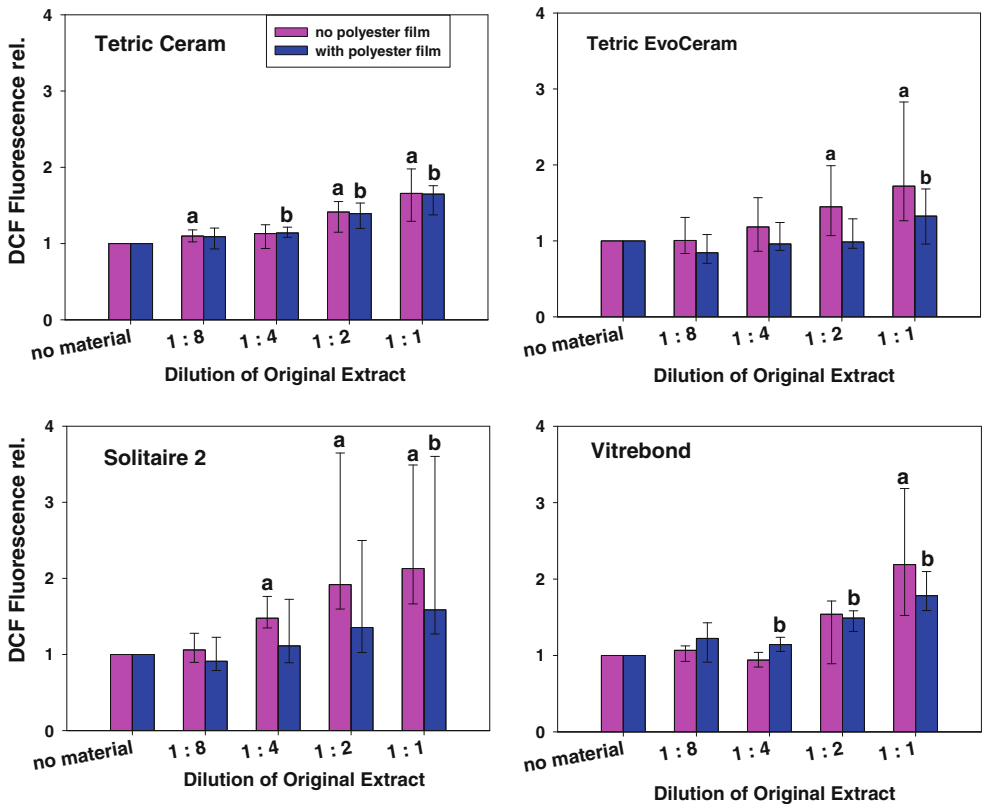

Fig. 1 Generation of reactive oxygen species (ROS) in human pulp-derived cells after exposure to methacrylate-based composite resins. The resin modified glass ionomer (RMGI) cement Vitrebond is included as a reference material. The production of ROS was measured using the oxidation-sensitive fluorescent probe 2′7′-dichlorodihydrofluorescin diacetate (H_2DCF–DA). The cell cultures were exposed to increasing dilutions of composite resins in cell culture medium for 1 h. Mean fluorescence intensities were obtained by histogram statistics using the WinMDI program (Version 2.8), and mean fluorescence intensities were normalized to untreated control cultures (no material) (=1.0). *Bars* represent median values plus 25% and 75% percentiles calculated from individual histograms (n=4); **a** indicates statistically significant differences between median ROS values detected in untreated and treated cell cultures exposed to extracts of specimens covered with a polyester film during light curing; **b** indicates statistically significant differences between median ROS values detected in untreated and treated cell cultures exposed to extracts of specimens prepared in the absence of a polyester film

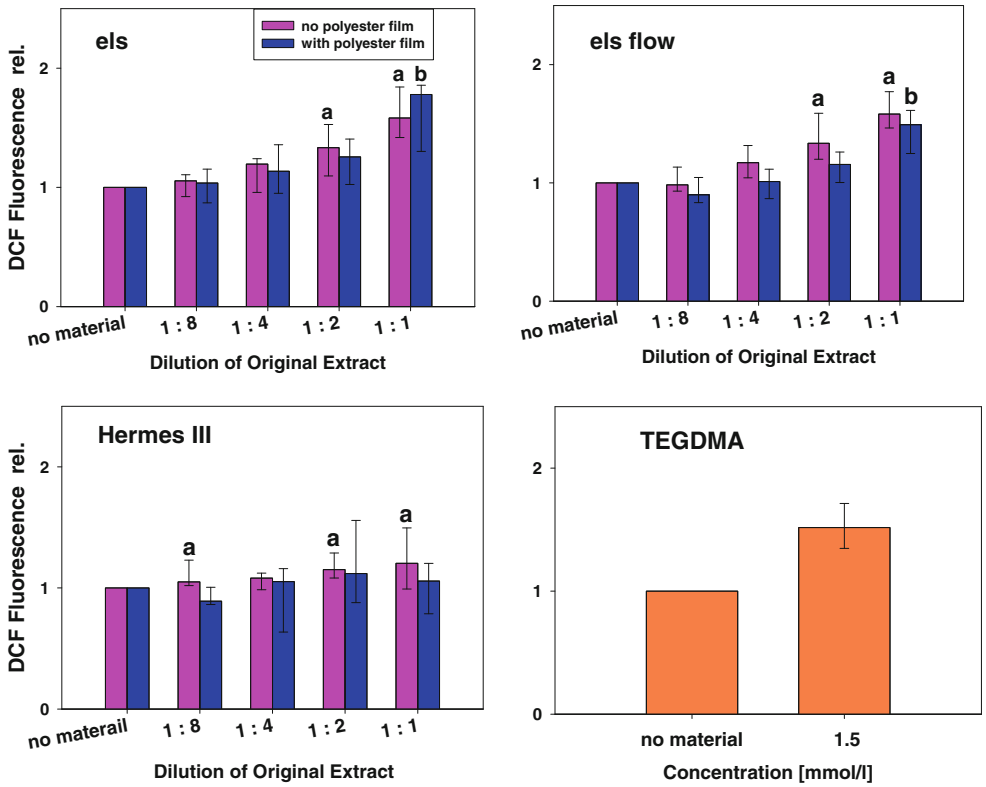

Fig. 2 Generation of reactive oxygen species (ROS) in human pulp-derived cells after exposure to extracts of methacrylate- (free of TEGDMA or HEMA) and silorane-based composite resins. The production of ROS was measured using the oxidation-sensitive fluorescent probe 2′7′-dichlorodihydrofluorescin diacetate (H_2DCF–DA). The cell cultures were exposed to increasing dilutions of composite resins in cell culture medium for 1 h, and 1.5 mmol/l TEGDMA was used as a control (n=12). Mean fluorescence intensities were obtained by histogram statistics using the WinMDI program (Version 2.8), and mean fluorescence intensities were normalized to untreated control cultures (no material) (=1.0). *Bars* represent median values plus 25% and 75% percentiles calculated from individual histograms (n=4); **a** indicates statistically significant differences between median ROS values detected in untreated and treated cell cultures exposed to extracts of specimens covered with a polyester film during light curing; **b** indicates statistically significant differences between median ROS values detected in untreated and treated cell cultures exposed to extracts of specimens prepared in the absence of a polyester film

absence of a polyester film, and similar amounts of ROS were detected in cell cultures exposed to extracts of the low viscous els flow (Fig. 2). The slight differences between median values of ROS production caused by extracts of els and els flow prepared from specimens that were covered with a polyester film during preparation were not significant. Interestingly, the generation of ROS in cell cultures exposed to extracts of the silorane-based composite resin Hermes III was very low, and a significant increase in ROS levels was not detected in the absence of a polyester film compared with untreated cells (Fig. 2). The resin monomer TEGDMA was used as a positive reference for ROS production. A concentration of 1.5 mmol/l TEGDMA increased the amount of ROS about 1.5-fold (Fig. 2).

Cytotoxicity of dental composite materials

The cytotoxicity of the same materials tested for ROS generation was analyzed for the detection of cell survival using a crystal violet assay. The lowest rates of cell survival were detected with extracts of Vitrebond, again included as a positive control material (Fig. 3). Different cytotoxic effects were detected with the various methacrylate- and silorane-based materials depending on the mode of sample preparation and the concentrations of extracts tested (Fig. 3). Undiluted extracts of Tetric Ceram and Tetric EvoCeram significantly reduced cell survival to 85% and 86% (p<0.003). Cell survival rates in extracts of samples covered with a polyester film during light curing slightly increased. However, the differences between median cell survival rates observed in cultures treated with extracts of both materials cured in the presence or absence of a polyester film were not significant. Likewise, undiluted extracts of els caused a decrease in cell survival to 89% compared with untreated controls (p≤0.008; Fig. 4). With original extracts of els flow, about 72% of the cell population survived in treated cultures (p≤0.001) when the samples were made in the absence of a polyester film.

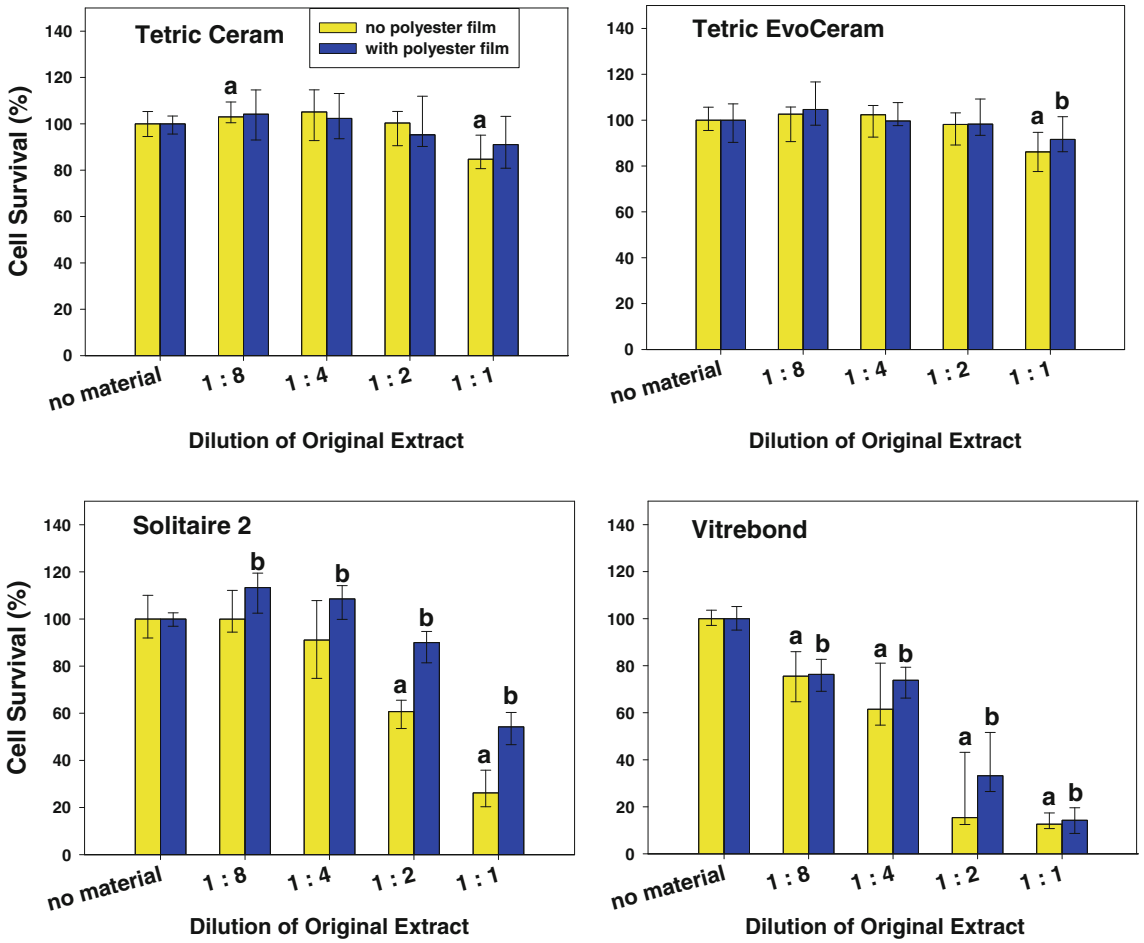

Fig. 3 Cell survival of human pulp-derived cells after exposure to extracts of methacrylate-based composite resins. The resin modified glass ionomer (RMGI) cement Vitrebond was included as a reference material. *Bars* represent median values plus 25% and 75% of cell survival data normalized to original optical density readings (absorbance at 600 nm) and calculated from four replicate cultures per concentration in at least four independent experiments (n=16). **a** Indicates statistically significant differences between median values detected in untreated and treated cell cultures exposed to extracts of specimens covered with a polyester film during light curing; **b** Indicates statistically significant differences between median values detected in untreated and treated cell cultures exposed to extracts of specimens prepared in the absence of a polyester film

Yet, cell survival significantly increased to 88% after exposure to extracts of els flow cured in the presence of a polyester film ($p \leq 0.004$; Fig. 4).

Undiluted extracts of Solitaire 2 made in the absence of a polyester film reduced cell survival to 26% compared with untreated cultures ($p \leq 0.000$; Fig. 3). However, after the application of the polyester film, a significant increase in cell survival was observed ($p < 0.0014$). Cell survival rates were significantly higher in cultures treated with extracts prepared from materials which were cured in the presence of a polyester film ($p < 0.004$). Nevertheless, Solitaire 2 was the most toxic material tested compared with original extracts of Tetric Ceram, Tetric EvoCeram, els and els flow in the absence of a polyester film ($p \leq 0.000$). In contrast to the methacrylate-based composite resins, Hermes III, a silorane-based composite resin, did not decrease pulp cell survival (Fig. 4). Noteworthy is that differences between medians of cell survival rates of original extracts of Hermes III, and all other materials tested here were statistically significant ($p \leq 0.01$). Therefore, the materials cytotoxicity based on cell survival detected in cultures exposed to undiluted extracts was ranked as follows from the most to the least toxic material: Solitaire 2>>els flow>Tetric Ceram=Tetric EvoCeram=els>Hermes III (Fig. 5a). Correlation analysis between cell survival (median values) and ROS production caused by the methacrylate- and silorane-based materials was found to be exponential. An increasing formation of ROS and a decrease in cell survival caused by materials not covered with a polyester film during preparation were positively correlated as indicated by a high correlation coefficient (r^2=0.95; Fig. 5b). A lack of such a high positive correlation, however, was detected when materials were covered with a polyester film (Fig. 5b).

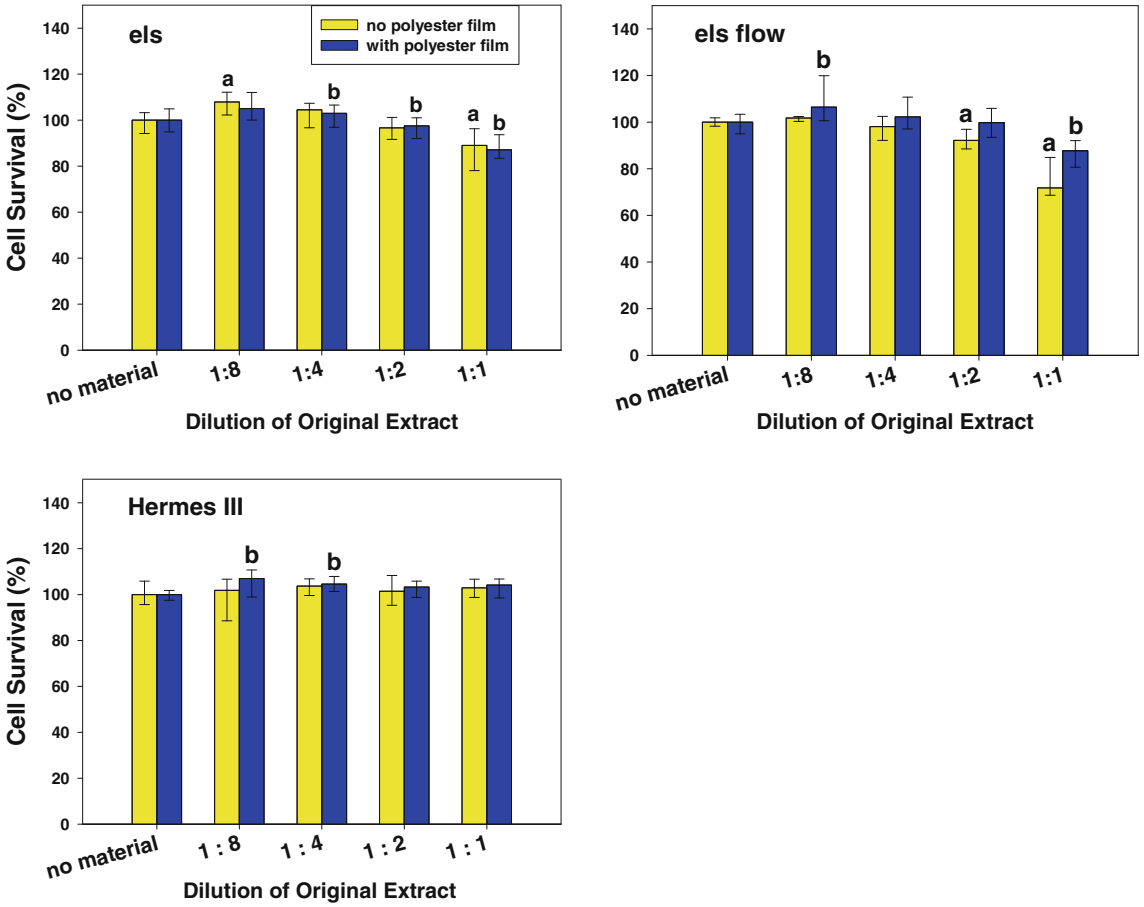

Fig. 4 Cell survival of human pulp-derived cells after exposure to extracts of methacrylate- (free of TEGDMA or HEMA) and silorane-based composite resins. *Bars* represent median values plus 25% and 75% of cell survival data normalized to original optical density readings (absorbance at 600 nm) and calculated from four replicate cultures per concentration in at least four independent experiments (n=12–16). **a** Indicates statistically significant differences between median values detected in untreated and treated cell cultures exposed to extracts of specimens covered with a polyester film during light curing; **b** Indicates statistically significant differences between median values detected in untreated and treated cell cultures exposed to extracts of specimens prepared in the absence of a polyester film

Discussion

The formation of ROS occurs in natural cellular processes, but also results from the exposure of cells to UV light, ionizing radiation (IR), and chemicals. Besides the risk of cellular damage caused by ROS generation, particularly the hydroxyl radical, which is the most abundant ROS, is discussed as an ideal mediator of signal transduction processes [23]. Dentine resin monomers induce oxidative stress through the formation of ROS beyond the redox-regulating capacities in cultured cells derived from various oral tissues [11, 16]. This imbalance in the cellular redox homeostasis, caused for instance by TEGDMA and HEMA, is at least in part a consequence of the depletion of the intracellular anti-oxidant system [24, 25]. Although it is well established that free monomers decrease the amount of reduced GSH, which protects redox-sensitive groups in lipids, nucleic acids, and proteins from oxidation, an increase in oxidized GSSG was not found. It is possible that GSH covalently binds to resin monomers similarly to the formation of adducts between HEMA and the pharmaceutical antioxidans *N*-acetylcysteine adduct [26]. An increase in the intracellular levels of ROS is associated with a modification in cellular transduction pathways regulating cell survival, proliferation, or apoptosis in the presence of small monomers like TEGDMA or HEMA at low concentrations [9, 16, 27, 28]. In this context, it is worth mentioning that, for instance, HEMA concentrations about 15–20 times lower than that available in the extracellular culture medium were found in 3T3 fibroblasts, and toxic effects were observed with intracellular HEMA concentrations as low as 0.2 mmol/L [26].

In the current study, alterations in the intracellular redox state of human pulp-derived cells as indicated by ROS production were determined after exposure to extracts of dental composite materials. DCF-DA (2'7'-

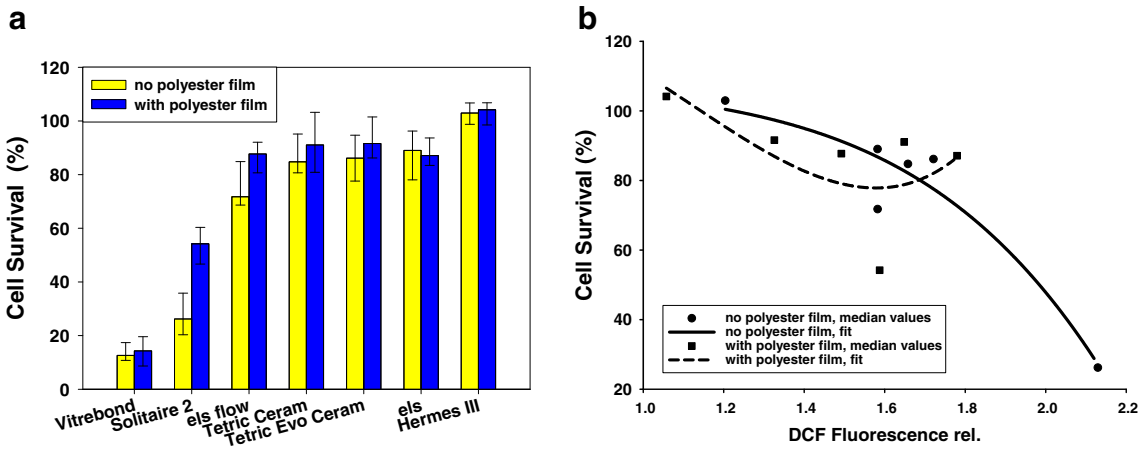

Fig. 5 **a** Cell survival of human pulp-derived cells after exposure to extracts of dental resin composites. *Bars* represent median values plus 25% and 75% of cell survival rates after a 24 h exposure of cell cultures to undiluted extracts of specimens prepared in the presence and absence of a polyester film. The values are taken from Figs. 3 and 4 (dilution 1:1) to present them here for comparison. **b** Correlations between median values of ROS production and cell survival rates analyzed using best fits; $y = a + bx^2 + ce^x$ is the corresponding equation for both fits between median values calculated from specimens prepared with and without polyester film. The correlation coefficient between median values calculated from specimens prepared without polyester film was $r^2 = 0.91$, and a correlation coefficient $r^2 = 0.42$ was calculated between median values obtained from specimens prepared with a polyester film

dichlorodihydrofluorescein diacetate) has been used as a detector of ROS by flow cytometry. The nonionic, nonpolar DCFH–DA is hydrolyzed enzymatically by intracellular esterases to the nonfluorescent DCFH, which is then oxidized to highly fluorescent DCF in the presence of ROS. Therefore, the intracellular DCF fluorescence can be used to quantify oxidative stress and compare ROS generation in treated and untreated cell cultures [29].

As expected, the resin monomer TEGDMA, a compound frequently used in methacrylate-based composite materials, enhanced ROS production similarly to previous observations [30]. In addition, all material extracts tested here affected the redox balance of human pulp-derived cells after only a short exposure period. The formation of ROS and the induction of cytotoxicity by extracts of methacrylate-based resin composites in a dose-dependent manner were very similar. Although light curing of the composite resins against a polyester film prior to extraction slightly reduced ROS production in some cases, these effects were not significant. Likewise, cell survival increased in human pulp-derived cells after exposure to extracts of composite specimens covered with a polyester film during preparation. These effects were most pronounced with extracts of composites containing TEGDMA or HEMA. The use of a polyester film prevents oxygen inhibition of the composite resin surface during light curing [5, 6]. Consequently, polymerization rates increase and a smaller amount of unreacted resin monomers is released from a composite into a solvent. In the absence of a polyester film, however, incomplete polymerization may occur, and hydrophilic monomers released from materials may reach pulp tissues depending on the remaining dentine thickness in concentrations effective to disturb cellular redox homeostasis [7]. Besides the induction of apoptosis, DNA damage and a delay of the cell cycle by even low concentrations of methacrylate-based resin monomers in primary human pulp cells, inhibition of the mineralization capacities of odontoblast-like cells was observed [17, 31, 32].

In this investigation, Solitaire 2 induced the highest amount of ROS among the methacrylate-based composite resins, and this oxidative stress is most likely related to the reduction in cell survival. A high positive correlation ($r^2 = 0.91$) was detected between all median values of ROS production and cell survival rates when specimens were prepared in the absence of a polyester film. This finding indicates that cytotoxic effects of dental composites depend on their capability to create reaction oxygen species, most likely because of an increased amount of free monomers released from an oxygen-inhibited surface layer of composite specimens [6]. However, no such correlation ($r^2 = 0.42$) was detected when specimens were prepared in the presence of a polyester film, suggesting that compounds other than methacrylic monomers added to the cytotoxicity of composite materials.

Cytotoxic effects of Solitaire 2 similar to the present observations were previously detected in different cell lines, and extracts of Solitaire 2 caused the least cell survival among several composite resin restorations [33]. Here, the cytotoxic effects of extracts of Solitaire 2 were drastically reduced when specimens were prepared in the presence of a polyester film. Cytotoxicity was most likely related to the release of monomers like TEGDMA. The concentration of

TEGDMA detected in undiluted extracts of Solitaire 2 correlated with cytotoxic concentrations of the TEGDMA monomer [33]. Even more, these monomer concentrations may be relevant in pulp tissue responses in a clinical situation [7]. Comparing Tetric Ceram and its replacement Tetric EvoCeram, the absence of the monomer TEGDMA in the new formulation had no significant effect on ROS production or cell survival under the current experimental conditions. Although the production of ROS by Tetric EvoCeram was slightly but not significantly reduced when specimens were covered with a polyester film during preparation, this protective effect on cell survival was not obvious with Tetric Ceram. Both materials were, however, equally effective in causing cell death as detected using a crystal violet assay. It is possible that HEMA is the cause of the cell responses observed in the presence of Tetric EvoCeram, since this small hydrophilic monomer was detected in Tetric EvoCeram extracts [34]. The observation that methacrylate-based monomers such as TEGDMA and HEMA caused decreased viability are consistent with earlier reports that showed cytotoxicity of biologically active monomers or additives of composite resin restorations [7, 10, 11, 33].

The materials els and els flow are based on methacrylate chemistry free of HEMA and TEGDMA according to the manufacturer's information, and TEGDMA was not extracted into aqueous and organic solvents as reported in another recent study [4]. Extracts from both materials increased the formation of ROS to some extent, and els and els flow were equally effective. Reduced cell survival was also observed. The decrease in cell survival caused by els flow extracts prepared from materials not covered with a polyester film is most likely due to incomplete polymerization of the higher amount of methacrylic monomers present in the flowable composite resin compared to els, as also suggested for the effects caused by other materials [35, 36]. Noteworthy is that the cytotoxicity of els flow extracts were clearly reduced when specimens were prepared in the presence of a polyester film.

Information on the biocompatibility of the silorane-based composite is minimal compared with the vast amount of data reported on classical methacrylate-based materials. Interestingly, the silorane-based composite resin Hermes III, which is an experimental formulation of Filtek silorane tested in the current study, led to no significant increase in ROS production. Moreover, unlike all other materials analyzed here, extracts of the material were not tested as being cytotoxic. It is most likely that the low solubility of silorane monomers in water adds to the absence of cell responses observed under the current experimental conditions. It has been recently reported that silorane monomers were released from a silorane dental material into an organic but not into an aqueous solution [37]. Yet, earlier investigations found no induction of genetic toxicity by silorane monomers [38, 39]. Observations in part similar to our present findings have been reported previously. While cytotoxic effects of the material were detected immediately after exposure in direct contact with cells, no cytotoxicity was discovered after aging of material specimens in artificial saliva. Thus far, no such increase in cell viability under these experimental conditions was detected with materials based on methacrylate chemistry [40]. Yet, cytotoxic effects of extracts of methacrylate-based materials tested here are still remarkably low compared with Vitrebond, a light curing glass ionomer cement.

In conclusion, the present study adds new information to our knowledge about the cytotoxicity of dental composite resin restorations based on different chemistries in 2D cultures of relevant target cells. Both traditional and new methacrylate-based composite resins disturbed the intracellular redox homeostasis to some extent and tested weakly cytotoxic under the current experimental conditions. Using a polyester film during polymerization generally inhibited cytotoxic effects as well as ROS production, although the differences were not statistically significant in most cases. No cytotoxicity, and only a very slight increase in ROS production, was detected with a silorane-based composite resin. The present findings also indicated a positive correlation between ROS production by composite resins and cytotoxicity as indicated in the crystal violet assay. These observations imply that, depending on the residual dentine layer in deep cavities in a clinical situation, biologically active resin monomers released from composite restorations may influence the dentine–pulp complex, for instance, its regenerative and reparative capacities. On the other hand, it was also assumed that the highest concentrations of TEGDMA released from composites into an organic solvent like methanol were much lower than required to cause cytotoxicity in gingival tissues [4]. Therefore, acute cytotoxicity in these tissues appears to be of minor relevance.

Acknowledgments This study was supported by the University of Regensburg Medical Center. The authors are grateful to Mrs. Christine Ross-Cavanna for a critical reading of the manuscript.

Conflict of interest The authors do not have any conflict of interests.

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