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Human gingival keratinocyte response to substances eluted from Silorane composite material reveal impact on cell behavior reflected by RNA levels and induction of apoptosis

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ABSTRACT

Objectives. The aim of this study was the characterization of siloran-derived composite eluates in conjunction with their putative impact on human gingival keratinocytes (HGK), i.e. levels of total RNA and induction of apoptosis compared to a methacrylate-based material.
Methods. Standardized Filtek™ Silorane specimens ($n = 20$) were subjected to scanning ion monitoring to detect monomer masses between 100 and 1000, after storage in human saliva, and 75% ethanol for up to 28 days. In order to evaluate the effect on cells, HGK were exposed to eluates from Filtek™ Silorane, Filtek™ Supreme XT and control medium for 1 and 4 days, prior to isolation of total RNA, and Annexin-5 fluorescence labeling indicating induction of apoptosis.
Results. Irrespective of the mode and storage time, SIM identified discrete peaks, corresponding to masses of "393" and "337". In response to both composite eluates, an effect on HGK was reflected by drastically reduced levels of isolated total RNA at each time period (after 1 day: control: 302 ng/μl; Filtek™ Silorane: 128 ng/μl, Filtek™ Supreme XT: 129 ng/μl and after 4 days: control: 528 ng/μl; Filtek™ Silorane: 162 ng/μl, Filtek™ Supreme XT: 166 ng/μl). Exposure to eluates from both composite materials yielded apoptosis induction in HGK, as demonstrated by a significant increase of cells exhibiting Annnexin-5 fluorescence.
Significance. Two distinct peaks were identified, which indicated the presence of corresponding substances. The composite-derived effects on HGK strongly suggest a negative impact on cells, as revealed by a clear reduction of total RNA levels, and significant increase in induction of apoptosis.

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1. Introduction

Due to the demands of esthetic restorative dentistry, several composite materials have been developed thus far. In this context, the degree of polymerization affects the physical properties and the clinical performance of resin composite materials [1,2], and therefore plays an important role in determining the ultimate success of the restoration [3]. In comparison with currently used composite materials based on methacrylate monomers, the innovative composite material, Filtek™ Silorane, exhibits less polymerization shrinkage. The hybrid Silorane comprises siloxane and oxirane functional moieties [4], and the highly hydrophobic nature of the Siloranes and the cycloaliphatic oxirane functional groups yield lower shrinkage [5]. The oxiranes, as cyclic esters, polymerize by a cationic ring-opening mechanism [5], thereby leading to reduced polymerization shrinkage.

The release of substances from dental composite materials after polymerization and their possible toxicity have been widely examined [6–8] during previous years. Monomers of the resin matrix have been shown to be eluted a long time after polymerization, e.g. 1 and 3 months or even 1 year after polymerization [9–13]. Several in vitro studies [14–18] have shown cytotoxic, genotoxic, mutagenic or estrogenic effects by some of the monomers released from the composite materials. However, limited information is available about the elution of substances from Silorane composite and its cell or tissue compatibility. As reported by Kopperud et al. [19], no substances were found to be eluted from Filtek™ Silorane in water, while Silorane monomers were found to be eluted in ethanol solution. Currently, only one study on cytotoxicity has been published by Krifka et al. [20], who, amongst others, investigated Silorane composite material-derived effects on human pulp-derived cells. The findings of this study [20] revealed no significant signs of cytotoxicity, while a slight increase in reactive oxygen species was detected. Following elution, composite substances not only affect the pulp, but also can exert putative hazardous effects on the periodontal tissues, including the gingival epithelium. Hence, composite compounds may affect cells regarding such facets as synthesis of RNA, and in addition to adhesion, proliferation, and differentiation, as well as apoptosis. In this context, early stages of the programmed cell death scenario can be detected by fluorescence dye-based Annexin-V labeling, rendering a definitive marker for induction of apoptosis [21,22].

Therefore, the aim of the present study was to identify substances released from the Silorane composite material Filtek™ Silorane following eluent exposure, i.e. human saliva and 75% ethanol. Thereafter, cell culture medium-based eluate effects of Filtek™ Silorane were evaluated and compared to the ones of the methacrylate-based composite material Filtek™ Supreme XT on the levels of total RNA and induction of apoptosis of human gingival keratinocytes.

2. Materials and methods

2.1. Composite materials

In the present study, Filtek™ Silorane (3 M ESPE Dental Products, Seefeld, Germany), a modern composite material, (shade A3) was evaluated, with respect to a possible release of monomers. According to the manufacturer's information the chemical composition of this materials is as follows: Silorane resin (3,4-epoxy-cyclohexyl-ethyl-cyclo-polymethyl-siloxane, bis-3,4-epoxy-cyclohexyl-ethyl-phenylmethyl-silane, initiating system (camphorquinone, iodonium salt and electron donor), quartz filler, yttrium fluoride, stabilizers and pigments.

Additionally, for the evaluation of the cell effects induced by Filtek™ Silorane, the nanohybrid methacrylate-based composite resin Filtek™ Supreme XT) was used as comparison. According to the manufacturer's information, this nanohybrid universal composite material contains methacrylate-based monomers (BisGMA, TEGDMA, UDMA, BisEMA).

2.2. Composite specimen's analysis by SIM

Sample for analysis were prepared using molds, supplied by Dentsply DeTrey (Konstanz, Germany), which allow for the production of standardized cylindrical specimens (diameter 4.5 mm and 2 mm thickness). The molds 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, Switzerland) was placed on top of them, in order to avoid the formation of an oxygen-inhibited superficial layer. Additionally, a glass slide was used, in order to flatten the surface. The specimens were polymerized using a halogen unit (Elipar® Highlight, 3 M ESPE, Seefeld, Germany) with a light intensity of 780–800 mW/cm^2. The spectral irradiance was determined with a visible curing light meter (Cure Rite; Dentsply, USA). The polymerization time was 40 s, according to the manufacturer's instructions. Two different eluents were used: human pooled saliva collected from people without composite restorations, and ethanol 75%. For each eluent, 10 Silorane specimens were prepared.

Directly after curing, each specimen was immediately immersed in 1 ml of the respective eluent, according to the group they belong to. The specimens were stored in a dark box at room temperature, and the eluent was replaced at day 1, days 7, and day 28, after polymerization. From the replaced eluent, solution eluates were prepared, and stored until analysis at 4 °C in the dark.

No information and no standards of the monomer components of the tested composite were available from the manufacturer. Consequently, a classical analysis using High Performance Liquid Chromatography coupled mass spectometry (LC–MS/MS) could not be applied. A triple quadrupole mass spectrometer (Model 1200L) from Varian Inc., was used and a SIM (scanning ion monitoring) on the specimens was performed. The eluates were scanned for any masses between 100 and 1000. Because of the lack of exact chemical information concerning the material, the masses identified were

compared to those published by Kopperud et al. [19], who obtained monomers with molecular masses of 371, 388, **393**, 737, 754, 759, 921, 938, 943, **337**, from the manufacturer of Silorane [19].

2.3. Cell culture

In the present study, immortalized human gingival keratinocytes (HGK) [23] were employed as paradigm of periodontal cells, and maintained in low-calcium keratinocyte growth medium (keratinocyte growth medium 2, KGM2, with provided supplements, Promocell, Heidelberg, Germany), containing 100 μg/ml kanamycin (Sigma, Mannheim, Germany). The cells were cultivated in wells of a 24-well plate (Falcon, BD Biosciences, Franklin Lakes, USA). Two different exposure times were applied in the present study. For the tested period of 1-day 1×10^5 cells/cm^2 in 500 μl native medium were seeded onto the wells, while for the period of 4 days 5×10^4 cells/cm^2 were seeded, to avoid advanced confluency in the test culture. The cells were kept under standard cell culture conditions (37 °C, 97% humidity and 5% CO_2), and were incubated until reaching 80% of confluency, to proceed eluate exposure.

2.4. Generation of HGK-compatible composite eluates and cell exposure

For this part of the study, 20 samples from each tested composite material were prepared as described above. The polymerization of the samples took place according to the manufacturers instructions. The samples of Filtek™ Supreme XT were cured for 20 s and the samples of Filtek™ Silorane for 40 s. To create cell-compatible composite eluates, each specimen was immersed in 1 ml medium KGM2. Half of the specimens were stored in a dark box at room temperature for 1 day, and the other half for 4 days. At the end of each storage period, the eluate, in which the composite samples (1 ml) were immersed, was applied to the respective HGK. The HGK were incubated for 1 day or for 4 days, according to the group they belong to. For each exposure period, equal amounts of HGK were cultivated in native medium as negative controls. After the respective time period, 8 repetitions from 2 independent biological replicates were pooled from each tested time period for total RNA extraction, respectively, in order to average out variations in cell numbers which could have otherwise resulted in variations in total RNA concentration. Additionally, 2 repetitions of the 2 biological replicates were used for Annexin-V detection by fluorescence microscopy, respectively.

2.5. Extraction of total RNA and measurement of concentration

After cultivation, the total RNA was isolated from the cell cultures using the RNeasy mini kit (Qiagen, Hilden, Germany), according to the manufacturer's instruction. The purified RNA of all samples was eluated with the same amounts of RNAse-free water. The specimens' derived RNA concentration and integrity was determined, using an automated electrophoresis system (Experion BioRad, München, Germany). Mean concentrations of RNA, measured from 8 repetitions of 2 independent biological replicates of both treated and untreated cells, were statistically analyzed using the Student's *t*-test for unequal variance ($N = 8 \pm$ SD).

2.6. Determination of apoptosis with fluorescence microscopy

After the exposure periods mentioned above, the eluates were removed, and HGK cultures were washed once with Annexin-V binding buffer (Invitrogen, Darmstadt, Germany). Thereafter, the cells were incubated with 500 μl Annexin-V binding buffer, containing 1 μl Annexin V-FITC detection reagent (Invitrogen, Darmstadt, Germany) for 5 min. The solutions were removed, and the cells were again washed with Annexin-V binding buffer. After this, they were fixed in 2% paraformaldehyde for 20 min. After washing twice with PBS, the cell nuclei were counterstained with 300 nM DAPI-solution. The cells were washed again twice with PBS, and once with distilled water, followed by mounting in Fluoromount-G mounting medium (Southern Biotech, Birmingham, USA), and evaluated by fluorescence microscopy (BZ-9000, Keyence Neu-Isenburg, Germany).

As a positive control for successful Annexin-V staining cell apoptosis was induced by incubating adherent cells with native medium containing 20% DMSO for 1 h at standard cell culture conditions followed by Annexin-V staining as described above.

To calculate the number of Annexin-V-positive cells, 5 representative images of 2-stained wells of the 2 independent biological replicates of both cultivation periods were taken into account in each case ($N = 10 \pm$ SD). The mean of Annexin-V-positive cells was calculated at the ratio of the total cell numbers. These values were statistically analyzed, using the Student's *t*-test for unequal variance.

3. Results

3.1. Analysis by SIM revealed detection of 2 peaks of discrete mass

In the context of putative material-derived effects, we first were interested in testing whether distinct substances could be detected in eluates, derived from Filtek™ Silorane. Due to the lack of Filtek™ Silorane monomer standards, we employed scanning ion monitoring (SIM) to qualitatively seek for eluted compounds between 100 and 1000 *m*/*z* values. Comparison of peak intensities allowed for semi-quantitative estimation (sqe) of eluted substances.

To detect early and late release of substances, the samples were scanned for the above-mentioned mass range, preceding incubation in saliva and 75% ethanol, and for periods of 1, 7, and 28 days, respectively. As exemplified in Fig. 1A (day 1) and B (day 7), SIM of Filtek™ Silorane stored in 75% ethanol, revealed a high peak at 337 (intensity: 26 mass counts) and a small peak at 393 (2.2 mass counts), at all measured time points. Based on the detected mass counts, sqe may indicate predominance of the 337-corresponding composite substance over the substance, which corresponds to 393. Intriguingly, both peaks were detectable in saliva (Fig. 2), more reflecting the physiological elution conditions of the oral cavity.

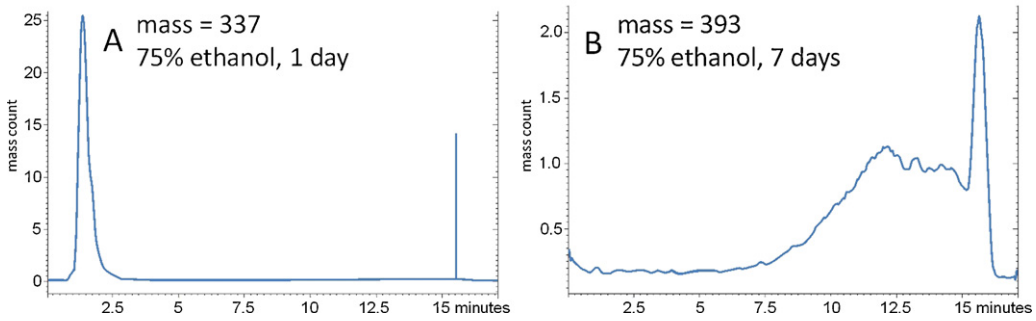

Fig. 1 – Exemplified mass graphs, as detected by scanning ion monitoring (SIM) of a large mass peak 337 after storage in ethanol 75% for 1 day (A), and small mass peak 393 after 7 days (B).

In saliva, exactly like in ethanol, both peaks exhibited clear intensity. The peak corresponding to the mass of 337 (Fig. 2A) was detectable exclusively at 28 days (15.5 mass counts) and the one applied for the 393 mass (Fig. 2B) was detected at day 1 (6.3 mass counts). Similar to ethanol, sqe suggested a predominance of 337 composite-derived substances in saliva as eluent, at day 28. This coincidence in the eluted compounds may suggest a possible presence of the corresponding substances in periodontal tissues and/or cells, respectively. With respect to saliva, the time dependent nature of detection may indicate that compound release in saliva is governed by the time scale.

3.2. *Response of HGK on the composite-eluates was characterized by diminished RNA levels and induction of apoptosis*

The findings on the release of substances from Filtek™ Silorane suggested putative effects on cell response. Since release of compounds could be detected at day 1 for the mass 393, it can not be excluded that effects on cell behavior may occur at rather early stages after composite polymerization, i.e. within the first week. Therefore in the present study we decided to expose HGK to composite eluates, at day 1 and day 4 of elution, and tested for levels of total RNA and induction of apoptosis. For cell compatibility with the eluates, keratinocyte culture medium was used as eluent. As is shown in Fig. 3, the response of HGK to both composite eluates revealed a drastic impact on total RNA levels, as detected by fluorescence dye-based electrophoresis RNA quantification. Both composite materials tested in the present study revealed similar effects on the HGK. With matched medium controls (Fig. 3, blue columns), exposure to eluates from both composite materials (Fig. 3, red and green columns) led to a similar reduction of RNA at day 1 (control: 302 ng/μl; Filtek™ Silorane: 128 ng/μl; Filtek™ Supreme XT: 129 ng/μl), and an even more pronounced reduction after 4 days of elution (control: 528 ng/μl; Filtek™ Silorane: 162 ng/μl; Filtek™ Supreme XT: 166 ng/μl). These findings point to consequences of composite eluate compounds on gingival epithelial kerationocyte RNA synthesis.

In conjunction with RNA levels, further cell response on material substances address apoptotic events. Therefore, HGK cultures were subjected to fluorescence dye-based Annexin-V-labeling, following eluate exposure. In order to discriminate Annexin-V-positive cells from the negative ones, they were labeled with a fluorescent Annexin-V antibody, while the cells' nuclei were counter stained by DAPI. As shown in Fig. 4, HGK cultures exposed to Filtek™ Silorane eluates clearly exhibited a significantly higher rate of cells, being in the early stage of apoptosis, i.e. approximately 4-fold at day 1 (Figs. 4A vs. B and 5, blue vs. red column). The same tendency was observed when HGK were exposed to eluates of Filtek™ Supreme XT. At day 1, almost 8% of the cells were in early stage of apoptosis (Figs. 4C and 5, green column. At the second tested time period the findings differed among the two tested composite materials. In the case of Filtek™ Silorane the exposed cells were fairly similar in their rate of apoptosis compared

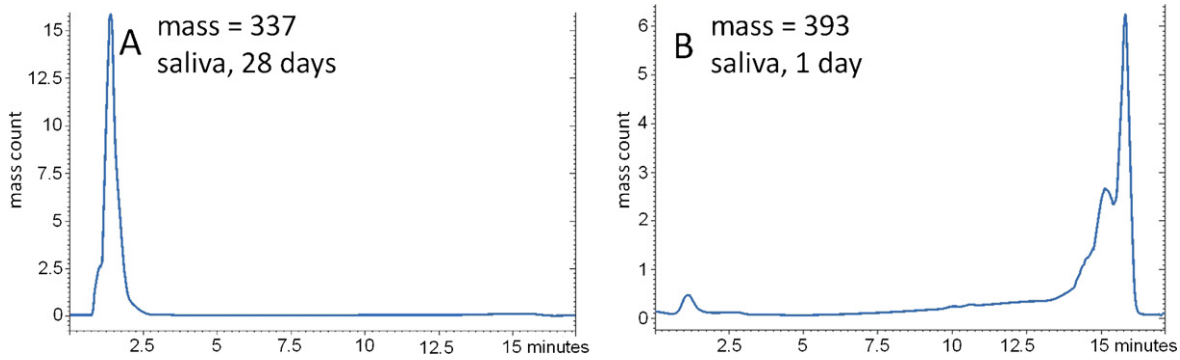

Fig. 2 – SIM reveals mass graphs of a high intensity, annotating to a mass peak of 337, following storage in saliva for 28 days (A), and of lower intensity, corresponding to the mass peak of 393, after 1 day (B).

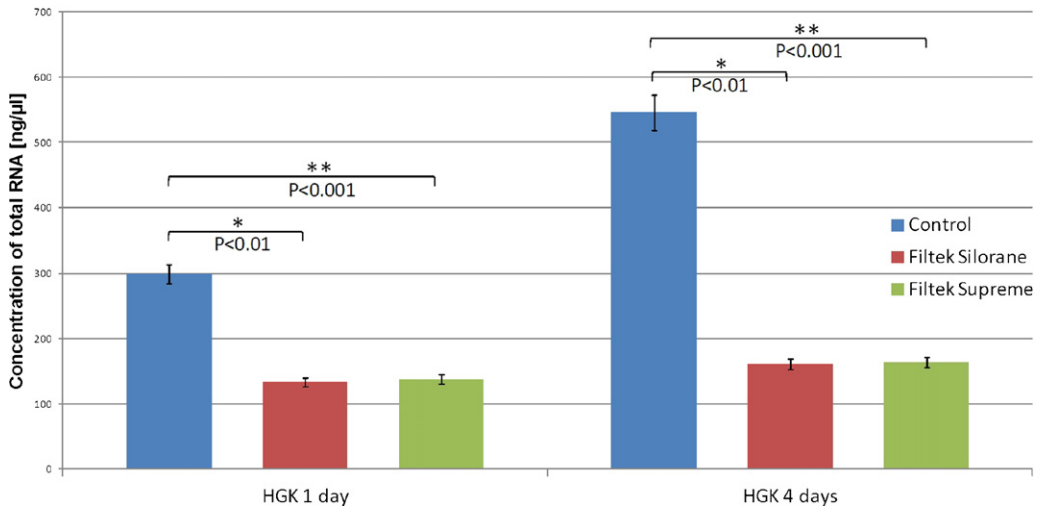

Fig. 3 – Mean total RNA concentrations extracted from 8-pooled wells of two biological replicates, respectively, of Filtek™ Silorane-exposed HGK (red columns), Filtek™ Silorane-exposed HGK (green columns), and non-exposed controls (blue columns). For both tested cultivation periods, the RNA concentrations extracted from the composite-exposed cells is significantly lower than with matched controls. Data represent the mean of total RNA of 8 repetitions of 2 independent biological replicates, respectively (N = 8 ± SD). Asterisks indicate significance ($P < 0.01$) and high significance ($P < 0.001$), analyzed by a Student's t-test for unequal variance.

with the ones of the control group (for comparison, Fig. 4D vs. E; and Fig. 5, blue vs. red column). In contrast to Filtek™ Silorane, almost 60% of the cells treated with the eluates from Filtek™ Supreme XT were found to be in early stage of apoptosis (Figs. 4F and 5, green column). As a positive control for successful staining for apoptotic cells, HGK were treated with 20% DMSO. They showed nearly 100% apoptotic cells (insert A1 in Fig. 4A). As already indicated by the RNA diminishment, eluate exposure may affect the cells. This is further backed up by the induction of apoptosis, which may have at its cause the substances corresponding to the respective peak. The feasibility of apoptotic events only at day 1 may indicate apoptosis as an early eluate-response-event in gingival keratinocytes.

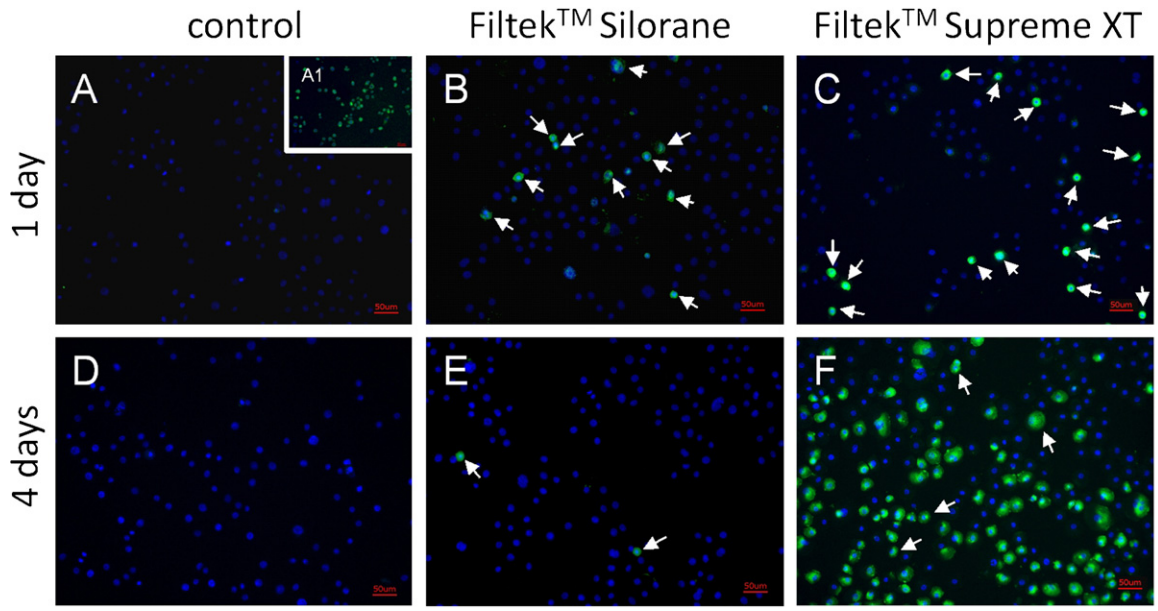

Fig. 4 – HGK are labeled with Annexin-V-FITC (green) to demarcate early apoptotic cells, while DAPI (blue) counter stain indicates total cell nuclei. Annexin-V-FITC-positive cells in HGK cultures of 1 and 4 days, exposed to untreated medium as control (A) and (D), and with substance-containing eluates, eluted from Filtek™ Silorane (B) and (E) and from Filtek™ Supreme XT (C) and (F). White arrows exemplify Annexin-V-positive cells, being in early stages of apoptosis. As a positive control, insert A1 in A shows successful staining for apoptotic cells, after treatment with DMSO.

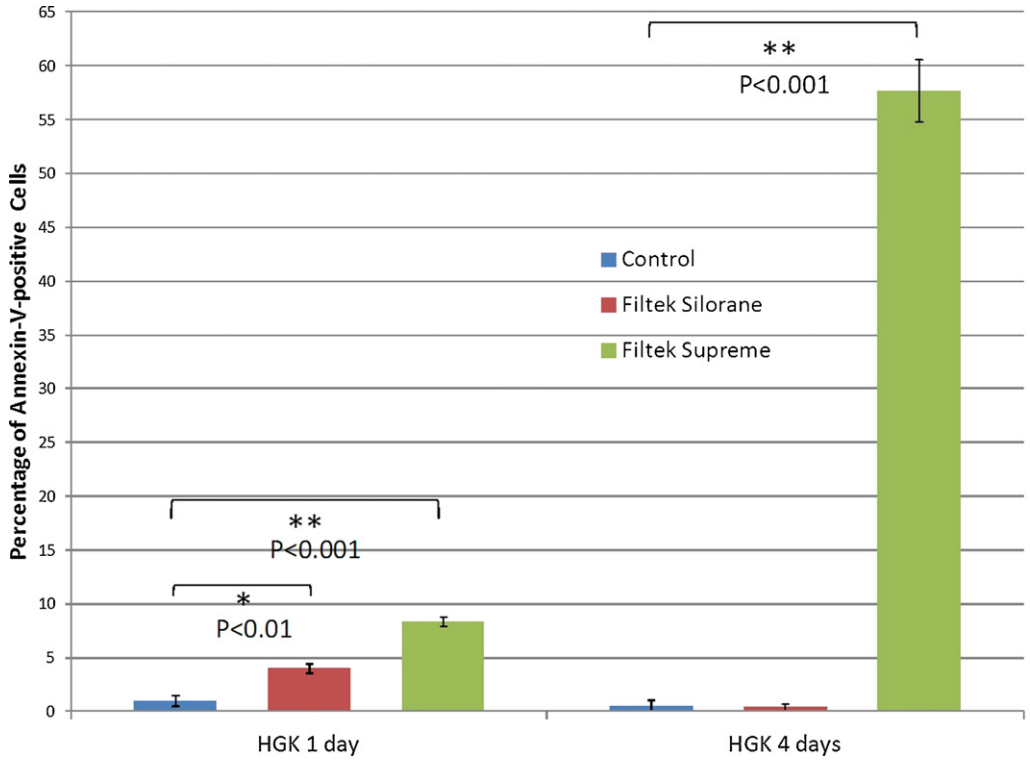

Fig. 5 – Percentage of Annexin-V-positive HGK, obtained from both time periods. After 1 day, a significant increase of Annexin-V-positive cells in exposed HGK cultures (blue and green columns) is found with matched controls (red columns). After 4 days Annexin-V-positive cells found for Filtek™ Supreme XT is significantly increased. Data represent the mean percentage of Annexin-V-positive cells obtained from 5 images of 2 repetitions of 2 independent biological replicates, respectively ($N = 10 \pm SD$). Asterisks indicate significance ($P < 0.01$) and high significance ($P < 0.001$), analyzed by a Student's t-test for unequal variance.

4. Discussion

In the context of esthetic restorative dentistry, an innovative feature of the composite material Filtek™ Silorane exists in the low degree of polymerization shrinkage. Moreover, this composite is assumed to be less cytotoxic, as indicated by the studies of Krifka et al. [20], presumably due to the lower rate of free monomers upon polymerization [19]. Despite these improvements in composite technology, it cannot be excluded that the potential release of remaining monomer substances may exert diffusion-related harmful effects on cells of periodontal tissues. Hence, our investigations aimed at the elucidation of putative Filtek™ Silorane-derived substance effects on periodontal cells from gingival epithelial tissue.

Regarding Filtek™ Silorane, we were first interested in analyzing whether composite compounds could be released and detected in response to different eluents. To gain insight in potential compound release, we conducted elution in eluents such as ethanol 75% rendering a harsh elution regime, but also more physiologic eluents, as reflected by saliva and cell-compatible cell culture medium, respectively. In previous studies [9–12,24–26] we could successfully detect the release of substances using several analytical methods, depending on the substances eluted. As shown in the past [24], scanning ion monitoring (SIM) has been successfully used, to detect standard-independent eluent-released composite substances in the corresponding eluates. In the present study, correlation of masses to eluate yielded the Filtek™ Silorane-derived substance peaks, which are shown in Figs. 1 and 2. In response to our eluents, i.e. ethanol and saliva, 2 distinct mass peaks, namely 337 and 393, could be detected by SIM, showing eluate-dependent intensities at the time points under study. Interestingly, our identified peaks were in concordance with mass peaks, identified in the study by Kopperud et al. [19], using standards of the Filtek™ Silorane composite.

According to the work of Kopperud et al. [19], the mass 393 of the positive ion was the dominant mass of a Silorane monomer bis-3,4-epoxycyclohexylethyl-phenylmethylsilane, while the mass 337 corresponded to the positive ion of the isopropyl-methyl-dephenyliodonium, which is the iodonium salt from the initiator system of Filtek™ Silorane.

In the present study, internal semi-quantitative estimation (sqe) comparison revealed differences in ethanol-derived peak intensity for the detected masses of 337 and 393, as indicated by the predominance of the 337 peak, irrespective of time. For saliva, differential peaks were detected dependent on time, whereat sqe comparison displayed the presence of a small 393 peak at day 1, while the peak corresponding to 337 was of high intensity. The time-dependent appearance of the mass peaks, observed in saliva may be due to the more physiologic, i.e.

aqueous character of this eluent. On the other hand, the constitutive peak presence in ethanol suggests that this eluent is more aggressive, thereby softening the network of composite materials, which determines the higher propensity for substance release [27].

In order to create cell compatible conditions for substances, potentially eluated from the tested composite materials, we consequently employed cell culture medium as eluent, and exposed HGK as paradigm of potentially composite-substance-addressed periodontal tissue cells to the corresponding eluates. In the present study a stable human gingival keratinocytes' cell line was used, in which tissue-specific properties are preserved [23], meaning that the number of biological replicates used are able to give valuable results.

In order to evaluate objectively the effect of Filtek™ Silorane on cells, untreated cell medium was used as negative control and Filtek™ Supreme XT for comparison as a representative methactrylate-based composite material. The choice of Filtek™ Supreme XT to represent the methacrylate-based composite materials was based on previous data [10,12,25,26]. In previous studies [10,12,25,26], we could show that Filtek™ Supreme XT was the composite material with the highest elution of monomers compared to other methacrylate-based materials, indicating higher toxicity. Brackett et al. [28] found in their study that materials with traditional compositions such as Filtek™ Supreme XT can be severely cytotoxic throughout an 8-week interval. According to Franz et al. [29] Filtek™ Supreme XT exhibits higher cytotoxicity on L-929 fibroblasts than the other composite materials tested. In the present study, we could observe clear composite-derived effects on cell behavioral features, such as levels of total RNA and apoptosis. Regarding the significant diminishment of total RNA levels, the comparable numbers of cell nuclei, visible in Fig. 4 at both, exposition to control medium and eluates, leads to the assumption that the substances released by cell culture medium as an eluate may affect RNA synthesis. Of significant interest is the finding that both composite materials showed similar effect on the RNA levels of HGK, while the Silorane technology is thought in the literature to be beneficial compared to methycrate-based composite material as far as their biocompatibility is concerned [5,20,30]. The release of substances from dental methacrylate-based composite materials and the oral and mucosal adverse reactions have been reposted in the past [10,13–15,17,30]. Such potential effects of Silorane-based materials have not been stated up to now. The similar results of Silorane-material in the present study with Filtek™ Supreme XT on the reduction of RNA amount, compared to untreated control, show some possible cytotoxic potential induced from both composite materials.

Depletion of RNA levels may not only occur as a consequence of material-related effects on cells' metabolism in general, but may also reflect other damage including apoptosis. Therefore, we aimed at detection of apoptotic events at very early stages by employing Annexin-V labeling of keratinocyte cultures exposed to eluates obtained from the composite materials. A positive control for Annexin-V labeling of HGK was also used in the study showing successful staining for apoptotic cells. In fact, we have observed significant Annexin V-positive label in both exposed cultures with matched controls at day 1, thereby strongly suggesting the presence of eluate-harboring substances, which obviously appear to be incompatible with cell survival. However, the findings of this test showed a more severe effect on HGK induced by the eluates from the Filtek™ Supreme XT compared to Filtek™ Silorane. The high release of substances from Filtek™ Supreme XT which is observed in previous studies [10,12,25,26] could explain the present findings, corresponding also with the literature [10,13–15,17,30] concerning the cytotoxic effects of methacrylate-based composite materials. Although in the case of Filtek™ Silorane the number of cells, exhibiting early apoptosis, namely 4%, appeared low at first sight, it is firstly important to note that the observed effects are significant, and secondly that it can not be excluded that the total number of Annexin V-demarcated cells would have been increased with prolonged eluate exposure periods, i.e. exceeding 1 day. On the other hand, the virtually low rate of 4% may also lead to the conclusion that 1 day of eluate incubation is sufficient, to drive a higher proportion of cells into later stages of the programmed cell death scenario, thus being already beyond the time slot of early Annexin V detection. Although not directly proven, our results provide evidence for the hypothesis that the mass peaks of 337 and 393 detected by SIM may be involved in causing of the observed cell effects.

The current number of compatibility studies on Filtek™ Silorane, their currently existing number is sparse, while Schweikl et al. [31] have reported low mutagenic potential for Siloranes, hereby proving DNA interference with substances of Filtek™ Silorane. In consequence, substance interference with DNA may also affect RNA, thereby also possibly explaining our lower levels of total RNA, seen in Filtek™ Silorane eluate treated keratinocyte cultures. Moreover, Krifka et al. [20] have published the lack of acute cytotoxicity, as seen for the Filtek™ Silorane derivate "Hermes III". Despite the obvious lack of acute cytotoxic effects, among others, including cell death via necrosis, it is of importance to note in this context that cytotoxicity-derived necrosis and apoptosis are two separate cell death events, based on completely different biological pathways. Thus, as cell damaging effects, they do not per se exclude each other. Hence, our novel findings on early apoptotic events offer no contradiction to the above-mentioned study by Krifka et al. [20].

5. Conclusion

Within the limitations of the present study, the above findings showed for the first time the presence of cell effects derived from substances, eluted from the composite Filtek™ Silorane. These effects were substantiated by evidence of the reduction in levels of total RNA, and of significant numbers of cells shown to be in early apoptotic stages. However, this effect concerning the early apoptosis was minimal compared to the effect observed on cells after exposure to eluates from Filtek™ Supreme XT. In terms of seeking causes for the observed composite-related material impact on periodontal gingival keratinocyte cells, our experiments provide evidence for substances annotating to the SIM-detected 337 and 393 mass peaks.

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