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Antioxidants as a novel dental resin-composite component: Effect on elution and degree of conversion

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ABSTRACT

Objective. Ascorbic acid (Asc) and N-acetylcysteine (NAC) were reported to reduce genotoxicity induced by dental (co)monomers and their epoxy metabolites. The aim of the present study was to investigate Asc or NAC as novel components in light-curable methacrylate based dental composites regarding their effects on degree of conversion (DC) and elution of composite components. Additionally, the release of Asc or NAC was determined.

Methods. Asc or NAC (1, 0.1, 0.01 or 0 wt%) was experimentally incorporated into the composites Venus®, Grandio® and Filtek™ Supreme XTE and polymerized according to the instruction of manufacturers. The samples were elussted in methanol and water. For each composite-antioxidant mixture and elution medium four samples (n = 4) were prepared. The eluates were analyzed by gas chromatography/mass spectrometry (GC/MS), high-performance liquid chromatography/ ultraviolett/diode array detection (HPLC/UV/DAD) and high-performance liquid chromatography/fluorescence detection (HPLC/FLD). DC of composite-antioxidant mixtures was measured in real-time with Fourier transform infrared spectroscopy (FTIR).

Results. The highest concentrations of eluted Asc were 313.98 μM (Venus®-1 wt% Asc; 1 day; methanol) and 245.34 μM (Filtek™ Supreme XTE-1 wt% Asc; 5 min; water). The highest concentrations of eluted NAC were 42.99 μM (1 day; Filtek™ Supreme XTE-1 wt% NAC; 1 day; methanol) and 108.11 μM (Filtek™ Supreme XTE-1 wt% NAC; 7 day; water). Triethylene glycol dimethacrylate (TEGDMA) elution was significantly increased in Venus®-1 wt% Asc and Grandio®-1 wt% Asc (1 day and 7 day methanol/water), compared to control. No significant difference was found for TEGDMA elution in Filtek™ Supreme XTE-1 wt% Asc/NAC. DC was significantly decreased compared to control (= composite without antioxidant) in Grandio® and Filtek™ Supreme XTE after 1, 0.1 and 0.01 wt% Asc incorporation and in Venus® after 1 and 0.1 wt% Asc incorporation. For composite-NAC mixtures, only DC of Grandio®-1 wt% NAC was significantly reduced.

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Significance. Incorporation of NAC (1 wt%), as a novel composite component, into Filtek™ Supreme XTE, had no effect on DC and composite component elution, and supplies sufficient amount of antioxidant which may reduce toxicity. Therefore, it represents a beneficial mixture.

1. Introduction

Methacrylate-based dental resins are frequently used in a clinical context because of their reliable aesthetic properties and physical performance. Light-cured resin composites consist of different (co)monomers and additives [1]. The polymerization of light-cured resin composites is incomplete and the residual (co)monomers and additives can be leachable [2]. They may contact pulp via dentinal tubules, then affect the activity of dental pulp cells or enter the intestine by swallowing, subsequently reaching the circulatory system and organs [3,4]. Additionally, allergic reactions such as asthma and contact dermatitis caused by methacrylates are also reported [5].

Previous studies have demonstrated that the (co)monomers TEGDMA and HEMA can be metabolized to the intermediate methacrylic acid (MA), and further to the epoxy metabolites 2,3-epoxy-2-methyl-propionicacid-methylester (EMPME) and 2,3-epoxy-2-methylpropionic acid (EMPA) [6–9]. These epoxy compounds are considered to be highly reactive molecules and regarded as mutagenic and carcinogenic agents [4,9–12]. Accordingly, teratogenic/embryotoxic effects were observed for EMPA and EMPME in the embryonic stem cells of mice [12]. Furthermore, EMPME and EMPA were reported to elicit severe cytotoxicity and higher rates of DNA double-strand breaks (DNA-DSBs) in human gingival fibroblasts (HGFs), compared to TEGDMA or HEMA addition alone [13,14]. DNA-DSBs can lead to carcinogenic and mutagenic effects [15].

Asc and NAC are regarded as radical scavengers [16]. It was shown that Asc and NAC can reduce the cytotoxicity induced by TEGDMA and HEMA [17,18]. Our previous studies have demonstrated that the addition of Asc or NAC to cell culture medium can reduce genotoxicity of dental (co)monomers and their epoxy metabolites, EMPA and EMPME [14,19,20]. Another study has reported that orally administered mixtures of antioxidants, including Asc and NAC, can reduce ionizing radiation-induced DSBs [21].

Recently, studies on incorporation of NAC into self-polymerizing poly-methyl methacrylate (PMMA)-based dental resin (two component system; typically used in prosthetic dentistry) resulted in a decrease of degree of conversion (DC), compared to untreated PMMA [22].

Till now, no data are available for the effect on DC and elution of composite components after incorporation of Asc or NAC into light-curable methacrylate based dental composites. Therefore, in the present study, Asc or NAC, as a novel composite component in light-curable methacrylate based dental composites, was investigated regarding their effects on DC and elution of composite components. Additionally, the release of Asc or NAC from new mixtures was determined.

2. Methods

The investigated resin-composites including manufacturers' data are listed in Table 1.

2.1. *Sample preparation*

2 g of each resin-composite (Table 1) were prepared by placing the uncured material on a glass plate in the dark, followed by addition of 1 wt% (20 mg), 0.1 wt% (2 mg), 0.01 wt% (0.2 mg) or 0 wt% (0 mg; control group) grounded fine powder of Asc (Sigma-Aldrich, China) or NAC (Sigma-Aldrich, China). Asc or NAC was experimentally incorporated into the uncured composite with a dental spatula for best possible homogenous distribution.

For high-performance liquid chromatography/ultraviolett/diode array detection (HPLC/UV/DAD) –, high-performance liquid chromatography/fluorescencedetection (HPLC/FLD) – and gaschromatography/massspectroscopy (GC/MS)-analyses corresponding composite-antioxidant mixture was polymerized in a Teflon mold (5 mm diameter and 2 mm thickness), placed on a plastic matrix strip (Frasaco, Tettnang, Germany). The uncured mixture was polymerized using a LED-light curing unit (LCU) (Elipar STM 10® high performance LED, 1200 mW/cm^2, 3M ESPE, Seefeld, Germany), according to the instructions of the manufacturers (Table 1). The irradiance of the LED-LCU was controlled with a radiometer (Demetron®, Kerr, USA) and was always between 1100 and 1200 mW/cm^2. The top surface of the composite sample was covered with a plastic strip during polymerization. After sample preparation, samples were transferred into brown glass vials (Macherey-Nagel, Düren, Germany) and 1 ml Methanol (GC Ultra Grade, RATISOLV® ≥99.9%, Roth, Karlsruhe, Germany) or 1 ml water (LC–MS-Grade, ROTISOLV®, Roth, Karlsruhe, Germany) was added. As internal standard (GC/MS analysis) caffeine (CF) solution (0.01 mg/ml) (HPLC ≥99.0%, Sigma-Aldrich, St. Louis, United States) was added. All samples were incubated at 37 °C in the dark. Quadruplets were prepared for each composite-antioxidant mixture and elution medium.

For GC/MS analysis the eluates were measured after incubation for 1 day and 7 day. Water samples were previously extracted with ethyl acetate (LC–MS-Grade, ROTISOLV® ≥99.9%, Roth, Karlsruhe, Germany) (1:1 v/v). To optimize layer separation, the samples were centrifuged at 2800 rpm for 10 min [23].

Measurements of Asc and NAC are not appropriate on GC/MS mode due to strong hydrophilic character and poor evaporability. Therefore, two different HPLC methods were applied:

Table 1 – Investigated dental materials, manufacturers, LOT numbers, types, shades, and polymerization times; composition of each material based on manufacturer's data.

Product name	Type	Manufacturer	LOT	Composition of materials based on manufacturer's data	Shade	Polymerization time
Venus®	Micro-hybrid	Heraeus Kulzer, Hanau, Germany	010504A	Bis-GMA, TEGDMA and contains 587 % filler (by volume), such as Barium Aluminium Fluoride glass; Highly dispersive Silicon Dioxide	A 2	20 s
Grandio®	Nano-hybrid	VOCO GmbH, Cuxhaven, Germany	1650433	Bis-GMA, TEGDMA	A 2	20 s
Filtek™ Supreme XTE	Nano-hybrid	3 M ESPE, Seefeld, Germany	N642628	Bis-GMA, UDMA, TEGDMA, PEGDMA, Bis-EMA; ZrO_2–SiO_2 cluster SiO_2 and ZrO_2 nanofiller	A 2	20 s

The concentrations of Asc in eluates of composite-Asc mixtures were quantified by HPLC/DAD/UV after incubation for 5 min, 1 day and 7 day.

The concentrations of NAC in eluates of composite-NAC mixtures were quantified by HPLC/FLD after incubation for 5 min, 1 day and 7 day.

2.2. *HPLC/UV/DAD analysis*

Eluates of composite-Asc mixtures were analyzed on a LaChrom HPLC/UV/DAD system (Merck, VWR, Darmstadt, Germany). Separations were carried out using a 5 μm, 100 × 4.6 mm Hypersil GOLD AX column and a 5 μm, 10 × 4 mm Hypersil GOLD AX guard column (Thermo Fisher Scientific, Dreieich, Germany). The column was operated at a flow rate of 0.5 ml/min with an isocratic mobile phase for 25 min, consisting of 65% acetonitrile (Merck, Darmstardt, Germany) and 35% 100 nM ammonium acetate buffer (pH 6.8) (Merck, Darmstardt, Germany). The chromatograms were recorded at 267 nm wavelength. The injection volume was 100, 200 or 400 μl.

Identification of Asc was achieved by comparing the retention time and UV-spectrum to an Asc reference standard. A calibration was performed by correlating peak areas of five different reference standard Asc concentrations (10, 20, 100, 200, 400 μM). Quantity of Asc from analyzed samples was calculated accordingly.

2.3. *HPLC/FLD analysis*

To quantify the concentration of NAC in the eluates of composite-NAC mixtures, a derivatization procedure was performed according to Ercal et al. [24] with modifications as described in the following: 100 μl sample eluate was added to 300 μl of a 1 mM N-(1-pyrenyl) maleimide (NPM) (Sigma-Aldrich, India) in Acetonitrile (LC–MS Grade, Roth, Karlsruhe, Germany) and incubated 23 °C for 5 min to form NPM-NAC adduct. To stop the reaction 5 μl of 2 M HCl was added. The resulting solution was analyzed on a LaChrom Elite HPLC/FLD system (VWR, Darmstadt, Germany). Separations were carried out using a 5 μm, 250 mm × 4 mm LiChrospher 60 RP-18 SelectB column and a 5 μm, 4 mm × 4 mm LiChroCart guard column filled with LiChrospher 100 RP-18 (Merck, Darmstadt, Germany). The column was operated at a flow rate of 0.7 ml/min with a gradient using water (HPLC-grade, Millipore, VWR, Darmstadt) with 1‰ Acetic acid (100%, Merck, Darmstadt, Germany)/1‰ Phosphoric acid (85%, Merck, Darmstadt, Germany) and Acetonitrile. Starting at 48%, Acetonitrile was linearly increased over 8 min up to 52% and held for another 12 min. Within 1 min, the eluent was changed to 55% Acetonitrile, held for 14 min, and returned to 48% Acetonitrile within 1 min. The column was reconditioned for at least 10 min. The chromatograms were recorded at an excitation wavelength of 330 nm and an emission wavelength of 380 nm. The injection volume was 50 μl.

Identification of NPM-NAC adduct was achieved by comparing the retention time to NPM-derivatized NAC reference standard. A calibration was performed by correlating peak areas of five different derivatized reference standard NAC concentrations at 50, 125, 250, 500, 1000 nM. Quantity of NAC from analyzed samples was calculated accordingly.

2.4. *GC/MS analysis*

The analysis of the eluates was performed on a Finnigan Trace GC ultra gas chromatograph connected to a DSQ mass spectrometer (Thermo Electron, Dreieich, Germany). A J&W VF-5 ms capillary column (length 30 m, inner diameter 0.25 mm; coating 0.25 m; Agilent, Böblingen, Germany) was used as the capillary column for gas chromatographic separation. Helium 5.0 was used as carrier gas at a constant flow rate of 1 ml/min. The temperature of the transfer line was 250 °C. For sample analysis 1 μl each was injected in splitless mode (splitless time 1 min, split flow 50 ml/min). For capillary transfer the programmable temperature vaporizing (PTV) inlet was heated from 30 °C to 320 °C (14.5 °C/s) and finally held for five min at this temperature. The GC oven was initially heated isothermally at 50 °C for 2 min, then increased to 280 °C (25 °C/min) and finally remained for five min at this temperature. The mass spectrometer (MS) was operated in the electron impact mode (EI) at 70 eV (ion source temperature: 240 °C). Samples were recorded in full scan mode (*m*/*z* 50–600).

Identification of the relevant compounds was achieved by comparing their mass spectra and retention times to the corresponding reference standards. For each reference standard compound a calibration was performed. The quantity of an identified analyte was calculated by correlating its characteristic mass peak area to the corresponding precompiled calibration curve (internal standard caffeine).

2.5. *Degree of conversion (DC)*

The measurements of the DC (n = 6) were performed in real time with an FTIR-Spectrometer with an attenuated total reflectance (ATR) accessory (Nexus, Thermo Nicolet, Madison, USA). The corresponding unpolymerized composite-antioxidant mixture (see Section 2.1) was put directly on the diamond ATR crystal in a Teflon mold (5 mm diameter and 2 mm thickness). The uncured mixture was polymerized using the same LED-LCU with same exposure time mentioned above. The FTIR spectra were recorded in real time for 5 min on the lower surface of the samples. DC was calculated by assessing the variation in peak height ratio of the absorbance intensities of methacrylate carbon to carbon (C—C) double bond peak at 1634 cm^{-1} by employing the aromatic C—C double bond peak at 1608 cm^{-1} as an internal standard during polymerization of the uncured material using Eq. (1).

$$DC_{peak}\% = \left(1 - \frac{\left(1634cm^{-1}/1608cm^{-1}\right)_{peak\ \ height\ \ after\ \ curing}}{\left(1634cm^{-1}/1608cm^{-1}\right)_{peak\ \ height\ \ before\ \ curing}}\right) \times 100 \quad (1)$$

2.6. *Calculations and statistics*

The results are presented as means (standard deviation, SD). The statistical significances ($p < 0.05$) of the differences in the experimental groups were asssnalyzed by one-way ANOVA and the post hoc test (Tukey's HSD test) [25].

3. Results

The results of HPLC, GC/MS and DC are shown in Fig. 1, and Tables 3–6. The samples of Venus® were not measurable after incorporation of 1 wt% NAC due to polymerization without photoinitiation. Therefore no data for HPLC, GC/MS and DC results of Venus®-1 wt% NAC are available.

3.1. *HPLC analysis*

Concentrations of Asc and NAC, released from composite-antioxidant mixtures are shown in Table 3.

3.1.1. *HPLC/UV/DAD*

Asc calibration on HPLC/UV/DAD resulted in a linear calibration curve (Eq. (2)) ($R^2 = 0.999$; limit of quantification (LOQ) = 2.05 μM)

$$y = 40704x - 5613282 \quad (2)$$

Table 2 – Detected eluted composite components.

Compound abbreviation	Compound
HEMA	2-Hydroxyethyl methacrylate
TEGDMA	Triethylene glycol dimethacrylate
CQ	Camphorquinone
DMABEE	4-Dimethylaminobenzoic acid ethyl ester
BHT	2,6-Di-t-butyl-4-methyl phenol
HMBP	2-Hydroxy-4-methoxy-benzophenone
TinP	2(2′-Hydroxy-5′-methylphenyl) benzotriazol
DDHT	Diethyl-2,5-dihydroxytrephthalate
CSA	Champhoric acid anhydride

For composite-Asc mixtures, Asc was detected in methanol and water eluates of all three investigated composites containing 1 wt% and 0.1 wt% Asc. While the concentrations in the eluates of composites containing 0.01 wt% were always lower than LOQ. The concentration of Asc (Venus®-1 wt% Asc) increased from 185.05 μM (5 min; methanol) to 313.98 μM (1 day; methanol) which was the highest concentration of eluted Asc in this study. While in Grandio®-Asc and Filtek™ Supreme XTE-Asc mixtures, the concentrations of eluted Asc decreased with increasing elution time (methanol and water). The highest concentration of eluted Asc in water eluate was 245.34 μM (5 min; Filtek™ Supreme XTE-1 wt% Asc).

3.1.2. *HPLC/FLD*

NAC calibration on HPLC/FLD resulted in a linear calibration curve (Eq. (3)) ($R^2 = 0.999$; LOQ = 0.03 μM)

$$y = 31708x + 5290208 \quad (3)$$

For composite-NAC mixtures, NAC was detected in methanol and water eluates of Grandio® and Filtek™ Supreme XTE containing 1, 0.1 and 0.01 wt% NAC, and Venus® containing 0.1 and 0.01 wt% NAC. The highest concentration of eluted NAC in methanol was 42.99 μM (1 d; Filtek™ Supreme XTE-1 wt% NAC). The highest concentration of eluted NAC in water (108.11 μM) was found for Filtek™ Supreme XTE-1 wt% NAC after 7 day elution, where the eluted concentrations of NAC increased. A NAC increase with increasing elution time was also found for Filtek™ Supreme XTE-0.1 wt% NAC in water.

3.2. *GC/MS analysis*

A total of nine released composite components (Table 2) were detected from investigated composites.

3.2.1. *Composite-Asc mixture*

3.2.1.1. *Venus®-Asc mixture (Table 4).* For Venus®-Asc misxture, CQ, CSA, BHT, TEGDMA, DDHT and HMBP were detected. TEGDMA elution was significantly increased in 1, 0.1 and 0.01 wt% of incorporated Asc after 1 and 7 day elution in methanol and water, compared to control.

3.2.1.2. *Grandio®-Asc mixture (Table 5).* For Grandio®-Asc mixture, HEMA, CQ, CSA, BHT, DMABEE, TEGDMA and TinP were detected. A significant decrease of elution was found for HEMA in 1, 0.1 and 0.01 wt% of incorporated Asc after 1

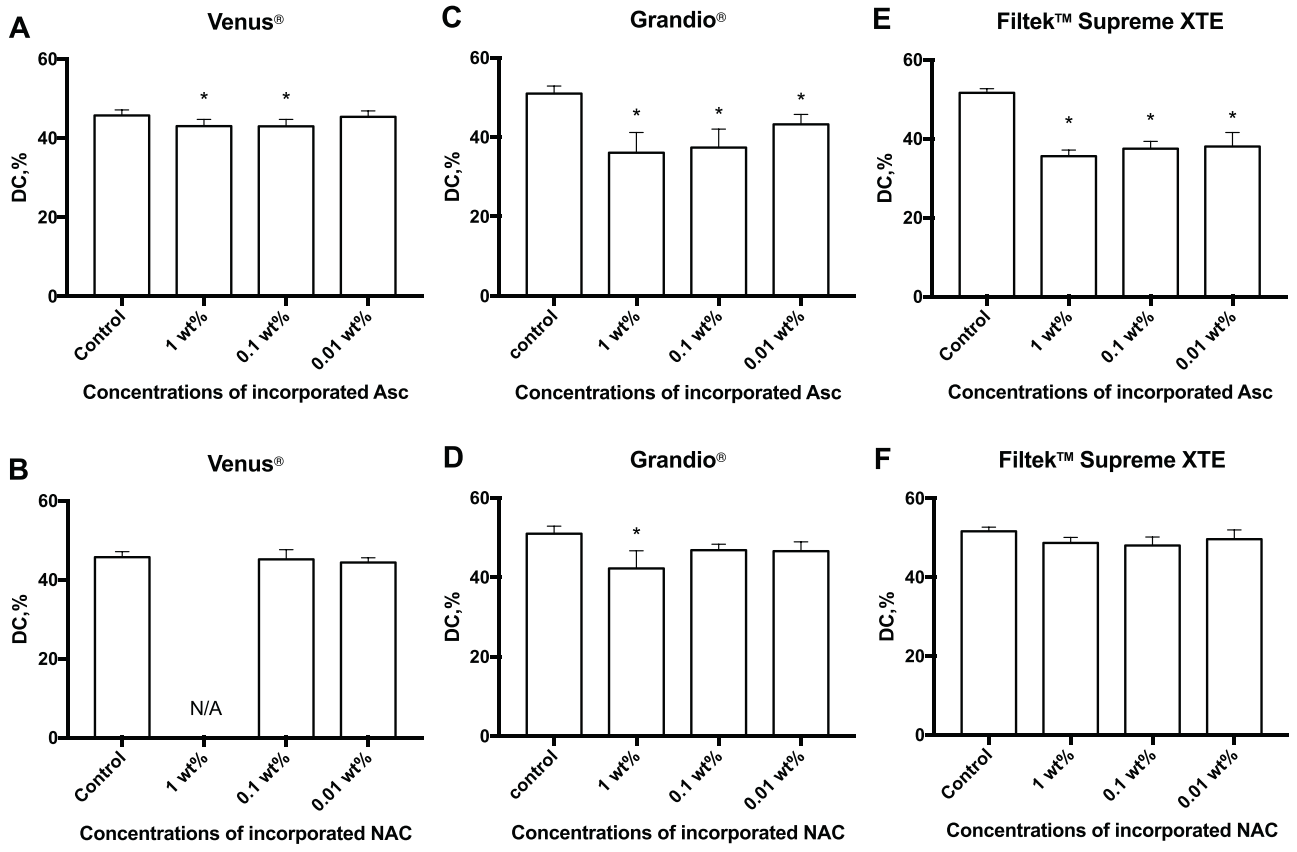

Fig. 1 – Degree of conversion of composite-antioxidant mixture. (A) Venus® -Asc (0.01, 0.1 and 1 wt%). (B) Venus® -NAC (0.01, 0.1 and 1 wt%). (C) Grandio® -Asc (0.01, 0.1 and 1 wt%). (D) Grandio® -NAC (0.01, 0.1 and 1 wt%). (E) Filtek Supreme XTE® -Asc (0.01, 0.1 and 1 wt%). (F) Filtek™ Supreme XTE-NAC (0.01, 0.1 and 1 wt%). Data are presented as mean ± SD, n = 6, *p < 0.05. N/A: no data available.

Table 3 – Elution of incorporated Asc or NAC from composite-antioxidant mixture after 5 min, 1 day and 7 day in methanol and water. Data are presented as mean (SD), n = 4. LOQ: limit of quantification. N/A: no data available.

Elution of Asc or NAC; mean (SD); μM		Venus®		Grandio®		Filtek™ Supreme XTE	
	Methanol	Water	Methanol	Water	Methanol	Water	
1 wt% Asc	5 min	185.05 (18.71)	119.46 (28.46)	195.51 (53.98)	132.69 (26.86)	286.39 (70.58)	245.34 (69.02)
	1 day	313.98 (17.80)	15.72 (5.95)	109.16 (44.70)	10.67 (3.23)	< LOQ	156.38 (50.67)
	7 day	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	18.39 (6.69)
0.1 wt% Asc	5 min	12.09 (3.71)	6.02 (1.42)	11.32 (4.73)	9.15 (1.45)	15.24 (5.57)	13.98 (3.31)
	1 day	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	7.04 (3.04)
	7 day	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
0.01 wt% Asc	5 min	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
	1 day	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
	7 day	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
1 wt% NAC	5 min	N/A	N/A	17.87 (1.67)	48.60 (6.34)	27.43 (8.33)	35.28 (9.41)
	1 day	N/A	N/A	6.72 (0.93)	35.69 (4.28)	42.99 (2.73)	89.39 (19.98)
	7 day	N/A	N/A	2.03 (0.47)	42.25 (5.36)	27.63 (6.87)	108.11 (16.14)
0.1 wt% NAC	5 min	7.20 (1.02)	2.05 (0.46)	5.59 (3.73)	2.18 (0.07)	3.90 (1.87)	2.61 (0.85)
	1 day	24.25 (3.20)	0.33 (0.13)	1.88 (0.68)	0.16 (0.04)	6.95 (3.35)	6.09 (1.53)
	7 day	4.45 (0.72)	0.19 (0.06)	0.45 (0.18)	0.11 (0.03)	4.08 (2.14)	8.20 (1.22)
0.01 wt% NAC	5 min	0.84 (0.14)	0.25 (0.04)	0.52 (0.16)	1.68 (0.91)	0.67 (0.35)	0.47 (0.22)
	1 day	0.20 (0.09)	0.18 (0.11)	< LOQ	< LOQ	< LOQ	< LOQ
	7 day	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ

Table 4 – Qualification and quantification of substances eluted from Venus® incorporated with 1, 0.1 and 0.01 wt% Asc or NAC for 1 and 7 day in methanol and water. Data are presented as mean (SD), n = 4.

TblColHeadVenus®; mean (SD); μM	1 day in methanol				7 day in methanol			
	Control	1 wt% Asc	0.1 wt% Asc	0.01 wt% Asc	Control	1 wt% Asc	0.1 wt% Asc	0.01 wt% Asc
CQ	16.42 (4.43)	19.18 (2.24)[b,c]	10.74 (1.98)[d]	10.23 (1.13)[a,d]	18.37 (3.39)	29.77 (4.29)[a,b,c]	13.68 (1.88)[d]	11.41 (1.40)[a,d]
CSA	9.52 (2.12)	3.70 (0.19)[a,b]	5.69 (0.61)[a]	6.54 (0.88)[a,d]	13.49 (2.18)	5.16 (0.37)[a,c]	10.08 (1.62)[a,b,d]	6.72 (0.74)[a,c]
BHT	2.89 (0.71)	2.92 (0.17)[b,c]	1.27 (0.11)[a,d]	1.19 (0.30)[a,d]	4.30 (0.58)	3.86 (0.45)[b,c]	2.14 (0.27)[a,d]	1.60 (0.35)[a,d]
TEGDMA	630.41 (34.93)	818.51 (44.49)[a]	831.40 (9.63)[a]	752.48 (83.52)[a]	652.86 (23.78)	1147.78 (41.93)[a,c]	1006.23 (86.75)[a,d]	1074.71 (40.55)[a]
DDHT	46.22 (9.65)	57.09 (1.80)[b,c]	45.00 (2.20)[d]	37.75 (2.95)[d]	34.87 (10.04)	92.76 (3.04)[a,b,c]	58.99 (2.55)[a,b,d]	20.05 (1.97)[a,c,d]
HMBP	184.05 (31.82)	179.54 (10.12)[b]	147.54 (10.75)	133.14 (10.77)[a,d]	286.46 (40.89)	324.70 (8.27)[b,c]	263.75 (18.47)[d]	243.58 (12.11)[d]
	1 day in water				7 day in water			
	Control	1 wt% Asc	0.1 wt% Asc	0.01 wt% Asc	Control	1 wt% Asc	0.1 wt% Asc	0.01 wt% Asc
CQ	1.52 (0.17)	2.07 (0.60)[c]	0.86 (0.19)[d]	1.39 (0.21)	2.92 (0.59)	3.76 (0.57)[b,c]	1.31 (0.21)[a,d]	0.98 (0.24)[a,d]
CSA	0.90 (0.13)	0.43 (0.09)[a]	0.54 (0.13)[a]	0.41 (0.04)[a]	1.24 (0.24)	0.61 (0.03)[a]	0.71 (0.17)[a]	0.72 (0.20)[a]
TEGDMA	148.87 (13.07)	191.00 (16.20)[a]	173.18 (9.80)[a]	182.66 (2.15)[a]	138.39 (23.73)	260.30 (9.97)[a,b,c]	224.84 (10.01)[a,d]	215.11 (16.83)[a,d]
	1 day in methanol				7 day in methanol			
	Control	1 wt% NAC	0.1 wt% NAC	0.01 wt% NAC	Control	1 wt% NAC	0.1 wt% NAC	0.01 wt% NAC
CQ	16.42 (4.43)	N/A	29.29 (0.94))[a,b]	15.32 (1.71)[c]	18.37 (3.39)	N/A	48.47 (3.78))[a,b]	24.93 (2.07)[c]
CSA	9.52 (2.12)	N/A	7.48 (0.62)	6.20 (0.63)[a]	13.49 (2.18)	N/A	13.01 (1.48)	10.30 (1.01)
BHT	2.89 (0.71)	N/A	5.61 (0.27))[a,b]	2.68 (0.11)[c]	4.30 (0.58)	N/A	9.03 (0.59)	4.41 (0.37)
TEGDMA	630.41 (34.93)	N/A	754.83 (40.41))[a,b]	548.13 (56.88)[c]	652.86 (23.78)	N/A	769.44 (26.02))[a,b]	620.63 (31.78)[c]
DDHT	46.22 (9.65)	N/A	65.97 (1.43))[a,b]	40.33 (1.46)[c]	34.87 (10.04)	N/A	80.67 (4.02))[a,b]	34.66 (1.17)[c]
HMBP	184.05 (31.82)	N/A	238.98 (13.98))[a,b]	159.85 (1.97)[c]	286.46 (40.89)	N/A	348.96 (25.12))[a,b]	277.87 (11.71)[c]
	1 day in water				7 day in water			
	Control	1 wt% NAC	0.1 wt% NAC	0.01 wt% NAC	Control	1 wt% NAC	0.1 wt% NAC	0.01 wt% NAC
CQ	1.52 (0.17)	N/A	2.55 (0.51))[a,b]	1.85 (0.15)[c]	2.92 (0.59)	N/A	4.67 (1.19)[a,b]	2.75 (0.36)[c]
CSA	0.90 (0.13)	N/A	1.51 (0.16)[a,b]	1.06 (0.05)[c]	1.24 (0.24)	N/A	1.74 (0.22)[a]	1.83 (0.18)[a]
TEGDMA	148.87 (13.07)	N/A	171.54 (6.91))[a,b]	141.77 (1.85)[c]	138.39 (23.73)	N/A	165.70 (8.98)	153.81 (4.59)

N/A: no data available.

[a] Significantly different to control.

[b] Significantly different to 0.01 wt% group.

[c] Significantly different to 0.1 wt% group.

[d] Significantly different to 1 wt% group, $p < 0.05$).

Table 5 – Qualification and quantification of substances eluted from Grandio® incorporated with 1, 0.1 and 0.01 wt% Asc or NAC for 1 and 7 day in methanol and water. Data are presented as mean (SD), n = 4.

Grandio®; mean (SD); μM	1 day in methanol				7 day in methanol			
	Control	1 wt% Asc	0.1 wt% Asc	0.01 wt% Asc	Control	1 wt% Asc	0.1 wt% Asc	0.01 wt% Asc
HEMA	13.25 (2.20)	4.53 (0.92)[a,b,c]	0.09 (0.05)[a,d]	0.08 (0.05)[a,d]	19.29 (2.45)	3.77 (0.81)[a,b,c]	0.43 (0.05)[a,d]	0.28 (0.14)[a,d]
CQ	5.57 (0.85)	20.69 (0.80)[a,b,c]	11.08 (4.57)[d]	8.55 (2.48)[d]	6.10 (0.71)	27.41 (1.38)[a,b,c]	12.38 (0.33)[a,d]	12.04 (3.27)[a,d]
CSA	2.12 (0.16)	2.76 (0.52)[c]	3.68 (0.54)[d]	4.56 (1.41)[a]	2.69 (0.19)	3.91 (1.06)	7.38 (2.73)[a]	4.85 (1.68)
BHT	9.13 (1.21)	20.24 (5.69)[a,b,c]	11.04 (2.06)[d]	10.67 (3.88)[d]	13.30 (1.26)	12.60 (1.82)	19.73 (8.75)	15.27 (5.25)
DMABEE	0.82 (0.22)	13.45 (2.40)[a,b,c]	6.60 (4.25)[a,d]	5.15 (1.42)[d]	1.78 (0.27)	19.74 (1.32)[a,b]	12.23 (8.69)[a]	9.37 (3.68)[d]
TEGDMA	198.13 (61.39)	708.12 (184.02)[a]	474.56 (104.78)	546.38 (150.28)[a]	326.05 (14.99)	672.21 (97.48)[a,b,c]	416.16 (36.98)[d]	472.84 (135.65)[d]
TinP	10.78 (7.57)	37.65 (9.98)[a,c]	18.42 (4.43)[d]	22.54 (7.13)	26.05 (3.14)	61.47 (17.35)[a,b,c]	26.54 (6.93)[d]	36.56 (12.71)[d]
	1 day in water				7 day in water			
	Control	1 wt% Asc	0.1 wt% Asc	0.01 wt% Asc	Control	1 wt% Asc	0.1 wt% Asc	0.01 wt% Asc
HEMA	1.40 (0.16)	0.03 (0.02)[a]	0.03 (0.01)[a]	0.03 (0.02)[a]	0.69 (0.11)	0.04 (0.03)[a]	0.02 (0.00)[a]	0.03 (0.03)[a]
CQ	1.28 (0.34)	5.24 (2.18)[a,b,c]	1.43 (0.49)[d]	1.84 (1.22)[d]	1.34 (0.39)	7.86 (1.99)[a,b,c]	0.95 (0.62)[d]	2.13 (1.56)[d]
CSA	0.76 (0.24)	1.28 (0.10)	1.09 (0.52)	0.80 (0.20)	1.12 (0.24)	1.40 (0.41)[c]	2.03 (0.06)[a,b,d]	1.38 (0.33)[c]
TEGDMA	91.41 (13.55)	167.68 (35.97)[a,b,c]	115.21 (13.05)[d]	119.49 (12.53)[d]	99.47 (9.58)	185.45 (32.56)[a,b,c]	116.25 (22.84)[d]	109.22 (29.41)[d]
	1 day in methanol				7 day in methanol			
	Control	1 wt%NAC	0.1 wt% NAC	0.01 wt% NAC	Control	1 wt% NAC	0.1 wt% NAC	0.01 wt% NAC
HEMA	13.25 (2.20)	13.32 (1.48)[c]	18.17 (0.84)[a,d]	15.79 (1.08)	19.29 (2.45)	15.37 (1.34)[c]	28.17 (12.02)[d]	19.24 (0.99)
CQ	5.57 (0.85)	11.45 (2.05)[a]	9.31 (0.27)[a]	9.59 (2.37)[a]	6.10 (0.71)	15.16 (2.07)[a]	15.20 (0.13)[a]	15.93 (3.24)[a]
CSA	2.12 (0.16)	0.79 (0.18)[a,b]	0.78 (0.17)[a,b]	1.95 (0.52)[c,d]	2.69 (0.19)	1.14 (0.13)[a,b]	1.30 (0.37)[a,b]	3.19 (0.32)[c,d]
BHT	9.13 (1.21)	7.21 (1.34)	12.45 (5.65)	9.53 (1.67)	13.30 (1.26)	8.03 (1.06)[a,b,c]	14.52 (0.91)[d]	14.98 (1.74)[d]
DMABEE	0.82 (0.22)	12.73 (2.09)[a,b,c]	6.95 (0.44)[a,d]	6.40 (1.40)[a,d]	1.78 (0.27)	18.86 (1.37)[a,b,c]	11.51 (0.51)[a,d]	9.11 (1.43)[a,d]
TEGDMA	198.13 (61.39)	216.25 (56.33)	272.27 (6.89)	266.42 (44.75)	326.05 (14.99)	238.80 (12.04)[a,b,c]	309.12 (5.06)[d]	299.37 (16.99)[d]
TinP	10.78 (7.57)	14.66 (4.23)[c]	24.51 (0.05)[a,d]	23.02 (1.19)[a]	26.05 (3.14)	25.88 (1.88)[b,c]	40.74 (2.13)[a,d]	34.42 (2.23)[a,d]
	1 day in water				7 day in water			
	Control	1 wt% NAC	0.1 wt% NAC	0.01 wt% NAC	Control	1 wt% NAC	0.1 wt% NAC	0.01 wt% NAC
HEMA	1.40 (0.16)	1.73 (0.14)[a,b,c]	1.40 (0.17)[d]	1.33 (0.06) [d]	0.69 (0.11)	2.19 (0.33)[a,b,c]	1.11 (0.27)[d]	1.44 (0.27)[a,d]
CQ	1.28 (0.34)	3.37 (1.07)[a,b,c]	1.38 (0.39)[d]	1.62 (0.96) [d]	1.34 (0.39)	6.82 (1.77)[a,b,c]	3.23 (0.86)[d]	3.47 (1.30)[d]
CSA	0.76 (0.24)	0.58 (0.12)	0.90 (0.16)[d]	0.37 (0.10) [a,c]	1.12 (0.24)	0.57 (0.18)[a]	0.85 (0.14)[b]	0.44 (0.07)[a,c]
TEGDMA	91.41 (13.55)	144.00 (5.98)[a,b,c]	111.17 (8.41)[a,d]	105.39 (6.65)[d]	99.47 (9.58)	125.49 (6.22)[a,b]	105.89 (17.08)	96.56 (11.01)[d]

[a] Significantly different to control.
[b] Significantly different to 0.01 wt% group.
[c] Significantly different to 0.1 wt% group.
[d] Significantly different to 1 wt% group, $p < 0.05$).

Table 6 – Qualification and quantification of substances eluted from Filtek™ Supreme XTE incorporated with 1, 0.1 and 0.01 wt% Asc or NAC for 1 and 7 day in methanol and water. Data are presented as mean (SD), n = 4.

Filtek™ Supreme XTE; mean (SD); μM	1 day in methanol				7 day in methanol			
	Control	1 wt% Asc	0.1 wt% Asc	0.01 wt% Asc	Control	1 wt% Asc	0.1 wt% Asc	0.01 wt% Asc
CQ	8.52 (0.21)	7.67 (2.17)	6.69 (1.21)	6.73 (0.26)	8.28 (0.25)	9.99 (1.07)	8.76 (3.24)	8.98 (0.97)
CSA	16.06 (0.02)	1.54 (0.40)[a,b]	1.45 (0.39) [a,b]	0.75 (0.13)[a,c,d]	16.00 (0.03)	2.83 (2.46) [a]	0.93 (0.22) [a]	0.48 (0.07) [a]
BHT	7.11 (0.32)	23.13 (8.54)[a,b,c]	11.72 (1.16)[d]	10.96 (0.72)[d]	10.76 (0.58)	32.60 (23.03)	13.61 (3.76)	13.98 (1.29)
TEGDMA	39.94 (4.04)	27.16 (8.17)	35.60 (9.60)	34.73 (4.73)	40.46 (4.42)	46.41 (4.16)	42.78 (10.13)	33.85 (2.21)
	1 day in water				7 day in water			
	Control	1 wt% Asc	0.1 wt% Asc	0.01 wt% Asc	Control	1 wt% Asc	0.1 wt% Asc	0.01 wt% Asc
CQ	2.67 (0.11)	3.00 (1.77)[c]	1.04 (0.18)[d]	1.49 (0.25)	2.71 (0.10)	4.70 (2.41)[c]	0.55 (0.16)[d]	2.24 (0.88)
CSA	2.61 (0.07)	1.18 (0.42)[a]	1.13 (0.27)[a]	0.75 (0.46)[a]	2.53 (0.08)	1.59 (0.50)[a,b]	0.84 (0.15)[a]	0.49 (0.25)[a,d]
TEGDMA	6.60 (0.30)	5.99 (1.59)[b,c]	13.43 (3.35)[a,d]	10.17 (1.07)[d]	4.09 (0.03)	12.81 (5.56)[a]	11.31 (4.76)	9.61 (1.82)
	1 day in methanol				7 day in methanol			
	Control	1 wt% NAC	0.1 wt% NAC	0.01 wt% NAC	Control	1 wt% NAC	0.1 wt% NAC	0.01 wt% NAC
CQ	8.52 (0.21)	8.82 (0.27)[c]	9.81 (0.60)[a,d]	9.14 (0.25)	8.28 (0.25)	8.38 (0.16)[b,c]	9.53 (0.48)[a,d]	9.07 (0.17)[a,d]
CSA	16.06 (0.02)	16.00 (0.02)	16.06 (0.03)	16.02 (0.01)	16.00 (0.03)	15.98 (0.03)	16.01 (0.03)	15.98 (0.01)
BHT	7.11 (0.32)	6.15 (0.37)[c]	8.94 (0.58)[a,b,d]	6.58 (0.71)[c]	10s.76 (0.58)	8.94 (0.29)[a],[3]	12.66 (1.36)[a,b,d]	10.17 (0.56)[c]
TEGDMA	39.94 (4.04)	35.93 (2.27)	43.75 (3.80)	39.16 (4.95)	40.46 (4.42)	36.52 (2.61)[c]	44.88 (4.24)[d]	40.44 (4.28)
	1 day in water				7 day in water			
	Control	1 wt% NAC	0.1 wt% NAC	0.01 wt% NAC	Control	1 wt% NAC	0.1 wt% NAC	0.01 wt% NAC
CQ	2.67 (0.11)	2.64 (0.14)	2.48 (0.04)	2.56 (0.03)	2.71 (0.10)	2.62 (1.31)	2.47 (0.04)	2.64 (0.05)
CSA	2.61 (0.07)	2.57 (0.03)	2.65 (0.09)	2.65 (0.07)	2.53 (0.08)	2.53 (1.26)	2.51 (0.03)	2.61 (0.08)
TEGDMA	6.60 (0.30)	6.66 (1.31)	7.48 (0.61)	7.22 (0.66)	4.09 (0.03)	5.08 (2.66)	7.42 (0.71)[a]	4.78 (0.20)

[a] Significantly different to control.
[b] Significantly different to 0.01 wt% group.
[c] Significantly different to 0.1 wt% group.
[d] Significantly different to 1 wt% group, $p < 0.05$).

and 7 day elution in methanol and water, compared to control. TEGDMA elution was significantly increased compared to control in 1 wt% Asc incorporation after 1 and 7 day elution in methanol and water; and 0.01 wt% Asc incorporation after 1 day methanol elution.

3.2.1.3. *Filtek™ Supreme XTE-Asc* (*Table 6*). For Filtek™ Supreme XTE-Asc mixture, CQ, CSA, BHT and TEGDMA were detected. TEGDMA elution was significantly increased for 0.1 wt% Asc incorporation after 1 day elution in water; and for 1 wt% Asc incorporation after 7 day elution in water, compared to control.

3.2.2. *Composite-NAC mixture*

3.2.2.1. *Venus®-NAC mixture* (*Table 4*). For Venus®-NAC mixture, CQ, CSA, BHT, TEGDMA, DDHT and HMBP were detected. TEGDMA elution was significantly increased for 0.1 wt% NAC incorporation after 1 day elution in water and 1 and 7 day in methanol, compared to control.

A significantly increased TEGDMA elution was found 0.1 wt%, compared to control.

3.2.2.2. *Grandio®-NAC mixture* (*Table 5*). For Grandio®-NAC mixture, HEMA, CQ, CSA, BHT, DMABEE, TEGDMA and TinP were detected. HEMA elution was significantly increased for 0.1% NAC incorporation after 1 day in methanol, 1 wt% NAC incorporation after 1 day and 7 day in water and 0.01 wt% NAC incorporation after 7 day in water, compared to control. A significant decrease of TEGDMA elution was found for 1 wt% of incorporated NAC after 7 day elution in methanol, compared to control. TEGDMA elution was significantly increased compared to control for 1 and 0.1 wt% NAC incorporation after 1 day elution in water; and for 1 wt% NAC incorporation after 7 day elution in water.

3.2.2.3. *Filtek™ Supreme XTE-NAC mixture* (*Table 6*). For Filtek™ Supreme XTE-NAC mixture, CQ, CSA, BHT and TEGDMA were detected. TEGDMA elution was significantly increased for 0.1 wt% NAC incorporation after 7 day elution in water, compared to control.

3.3. DC

For composite-Asc mixture, DCs of Grandio® and Filtek™ Supreme XTE with 1, 0.1 and 0.01 wt% Asc were significantly decreased (Fig. 1C, E), compared to control. DCs of Venus® with 1 and 0.1 wt% Asc were significantly decreased (Fig. 1A), compared to control.

For composite-NAC mixture, DC of Grandio® with 1 wt% NAC was significantly decreased (Fig. 1D), compared to control. No significant DC change was found for Filtek™ Supreme XTE with 1, 0.1 and 0.01 wt% NAC (Fig. 1F), compared to control.

4. Discussion

The effects of Asc or NAC after incorporation (1, 0.1 and 0.01 wt%) into three different composites (Venus®, Grandio® and Filtek™ Supreme XTE) were determined on the DC, on the elution of composite components and on the release of Asc or NAC.

These investigated composites were selected because previous studies have shown various compositions and high releases of methacrylates and additives [26–28].

From all investigated composites only Venus® samples were not measurable after incorporation of 1 wt% NAC. These results indicate that only for Venus® the incorporation of 1 wt% NAC may lead to an unstable composite mixture which is able to polymerize without photoinitiation.

4.1. *Effect of Asc/NAC on DC*

Photoinitiated polymerization occurs by a chain reaction between the free radicals formed by the photoinitiating system and the monomers [29]. CQ, a photoinitiator, has been widely used for light-cured dental composite [29]. Photopolymerization is initiated by CQ/amine photoinitiating system. In this process, aminoalkyl radical, a key radical initiating polymerization, was produced via photoinduced electron transfer [29]. In addition, a direct hydrogen atom abstraction of triplet state of CQ on the monomer may also form reactive radicals [29]. Hence, the significantly reduced DC after incorporation of Asc or NAC into composites in this study might be due to these antioxidants which can scavenge initiating radicals [30], consequently suppressing the initiating of chain polymerization. Additionally, the efficiency of photopolymerization also depends on the steric structure of the amine-derived radicals, which must approach the reactive unsaturated bond in a monomer [31]. Therefore the incorporation of Asc or NAC might interfere the diffusion of electrons, which impedes the formation of exciplex [32] or further polymerization.

A significant lower DC was found for all investigated composites containing 1, 0.1 and 0.01 wt% Asc (except for Venus®-0.01 wt% Asc), compared to control. While for NAC incorporation, no significant change of DC was detected for Venus® and Grandio® containing 0.1 wt% and 0.01 wt% NAC, and for Filtek™ Supreme XTE with 1 wt% NAC. This indicates that Asc has a stronger influence on DC than NAC. In addition to a stronger ability of scavenging radicals and interfering the diffusion of electrons, another explanation might be that Asc possesses chain-breaking properties by inhibiting free-radical-mediated chain reactions [33].

4.2. *Elution of composite components and effect of Asc/NAC*

For the GC/MS analysis, methanol and water were used as elution media for 1 day and 7 day elution experiments according to our previous studies [23,26].

TEGDMA is a (co)monomer frequently used in composites to enrich the organic resin matrix of composites with a maximum of inorganic filler particles [34]. A significant increase of eluted composite components (e.g. TEGDMA), compared to control, can cause adverse effects [13,35]. Our previous study revealed that an exposure with TEGDMA at concentrations of 360 μM induces 4-fold higher number of DNA-DSBs-foci in HGFs compared to negative control [13]. TEGDMA was detected in the eluates of all investigated composites. The highest TEGDMA concentration in methanol

eluates was 1148 μM (Venus® -1 wt% Asc, 7 day). This is about 3 times higher than the cited genotoxic concentration. However, methanol eluates represent the maximal elutable concentration of (co)monomers and additives [37]. However, water eluates allow the utmost physiological comparison to dentinal fluid and human saliva [27,38]. In comparison, in this study highest concentration of TEGDMA in water was 260 μM (Venus® -1 wt% Asc, 7 day). This is 1.4 times lower than the cited genotoxic concentration.

HEMA is used in dental composites as a (co)monomer of the organic resin matrix due to its hydrophilic application. In previous studies for HEMA a genotoxic concentration at 1100 μM was found in HGFs [13]. HEMA was detected only in the eluates of Grandio. The highest HEMA concentration with 28 μM was detected in the methanol eluate of Grandio-0.1 wt% NAC after 7 day. This is 39 times lower than the cited genotoxic concentration.

Incorporation of Asc into Venus® (1, 0.1 and 0.01 wt%, 1 day/7 day) and Grandio® (1 wt%, 1 day/7 day), and incorporation of NAC into Venus® (0.1 wt%, 1 day) led to a significant increase of TEGDMA in methanol and water eluates, compared to control. These results indicate that the incorporation of Asc or NAC into some composites can promote the release of TEGDMA and accordingly may induce adverse (side) effects (e.g. methacrylate allergy, cytotoxic/genotoxic effects [13,35]).

However, our data also showed that the incorporation of Asc or NAC at specific wt% did not influence TEGDMA release (e.g. Filtek™ Supreme XTE-1, 0.1 and 0.01 wt% Asc or NAC 1 and 7 day in methanol). In Grandio® a significant reduced release of TEGDMA after incorporation of 1 wt% NAC (7 day, methanol) and a significant reduced release of HEMA after incorporation of Asc (1, 0.1, 0.01 wt%, methanol / water, 1 day/7day) could be detected. These results might be explained by the interaction of Asc or NAC with components of the composite and therefore less HEMA or TEGDMA is elutable as a positive effect.

Our previous study demonstrated that a lower DC is accompanied by a higher amount of eluted composite components [40] as a negative effect. However, molecular weight and hydrophobicity of (co)monomers as well as the filler content play also a role on elution mechanism. Therefore, the correlation between DC and component elution remains to be further illustrated [41,42]. In the present study, no relation between DC and the elution of composite components after the incorporation of Asc or NAC at any wt% could be found.

4.3. *Release of Asc or NAC from composite-antioxidant mixtures*

Asc can diminish DNA lesion by scavenging reactive species directly and reducing their formation, or preventing proteins that repair DNA against radical attack [43]. NAC can scavenge free radicals through thiol side-chain directly as well as by simultaneous increase of intracellular glutathione (GSH) content [44]. Additionally, evidence shows that NAC reduces the availability of free dental resin monomers by reacting with the methacrylic group through Michael-type addition [45,46]. Both Asc and NAC were reported to prevent the formation of DNA adducts [47,48].

In our previous studies cytotoxicity and genotoxicity of dental (co)monomers (e.g. TEGDMA) and their epoxy metabolites (e.g. EMPA) have been found [13,14]. Furthermore, we reported that cell culture medium eluates of dental composites containing total elutable components also induced DNA-DSBs [28]. Recently, we demonstrated that the presence of Asc or NAC significantly reduced cytotoxicity and genotoxicity induced by dental (co)monomers and their epoxy metabolites [14,17,19,20]. Concentrations higher than 50 μM Asc or NAC caused a significant reduction of DNA-DSBs induced by dental (co)monomer intermediates and their epoxy metabolites, compared to control in HGFs [14]. Therefore these concentrations play a key role in the reduction of cell toxicity.

Based on this 50 μM Asc/NAC [14], the present study was designed for the incorporation of NAC or Asc starting with 0.01 wt%, which corresponds to a calculated maximum elutable concentration of about 57 μM Asc or 61 μM NAC. However, elution mechanism depends on the molecular weight, hydrophobicity, filler content, investigated material and the final network characteristics of the resin-matrix [41,42,49–51]. Consequently, in the present study concentrations of released Asc or NAC were always lower than the calculated corresponding maximal elutable Asc or NAC concentration.

In the present study, elution of Asc or NAC at 5 min, 1 day and 7 day was determined to estimate the maximum available concentration of released antioxidant in eluates. However, degradations of Asc and NAC in solutions were reported in other studies [52,53]. This is in accordance with our data because a degradation of eluted Asc and NAC in methanol and water was observed. Nevertheless, an increased release of antioxidants can also be found for Venus® -1 wt% Asc (methanol) and Filtek™ Supreme XTE-1 wt% NAC (water) with increasing elution time.

As described above, concentrations higher than 50 μM Asc or NAC can significantly reduce DNA-DSBs [14]. In the present study, NAC or Asc concentrations over this value were found in methanol or water eluates of Venus® -1 wt% Asc, Grandio® -1 wt% Asc, Filtek™ Supreme XTE-1 wt% Asc and Filtek™ Supreme XTE-1 wt% NAC. Hence, it can be concluded that these composites containing 1 wt% Asc or 1 wt% NAC might offer sufficient amounts of eluted antioxidants to reduce genotoxicity induced by dental composite (co)monomers, their metabolization intermediates and epoxy metabolites in human oral cells.

4.4. *Risk assessment*

Among all investigated composite-antioxidant mixtures, only Venus®, Grandio® and Filtek™ Supreme XTE (each 1 wt% Asc) and Filtek™ Supreme XTE (1 wt% NAC) can offer sufficient amounts of eluted antioxidants to reduce DNA-DSBs [14]. However, the incorporation of 1 wt% Asc into Venus®, Grandio® and Filtek™ Supreme XTE showed a significant decreased DC and a significant increased elution of TEGDMA. The increase of composite component elution (e.g. TEGDMA) can lead to adverse effects [13,35]. Only Filtek™ Supreme XTE (1 wt% NAC) showed no significant change as well on DC as on composite component elution and supplies sufficient amount

of antioxidant to reduce toxicity. Therefore, Filtek™ Supreme XTE-1 wt% NAC represents a beneficial mixture.

If we assume the methanol elution as a worst-case scenario, in the present study for Filtek™ Supreme XTE-1 wt% NAC (1 day methanol elution), the total concentration of eluted TEGDMA from 10 filling (100 mg each) would reach about 360 μM, which can induce DNA-DSBs [13]. Similarly, the maximum concentration of released NAC from one filling was 43 μM (Filtek™ Supreme XTE-1 wt% NAC, 1 day, methanol). For 10 fillings this would result in 430 μM total released NAC. This is 9-fold higher than 50 μM NAC, which can reduce DNA-DSBs [14]. Therefore, the incorporation of 1 wt% NAC into Filtek™ Supreme XTE would be a useful step to reduce dental (co)monomer induced DNA-DSBs. However, it is emphasized that these data may not be easily transformed into the clinical situation, because in the physiological situation the continuous formation of saliva may lead to a dilution of released composite components.

5. Conclusion

Incorporation of NAC (1 wt%), as a novel composite component, into Filtek™ Supreme XTE, had no effect on DC and composite component elution and supplies sufficient amount of antioxidant which may reduce toxicity. Therefore, it represents a beneficial mixture.

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REFERENCES

[1] Geurtsen W, Lehmann F, Spahl W, Leyhausen G. Cytotoxicity of 35 dental resin composite monomers/additives in permanent 3T3 and three human primary fibroblast cultures. J Biomed Mater Res 1998;41:474–80.
[2] Ferracane JL, Condon JR. Rate of elution of leachable components from composite. Dent Mater 1990;6:282–7.
[3] Geurtsen W. Biocompatibility of resin-modified filling materials. Crit Rev Oral Biol Med 2000;11:333–55.
[4] Reichl FX, Durner J, Hickel R, Kunzelmann KH, Jewett A, Wang MY, et al. Distribution and excretion of TEGDMA in guinea pigs and mice. J Dent Res 2001;80:1412–5.
[5] Lindstrom M, Alanko K, Keskinen H, Kanerva L. Dentist's occupational asthma, rhinoconjunctivitis, and allergic contact dermatitis from methacrylates. Allergy 2002;57:543–5.
[6] Reichl FX, Durner J, Hickel R, Spahl W, Kehe K, Walther U, et al. Uptake, clearance and metabolism of TEGDMA in guinea pigs. Dent Mater 2002;18:581–9.
[7] Reichl FX, Durner J, Kehe K, Manhart J, Folwaczny M, Kleinsasser N, et al. Toxicokinetic of HEMA in guinea pigs. J Dent 2002;30:353–8.
[8] Reichl FX, Seiss M, Buters J, Behrendt H, Hickel R, Durner J. Expression of CYP450-2E1 and formation of 2,3-epoxymethacrylic acid (2,3-EMA) in human oral cells exposed to dental materials. Dent Mater 2010;26:1151–6.
[9] Seiss M, Nitz S, Kleinsasser N, Buters JT, Behrendt H, Hickel R, et al. Identification of 2,3-epoxymethacrylic acid as an intermediate in the metabolism of dental materials in human liver microsomes. Dent Mater 2007;23:9–16.
[10] Eckhardt A, Gerstmayr N, Hiller KA, Bolay C, Waha C, Spagnuolo G, et al. TEGDMA-induced oxidative DNA damage and activation of ATM and MAP kinases. Biomaterials 2009;30:2006–14.
[11] Kleinsasser NH, Wallner BC, Harreus UA, Kleinjung T, Folwaczny M, Hickel R, et al. Genotoxicity and cytotoxicity of dental materials in human lymphocytes as assessed by the single cell microgel electrophoresis (comet) assay. J Dent 2004;32:229–34.
[12] Schwengberg S, Bohlen H, Kleinsasser N, Kehe K, Seiss M, Walther UI, et al. In vitro embryotoxicity assessment with dental restorative materials. J Dent 2005;33:49–55.
[13] Urcan E, Scherthan H, Styllou M, Haertel U, Hickel R, Reichl FX. Induction of DNA double-strand breaks in primary gingival fibroblasts by exposure to dental resin composites. Biomaterials 2010;31:2010–4.
[14] Yang Y, He X, Shi J, Hickel R, Reichl FX, Hogg C. Effects of antioxidants on DNA double-strand breaks in human gingival fibroblasts exposed to dental resin co-monomer epoxy metabolites. Dent Mater 2017;33:418–26.
[15] Mahaney BL, Meek K, Lees-Miller SP. Repair of ionizing radiation-induced DNA double-strand breaks by non-homologous end-joining. Biochem J 2009;417:639–50.
[16] Kurebayashi H, Ohno Y. Metabolism of acrylamide to glycidamide and their cytotoxicity in isolated rat hepatocytes: protective effects of GSH precursors. Arch Toxicol 2006;80:820–8.
[17] Walther UI, Siagian II, Walther SC, Reichl FX, Hickel R. Antioxidative vitamins decrease cytotoxicity of HEMA and TEGDMA in cultured cell lines. Arch Oral Biol 2004;49:125–31.
[18] Spagnuolo G, D'Anto V, Cosentino C, Schmalz G, Schweikl H, Rengo S. Effect of N-acetyl-L-cysteine on ROS production and cell death caused by HEMA in human primary gingival fibroblasts. Biomaterials 2006;27:1803–9.
[19] Lottner S, Shehata M, Hickel R, Reichl FX, Durner J. Effects of antioxidants on DNA-double strand breaks in human gingival fibroblasts exposed to methacrylate based monomers. Dent Mater 2013;29:991–8.
[20] Styllou P, Styllou M, Hickel R, Hogg C, Reichl FX, Scherthan H. NAC ameliorates dental composite-induced DNA double-strand breaks and chromatin condensation. Dent Mater J 2017;36:638–46.
[21] Kuefner MA, Brand M, Ehrlich J, Braga L, Uder M, Semelka RC. Effect of antioxidants on X-ray-induced gamma-H2AX foci in human blood lymphocytes: preliminary observations. Radiology 2012;264:59–67.
[22] Jiao Y, Ma S, Li J, Shan L, Yang Y, Li M, et al. The influences of N-acetyl cysteine (NAC) on the cytotoxicity and mechanical properties of poly-methylmethacrylate (PMMA)-based dental resin. PeerJ 2015;3:e868.
[23] Rothmund L, Reichl FX, Hickel R, Styllou P, Styllou M, Kehe K. Effect of layer thickness on the elution of bulk-fill composite components. Dent Mater 2017;33:54–62.
[24] Ercal N, Oztezcan S, Hammond TC, Matthews RH, Spitz DR. High-performance liquid chromatography assay for N-acetylcysteine in biological samples following derivatization with N-(1-pyrenyl)maleimide. J Chromatogr B: Biomed Appl 1996;685:329–34.
[25] Field AP. Discovering statistics using SPSS: (and sex, sdrugs and rock 'n' roll). 3rd ed. Los Angeles: SAGE Publications; 2009.

[26] Schuster L, Rothmund L, He X, Van Landuyt KL, Schweikl H, Hellwig E, et al. Effect of opalescence(R) bleaching gels on the elution of dental composite components. Dent Mater 2015;31:745–57.
[27] Sevkusic M, Schuster L, Rothmund L, Dettinger K, Maier M, Hickel R, et al. The elution and breakdown behavior of constituents from various light-cured composites. Dent Mater 2014;30:619–31.
[28] Yang Y, Reichl FX, Shi J, He X, Hickel R, Hogg C. Cytotoxicity and DNA double-strand breaks in human gingival fibroblasts exposed to eluates of dental composites. Dent Mater 2018;34:201–8.
[29] Jakubiak J, Allonas X, Fouassier J, Sionkowska A, Andrzejewska E, Linden L, et al. Camphorquinone–amines photoinitating systems for the initiation of free radical polymerization. Polymer 2003;44:5219–26.
[30] Gutteridge JM. Biological origin of free radicals, and mechanisms of antioxidant protection. Chem Biol Interact 1994;91:133–40.
[31] Mateo J, Bosch P, Lozano A. Reactivity of radicals derived from dimethylanilines in acrylic photopolymerization. Macromolecules 1994;27:7794–9.
[32] Stansbury JW. Curing dental resins and composites by photopolymerization. J Esthet Dent 2000;12:300–8.
[33] Foy CJ, Passmore AP, Vahidassr MD, Young IS, Lawson JT. Plasma chain-breaking antioxidants in Alzheimer's disease, vascular dementia and Parkinson's disease. QJM 1999;92:39–45.
[34] Hickel R, Dasch W, Janda R, Tyas M, Anusavice K. New direct restorative materials. FDI commission project. Int Dent J 1998;48:3–16.
[35] Goldberg M. In vitro and in vivo studies on the toxicity of dental resin components: a review. Clin Oral Investig 2008;12:1–8.
[37] Tanaka K, Taira M, Shintani H, Wakasa K, Yamaki M. Residual monomers (TEGDMA and Bis-GMA) of a set visible-light-cured dental composite resin when immersed in water. J Oral Rehabil 1991;18:353–62.
[38] Rothmund L, Shehata M, Van Landuyt KL, Schweikl H, Carell T, Geurtsen W, et al. Release and protein binding of components from resin based composites in native saliva and other extraction media. Dent Mater 2015;31:496–504.
[40] Durner J, Obermaier J, Draenert M, Ilie N. Correlation of the degree of conversion with the amount of elutable substances in nano-hybrid dental composites. Dent Mater 2012;28:1146–53.
[41] Lempel E, Czibulya Z, Kovacs B, Szalma J, Toth A, Kunsagi-Mate S, et al. Degree of conversion and BisGMA, TEGDMA, UDMA elution from flowable bulk fill composites. Int J Mol Sci 2016;17.
[42] Alshali RZ, Salim NA, Sung R, Satterthwaite JD, Silikas N. Analysis of long-term monomer elution from bulk-fill and conventional resin-composites using high performance liquid chromatography. Dent Mater 2015;31:1587–98.
[43] Padayatty SJ, Katz A, Wang Y, Eck P, Kwon O, Lee JH, et al. Vitamin C as an antioxidant: evaluation of its role in disease prevention. J Am Coll Nutr 2003;22:18–35.
[44] Krifka S, Spagnuolo G, Schmalz G, Schweikl H. A review of adaptive mechanisms in cell responses towards oxidative stress caused by dental resin monomers. Biomaterials 2013;34:4555–63.
[45] Schweikl H, Schmalz G. Triethylene glycol dimethacrylate induces large deletions in the hprt gene of V79 cells. Mutat Res 1999;438:71–8.
[46] Pei Y, Liu H, Yang Y, Yang Y, Jiao Y, Tay FR, et al. Biological activities and potential oral applications of N-acetylcysteine: progress and prospects. Oxid Med Cell Longev 2018;2018.
[47] Grosse Y, Chekir-Ghedira L, Huc A, Obrecht-Pflumio S, Dirheimer G, Bacha H, et al. Retinol, ascorbic acid and alpha-tocopherol prevent DNA adduct formation in mice treated with the mycotoxins ochratoxin A and zearalenone. Cancer Lett 1997;114:225–559.
[48] Reliene R, Fischer E, Schiestl RH. Effect of N-acetyl cysteine on oxidative DNA damage and the frequency of DNA deletions in atm-deficient mice. Cancer Res 2004;64:5148–53.
[49] Pongprueksa P, De Munck J, Duca RC, Poels K, Covaci A, Hoet P, et al. Monomer elution in relation to degree of conversion for different types of composite. J Dent 2015;43:1448–55.
[50] Al-Ahdal K, Ilie N, Silikas N, Watts DC. Polymerization kinetics and impact of post polymerization on the degree of conversion of bulk-fill resin-composite at clinically relevant depth. Dent Mater 2015;31:1207–13.
[51] Durner J, Stojanovic M, Urcan E, Spahl W, Haertel U, Hickel R, et al. Effect of hydrogen peroxide on the three-dimensional polymer network in composites. Dent Mater 2011;27:573–80.
[52] Yuan J-P, Chen F. Degradation of ascorbic acid in aqueous solution. J Agric Food Chem 1998;46:5078–82.
[53] Rosati E, Sabatini R, Ayroldi E, Tabilio A, Bartoli A, Bruscoli S, et al. Apoptosis of human primary B lymphocytes is inhibited by N-acetyl-L-cysteine. J Leukoc Biol 2004;76:152–61.