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Long-term elution of monomers from resin-based dental composites

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ABSTRACT

Objective. To bridge the gap between the current alarming literature on resin-based dental materials and the limited clinical observations, more precise knowledge on the actual quantity of released compounds should be acquired. The objective of this study was to quantify the long-term elution of various compounds from resin-based dental composites during one year.

Methods. Eight materials were investigated: G-aenial Anterior, G-aenial Posterior, Venus, Venus Pearl, Venus Diamond, Ceram X mono, Dyract and Filtek Supreme XTE. Cylindrical specimens (6 mm diameter, 2 mm thickness) were immersed in 1 mL of three different extraction solutions (water, artificial saliva or ethanol) and stored in the dark at 37 °C. Every week, the extraction solution was refreshed. The samples were analyzed using ultra-performance liquid chromatography-tandem mass spectrometry.

Results. BisEMA3, BisEMA6, BisEMA10, BisGMA, CQ, HEMA, TCD-DI-HEA, TEGDMA, and UDMA were quantified in the samples. Depending on the composite and the extraction solution, certain monomers (BisGMA, HEMA and UDMA) were able to continuously elute from the materials, up until 52 weeks after initial immersion. Monomer elution was clearly higher when ethanol was used as extraction solution. It could be demonstrated that the tested composites continued to release small quantities of monomers over longer periods when a continuous refreshing protocol is followed.

Significance. Even if monomer elution may not lead to a risk at short term, the potential long-term toxicity should be further investigated. Long-term elution and subsequent chronic exposure to monomers from resin-based dental materials should not be neglected when assessing the overall human health risks.

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1. Introduction

In clinical practice, resin-based dental composites seem to be a biological and functional acceptable substitute for amalgam. Conversely, there are *in-vitro* studies that indicate that several of the compounds eluted from resin-based dental materials may have biotoxic effects, such as allergenic potential [1], cytotoxicity at high concentrations [2], disrupting of vital cell functions at sub-cytotoxic concentrations [3] and even induction of DNA damage [4,5]. Even though currently there is no direct evidence that composite restorations may hold serious health hazards, it should be kept in mind that certain adverse effects may appear in the long term (even after several decades). However, good knowledge on the long-term release from resin-based materials is primordial to evaluate potential toxicological effects.

The release of ingredients from resin-based dental materials has already been extensively investigated *in vitro* by immersing a composite sample in an extraction solution, such as water or an organic solvent [6]. Typically, the release after 24 h or 1 week is determined, but few studies also incubated the samples for longer periods (up to one month, three months and even one year, respectively) [7–9]. Since composite materials are expected to remain in the mouth for many years, extended storage periods in *in-vitro* studies are indeed more suitable to investigate the release of various ingredients from composites. However, in the available long-term studies, the samples were left undisturbed in the incubator and the solvent was not refreshed in between. In a recent study by Cokic et al., it was shown that release kinetics in *in-vitro* experiments are also influenced by saturation of the extraction solvent by the leached monomers and compounds, which may result in reduced release [10]. In the mouth, the overall elution of compounds may thus be larger than expected based on these classic *in-vitro* elution studies, since saturation can never be reached due to the continuous removal of the eluates with saliva (or pulpal fluid) [6,10]. It is therefore recommended to refresh the extraction medium after equal time intervals to avoid solvent saturation by the leached components.

The aim of the present study was to evaluate the long-term release of compounds from eight resin-based dental composite materials over a period of one year. Composite specimens were immersed in three different extraction solutions (water, artificial saliva and ethanol) during a period of 52 weeks, while the extraction solutions were refreshed weekly. The release of compounds from the composites was quantified by ultra-high performance liquid chromatography–tandem mass spectrometry (UHPLC–MS/MS) following a previously optimized protocol [11].

2. Materials and methods

2.1. Materials

Absolute ethanol was purchased from Fisher Scientific (Aalst, Belgium). Urea was obtained from GE Healthcare Europe GmbH (Diegem, Belgium). Acetic acid (LC–MS grade), alpha-amylase, ammonium acetate (LC–MS ultra), ammonium formate (LC–MS grade), bisphenol A-d16 (d16-BPA), deuterium, diethyl phthalate-3,4,5,6-d4 (d4-DEP), formic acid (LC–MS grade), methanol (LC–MS grade), mucin from porcine stomach, potassium thiocyanate, sodium phosphate monobasic dehydrate, uric acid were purchased from Sigma-Aldrich (Diegem, Belgium). Potassium chloride, sodium chloride and water HiPerSolv CHROMANORM for HPLC were obtained from VWR (Haasrode, Belgium).

2.2. Sample preparation

The monomer elution from eight different resin-based dental composites was evaluated: G-aenial Anterior and G-aenial Posterior (GC Europe, Leuven, Belgium), Venus, Venus Pearl and Venus Diamond (Heraeus Kulzer, Hanau, Germany), Ceram X mono and Dyract (Dentsply DeTrey GmbH, Konstanz Germany), and Filtek Supreme XTE (3M ESPE Dental Products, Seefeld, Germany). The composition of these materials, as given by the manufacturers, is summarized in Table 1.

Cylindrical specimens were prepared in standardized white Teflon molds (6 mm internal diameter, 2 mm thickness) (n = 18 for each composite). The composites were cured for 20 s with the polywave LED light-curing unit Bluephase G2 (Ivoclar-Vivadent, Schaan, Liechtenstein) in 'high' power mode. The mean and maximum irradiance of the Bluephase G2 curing unit was 1367 mW/cm^2 and 1430 mW/cm^2, and the total energy delivered was 5.6 J/cm^2 and 22.8 J/cm^2 in the 380–420 nm and 420–540 nm regions, respectively, as measured by the MARC resin calibrator (Bluelight Analytics, Halifax, NS, Canada). A glass cover was placed below and on top of the sample to prevent the formation of an oxygen inhibition layer, to ensure smooth surfaces and to avoid excess of material. The light-curing unit was fixed in a three-prong extension clamp to standardize the distance between the light-curing tip and the sample at approximately 1 mm.

2.3. Evaluation of the degree of conversion

For the measurement of the degree of conversion (DC), immediately after polymerization, four Raman spectra were acquired from the middle area of the top and bottom surface of the cylindrical specimens (n = 5) using micro-Raman spectroscopy (Senterra, Bruker, Ettlingen, Germany). The surface was excited with a near-infrared (785 nm) laser of 50 mW laser power and analyzed through a 100× objective and 50 × 1000-μm pin-hole aperture. The collected spectra ranged from 50 to 3500 cm^{-1} with a resolution of 9–15 cm^{-1}. The integration time of each spectrum was set to 20 s with 3 co-additions. The CCD detector to obtain the micro-Raman spectra possessed a 1024 × 256 pixel resolution, and was cooled down thermo-electrically to a temperature of −65 °C. Results were processed using OPUS 7.0 software (Bruker, Ettlingen, Germany). The DC was calculated as the ratio of peak intensities of the aliphatic 1640 cm^{-1} and aromatic 1610 cm^{-1} peaks in cured and uncured materials. The following formula was used: $DC(\%) = [1 - (R_{cured}/R_{uncured})] \times 100$, where R is the ratio of intensities of the 1640 cm^{-1} and 1610 cm^{-1} peaks in the spectra of the cured or uncured specimens.

Table 1 – The different resin-based dental composites examined in the present study.

Composite	Type	Shade	Resin matrix	Filler	Manufacturer
G-aenial Anterior	Micro-hybrid composite	A3	CQ UDMA	73 wt%/64 vol.% Prepolymerized fillers (16–17 μm; silica, strontium and lanthanoid fluoride), 850 nm silica glass, 16 nm fumed silica	GC Europe, Leuven, Belgium
G-aenial Posterior	Micro-hybrid composite	P-A3	BisEMA CQ UDMA	77 wt%/65 vol.% Prepolymerized fillers (16–17 μm; silica, strontium and lanthanoid fluoride), fluoroaluminosilicate, and 16 nm fumed silica	GC Europe, Leuven, Belgium
Venus	Nano-hybrid composite	A3	BisGMA TEGDMA	77 wt%/61 vol.% Ba–Al–F-glass (0.7 μm; max. < 2 μm), highly dispersive SiO_2 (0.04 μm)	Heraeus Kulzer, Hanau, Germany
Venus Pearl	Nano-hybrid composite	A3	CQ TCD-DI-HEA UDMA	82 wt%/64 vol.% Ba–Al–F-glass, SiO_2 nanofiller (5 nm), highly discrete nanoparticles (5 nm–20 μm), pigments	Heraeus Kulzer, Hanau, Germany
Venus Diamond	Nano-hybrid composite	A3	TCD-DI-HEA UDMA	80 wt%/59 vol.% Ba–Al–F-glass, pre-polymerized filler (<5 μm), SiO_2 nanofiller (5 nm), highly discrete nanoparticles (5 nm–5 μm)	Heraeus Kulzer, Hanau, Germany
Ceram X mono	Nano-hybrid composite	M2	BisGMA CQ TEGDMA UDMA	76 wt%/57 vol.% Ba–Al–borosilicate glass (1–1.5 μm), methacrylate functionalised silicon dioxide nanofiller (10 nm) iron oxide pigments, titanium oxide pigments, aluminum sulfo silicate pigments	Dentsply DeTrey GmbH, Konstanz Germany
Dyract	Compomer	A2	UDMA	73 wt%/47 vol.% Strontium–Al–Na–fluoro-P–silicate–glass, strontium, fluoride iron oxide pigments	Dentsply DeTrey GmbH, Konstanz Germany
Filtek Supreme XTE	Nano-filled composite	A3	BisEMA BisGMA TEGDMA UDMA	78.5 wt%/63.3 vol.% SiO_2 (20 nm) and ZrO_2 (4–11 nm) nanofiller, aggregated ZrO_2/SiO_2 cluster filler	3M ESPE Dental Products, Seefeld, Germany

2.4. *Elution experiment*

After polymerization, the specimens were weighed and immediately immersed in 1 mL of extraction solution (water, artificial saliva, or ethanol) in glass vials that were firmly closed with aluminum crimp caps with moulded septa of butyl/PTFE. The ratio between the sample and the extraction solution volume was greater than 1:10 and the samples were fully immersed, which is in line with the requirements of ISO 10993-13 [12]. The artificial saliva was prepared according to the protocol described by Denys et al. [13]. The samples were stored in the dark at 37 °C and the extraction solution was renewed every week during a period of one year. Samples were stored at −80 °C until analysis. To avoid contamination, care was taken to use only glass pipettes and glass containers.

2.5. *Analytical procedure for the quantification of target compounds*

Although the extraction solutions were refreshed every week during a period of one year and extraction samples of each week were stored, only the samples of week 1, 2, 3, 4, 6, 8, 10, 12, 14, 16, 20, 24, 28, 32, 36, 40, 44, 48 and 52 were analyzed using UHPLC–MS/MS. The analysis was performed according to a previously described protocol [11]. Briefly, aliquots of the samples diluted to reach a mixture of ethanol/water (1:1, v/v) or ethanol/artificial saliva (1:1, v/v). For the artificial saliva samples, 0.1% formic acid was added. A Waters® Micromass Quattro Premier Mass Spectrometer (Waters, Milford, MA, USA) equipped with ESI was used for all sample analysis. Samples (10 μL) were injected on to an Acquity UHPLC BEH C18 column (50 mm × 2.1 mm, 1.7 μm; Waters). Chromatographic separation was achieved using a mixture of 2 mM ammonium acetate buffer, pH 5.6 (A) and methanol (B), upon the following gradient: 0–0.2 min, 15% B; 0.2– 0.4 min, 15–60% B; 0.4–0.6 min, 60% B; 0.6–1.4 min, 60–95% B; 1.4–2.5 min, 95% B; 2.5–3.0 min, 95–15% B; 3.0–3.5 min, 15% B. The lower limits of quantification (LLOQ) in the present study were: 2 ng/mL BisEMA3, 2 ng/mL BisEMA6, 5 ng/mL BisEMA10, 10 ng/mL BisGMA, 5 ng/mL BisPMA, 50 ng/mL BPA, 50 ng/mL CQ, 200 ng/mL HEMA, 5 ng/mL TCD-DI-HEA, 5 ng/mL TEGDMA, 5 ng/mL UDMA.

2.6. *Statistical data analysis*

GraphPad Prism software, Version 8, (GraphPad Software, San Diego CA, USA) was used for statistical analysis. The significance of the differences in DC values between the top and bottom surfaces of the specimens and between the different composites was determined with paired t-test and one-way ANOVA in combination with Tukey *post-hoc* test, respectively, at a significance level of $\alpha = 0.05$. Two-way ANOVA in combination with Tukey *post-hoc* analysis was used to compare the cumulative elution after 52 weeks between the three different extraction solutions and between the eight different composite materials. The significance level α was set at 0.05.

3. Results

3.1. Degree of conversion

The DC of the eight composite materials was measured at the top and bottom surface of the 2-mm thick cylindrical specimens after illumination for 20 s using a polywave LED light-curing unit (Fig. 1). For all composites, the mean DC varied between 53% and 79%. A significantly higher DC at the top surface compared to the bottom surface was observed for the composites Venus ($p < 0.0001$), Venus Diamond ($p = 0.04$), Dyract ($p = 0.03$) and Filtek Supreme XTE ($p = 0.003$). For the top surface, Venus Pearl (78.9 ± 11.4%) and Venus Diamond (78.9 ± 7.7%) showed significant higher DC compared to all the other composite materials, except for G-aenial Anterior (66.5 ± 6.0%). No significant difference between the other composites was observed. For the bottom surface, Venus Pearl (76.3 ± 11.6%) and Venus Diamond (74.0 ± 11.0%) showed significant higher DC compared to the other composites. G-aenial Posterior (53.1 ± 3.8%) and Ceram X mono (53.5 ± 1.9%) showed significantly lower DC values.

3.2. Elution of monomers

The cumulative elution of the different compounds from the eight composite materials in the three different extraction solutions (expressed in log(nmol)) during the period of 52 weeks is shown in Fig. 2. Actual elution amounts (for each week, expressed in nmol) can be found in Supplementary Table 1. From the 11 compounds included in the analytical UHPLC–MS/MS method, only the compounds BisEMA3, BisEMA6, BisEMA10, BisGMA, CQ, HEMA, TCD-DI-HEA, TEGDMA, and UDMA could be quantified. BisPMA and BPA levels were lower than the method LLOQ. Depending on the composite and the extraction solution, certain monomers (especially BisGMA, HEMA and UDMA) were able to continuously elute from the materials, up until a period of 52 weeks after polymerization and initial immersion into the solvent.

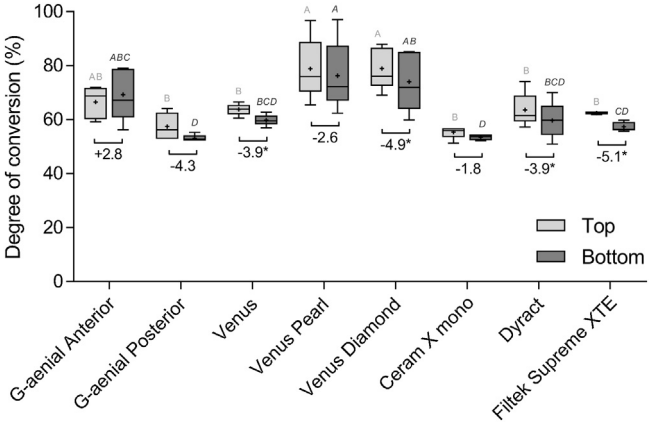

Fig. 1 – Degree of conversion (DC) (expressed in percentage) of the eight composite materials measured at four different spots of the top and bottom surface of the 2-mm thick cylindrical specimens. Results are presented in boxplots with the horizontal line in the box representing the median, the plus-sign representing the mean value, the boxes representing the first quartile to the third quartile and the whiskers representing the maximum and the minimum value (n = 5). The differences between top and bottom are indicated in percentage. Asterisks * indicate statistical significant differences between top and bottom DC (paired t-test, $p < 0.05$). The same letters (normal for top, italic for bottom) indicate no statistically significant difference (one-way ANOVA + Tukey *post-hoc*, $p < 0.05$).

The cumulative monomer elution from the different composites after 52 weeks of immersion in water, artificial saliva and ethanol and statistical comparison between the three different extraction solvents is shown in Supplementary Table 2. Monomer elution was significantly higher when pure ethanol was used as extraction solution compared to water and artificial saliva, with the exception of CQ elution from Venus Diamond and Filtek Supreme XTE, TEGDMA elution from Filtek Supreme XTE and UDMA elution from Venus, Ceram X mono and Dyract. In general, TEGDMA and UDMA elution in water seemed higher compared to elution in artificial saliva, however, this difference was not always significant. The comparison of the cumulative monomer elution after 52 weeks from the eight different composites is shown in Supplementary Table 3 and will be discussed in the next sections for each monomer separately.

3.2.1. BisEMA3, BisEMA6 and BisEMA10

The different variants of BisEMA were predominantly observed in the ethanol samples (G-aenial Anterior, G-aenial Posterior, Ceram X mono, Filtek Supreme XTE), while only small amounts (below 0.15 nmol) were found after the first week of immersion in the water and artificial saliva. Only Filtek Supreme XTE released BisEMA in artificial saliva.

BisEMA3 elution in ethanol attenuated after week 8–10 but still continued in low amounts until the end of the experiment (ranging from 0.02 ± 0.01 nmol to 0.47 ± 0.11 nmol at week 52) for G-aenial Anterior, G-aenial Posterior and Filtek Supreme XTE. For Ceram X mono, BisEMA3 elution attenuated around week 18. However, the eluted amounts stayed at a relative high level of approximately 1 nmol per week (1.39 ± 0.12 nmol at week 52).

3.2.2. BisGMA

The composites Venus and Filtek Supreme XTE released BisGMA in all three extraction solutions. Elution in water stopped after 8 weeks for Filtek Supreme XTE, but continued until week 52 for Venus. In artificial saliva, elution stopped after 8 and 4 weeks, for Venus and Filtek Supreme XTE, respectively. In ethanol, BisGMA elution from these two composites continued until week 52. In water and ethanol, BisGMA elution was significantly higher in Venus compared to Filtek Supreme XTE.

3.2.3. CQ

The photo-initiator CQ was leached from all eight composites tested mainly during week 1 and 2. Highest elution was observed with ethanol as extraction solution (ranging from 9.69 ± 1.83 nmol to 62.53 ± 12.3 nmol after week 1 for Venus Diamond and Dyract, respectively). Only in ethanol samples,

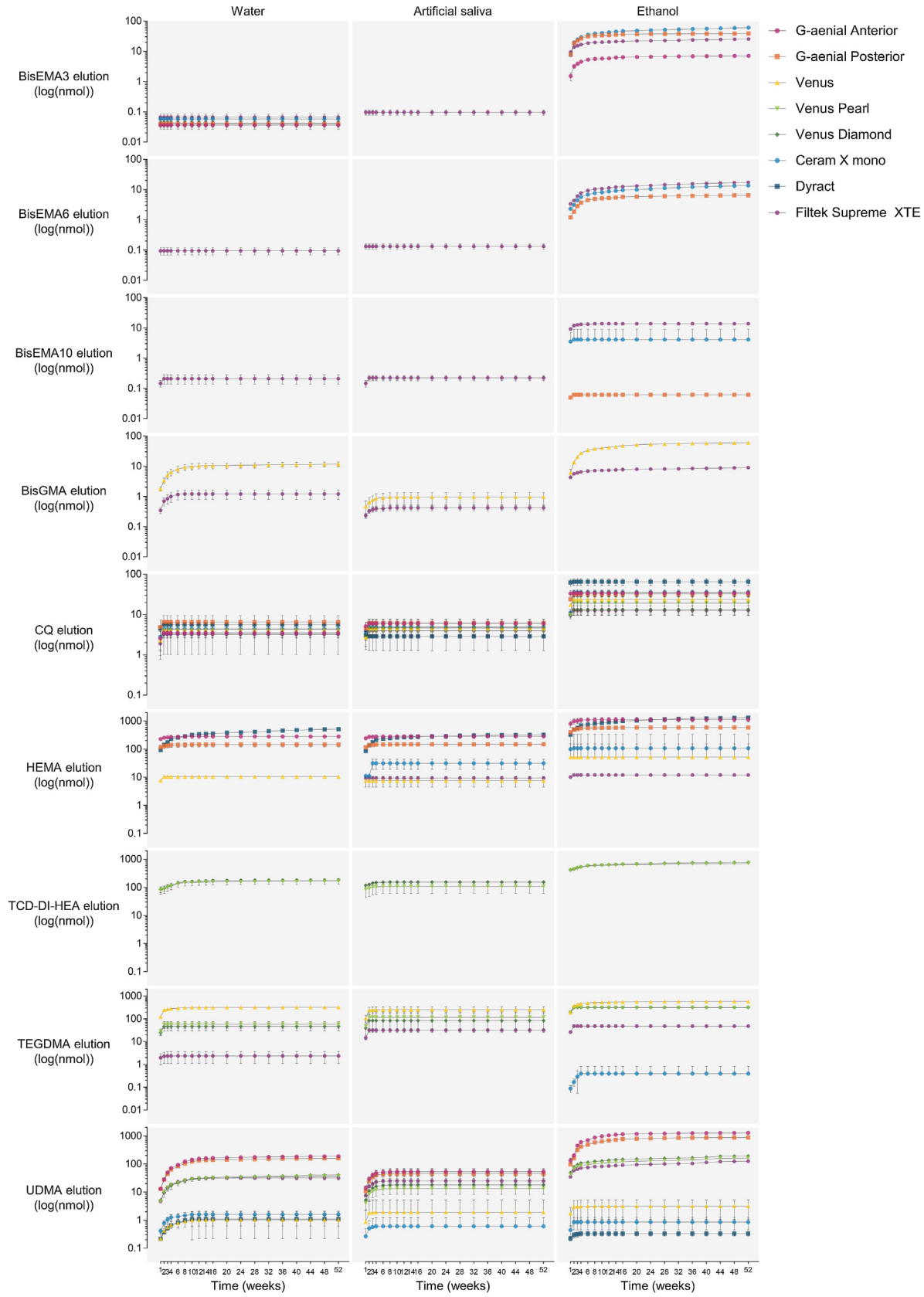

Fig. 2 – Cumulative elution (expressed in log(nmol)) of the different monomers from the eight composites found in water, artificial saliva or ethanol samples as observed during 52 weeks. Results are presented as mean ± SD (n = 6).

Table 2 – Estimated daily intake (EDI) of the different monomers (ng/kg bw/day) for adults and children after acute and chronic exposure due to replacement of total crowns of different teeth.

Typical restorations		SA (mm^2)	Estimated daily intake (ng/kg bw/day)							
			Acute exposure (week 1)				Chronic exposure (week 2–4)			
			BisGMA	HEMA	TEGDMA	UDMA	BisGMA	HEMA	TEGDMA	UDMA
Adults (70 kg)										
Front teeth	Central incisor	223	3.02	53.88	147.54	32.36	1.16	27.62	66.10	25.55
	Lateral incisor	178	2.41	43.00	117.77	25.83	0.93	22.05	52.76	20.39
	Canine	210	2.84	50.74	138.94	30.47	1.10	26.01	62.24	24.06
Premolars	First premolar	203	2.75	49.04	134.31	29.46	1.06	25.15	60.17	23.26
	Second premolar	191	2.59	46.15	126.37	27.72	1.00	23.66	56.61	21.88
Molars	First molar	315	4.27	76.10	208.41	45.71	1.64	39.02	93.36	36.09
	Second molar	276	3.74	66.68	182.60	40.05	1.44	34.19	81.81	31.62
	Third molar	247	3.34	59.67	163.42	35.84	1.29	30.60	73.21	28.30
4 quadrants		7372	99.83	1781.07	4877.36	1069.74	38.46	913.17	2185.03	844.63
Children (20 kg)										
Front teeth	Central incisor	223	10.57	188.57	516.38	113.26	4.07	96.68	231.34	89.42
	Lateral incisor	178	8.44	150.52	412.18	90.40	3.25	77.17	184.65	71.38
	Canine	210	9.95	177.58	486.28	106.66	3.83	91.04	217.85	84.21
Premolars	First premolar	203	9.62	171.66	470.07	103.10	3.71	88.01	210.59	81.40
	Second premolar	191	9.05	161.51	442.28	97.01	3.49	82.81	198.14	76.59
Molars	First molar	315	14.93	266.36	729.42	159.98	5.75	136.57	326.78	126.32
	Second molar	276	13.08	233.38	639.11	140.18	5.04	119.66	286.32	110.68
	Third molar	247	11.71	208.86	571.96	125.45	4.51	107.09	256.23	99.05
4 quadrants		7372	349.40	6233.73	17070.77	3744.10	134.61	3196.10	7647.62	2956.20

Abbreviations: SA: surface area.

significant differences in 52-week cumulative elution between composites could be observed.

3.2.4. *HEMA*

Compared to all eluted monomers, HEMA was released in the highest quantities (ranging from 85.76 ± 9.33 nmol in artificial saliva to 803.03 ± 204.94 nmol in ethanol for Dyract and G-aenial Anterior, respectively). For G-aenial Anterior and G-aenial Posterior, elution stopped after week 2 and 12 for artificial saliva and water, respectively. For ethanol, elution continued until week 16 and 20 for G-aenial Anterior and G-aenial Posterior, respectively. For the compomer Dyract, HEMA elution continued up to week 52 in all three extraction solutions (ranging from 2.74 ± 1.57 nmol to 13.95 ± 2.42 nmol for artificial saliva and ethanol at week 52). In all three extraction solutions, 52-week cumulative HEMA elution was significantly different between the three composites, except for G-aenial Anterior and Dyract in artificial saliva.

3.2.5. *TCD-DI-HEA*

Elution of TCD-DI-HEA from Venus Pearl and Venus Diamond was found in all three extraction solutions. In artificial saliva, elution stopped after 10 weeks of immersion, while elution in water and ethanol continued. Moreover, the release in ethanol stayed higher than 6 nmol per week. Only in ethanol, Venus Diamond released significantly more TCD-DI-HEA compared to Venus Pearl ($p < 0.001$).

3.2.6. *TEGDMA*

For the composite Venus, TEGDMA elution attenuated around week 8 but persisted until week 52 in water and ethanol, while in artificial saliva, elution stopped after 6 weeks of immersion. TEGDMA release from Ceram X mono and Filtek Supreme XTE was only observed during the first month of immersion. TEGDMA elution was the highest for Venus, followed by Venus Pearl, Venus Diamond, Filtek Supreme XTE and Ceram X mono.

3.2.7. *UDMA*

In ethanol, UDMA elution continued until week 52 for G-aenial Anterior, G-aenial Posterior, Venus Pearl, Venus Diamond and Filtek Supreme XTE (ranging from 2.20 ± 0.68 nmol to 7.66 ± 0.47 nmol for Filtek Supreme XTE and G-aenial Anterior, respectively). For Venus, Dyract and Ceram X mono only small eluted quantities of UDMA were observed until 12 weeks after immersion in water. In artificial saliva, for all composite materials, UDMA elution practically stopped after week 8–12.

3.3. *Estimation of the potential exposure to monomers from dental composite*

With the highest elution results in artificial saliva during the first four weeks (BisGMA from Venus, HEMA from Dyract, TCD-DI-HEA from Venus Diamond, TEGDMA from Venus, UDMA from G-aenial Anterior), the estimated daily intake (EDI) of monomers released from total crown restorations was calculated based on the average total crown surface areas [6] and the assumption that the body weight of adults and children was 70 kg and 20 kg, respectively (Table 2). Patients with total wear (tooth loss due to attrition, abrasion and erosion) typically require full crown restorations of all teeth. In this worst-case scenario, the total exposed surface area (including all 32 teeth) would be 7372 mm^2.

4. Discussion

In the present study, the elution of various compounds from eight different resin-based dental composites over a period of one year was quantified with UHPLC–MS/MS. Thanks to a set-up with equal-interval solvent change, we were able to demonstrate that even after a period of one year, some monomers continue to be released. The type of eluted monomer and the quantity released varied considerably depending on the composite material and the extraction solution used. In general, our results show an acute release phase after the first 24 h, which is in correspondence with many previous studies. However, in contrast to previous studies, the release did not stop after the acute phase, but small quantities of certain compounds continued to leach, even up to 52 weeks depending on the composite and the extraction solution. Manojlovic et al. already explained this leaching pattern by a first high release of compounds at the surface of the specimen, followed by slower release of compounds within the specimen, which can be released only after swelling of the polymer chains [14]. However, it was until now not known that release could continue for at least one year.

The samples were analyzed using UHPLC–MS/MS. The use of liquid chromatography in combination with tandem mass spectrometry allows the quantification of a specific compound based on the selective separation of the different compounds in the sample and the fragmentation of preselected precursor ions into product ions. Hyphenated techniques (chromatographic techniques combined with spectroscopic techniques) are required to target multiple analytes and to provide reliable results within a short time period with low quantification limits. The use of ultra-UHPLC in combination with a tandem MS detector appears to be adequate to fulfill these requirements in terms of speed, sensitivity, and selectivity.

As described by Ferracane [15], several factors can influence the elution of different compounds from resin-based dental materials. First, the amount of compounds released is directly related to the DC, which varies between 50% and 70% and reaches a maximum after 24 h due to a post-cure process (*i.e.* in-the-dark polymerization) [16–19]. Second, the type of the extraction solution can affect the elution. Third, the size and the chemical nature of the released components play a role. Additionally, the physical and mechanical characteristics of the composite resins are related to the filler content, filler size and the distribution of the filler particles. Therefore, the composition (filler content) of composites can directly influence the elution process [8].

The DC of the composite specimens was measured using micro-Raman spectrometry. Micro-Raman does not require specific specimen preparation and allows a non-destructive analysis. The DC measured at the top and the bottom surface of the tested composites varied between 53% and 79%, which is in line with the normal range for most commercial dental composites. Only for the composites Venus, Venus Diamond, Dyract and Filtek Supreme XTE, the DC measured at the top surface was significantly higher compared to the DC at the bottom surface, indicating that the instructions for use should maybe instruct smaller layers of composite or longer lighting time.

The surface area of the composite specimens used in this study (approximately 94.25 mm^2) is comparable with the average estimates of the surface area of a large incisal restoration, a cusp restoration of the premolars or a mesial-occlusal-distal box restoration of the molars with an average estimated surface area of 95 mm^2 [6].

Water and artificial saliva were used to simulate the physiological condition. It should be mentioned that human saliva is expected to be more reactive compared to artificial saliva due to the presence of certain bacterial enzymes such as cholinesterases, which are able to degrade the composite materials [20]. However, the artificial saliva used in this experiment contained alpha-amylase, also present in human saliva. It has also been demonstrated by Rothmund et al. that proteins present in human saliva are able to bind to the monomers, which could have an effect on the release of monomers from composites [21]. Ethanol was used as an aggressive organic solution and represented the worst-case scenario for the estimation of the total amount of compounds that might leach out from the composite materials. Nevertheless, the US FDA recommends the use of 75% ethanol-water solution as a food/oral simulating liquid and thus clinically relevant [22]. Ethanol is able to penetrate into the resin matrix and swell the polymer network, promoting the release of the unreacted monomers [23]. The release of all detected compounds waned earlier in artificial saliva compared to water and pure ethanol. In general, monomer elution with artificial saliva stopped after week 8–10, while for certain compounds elution in ethanol continued during the entire experiment. A plausible hypothesis for this phenomenon may be the formation of a 'salivary pellicle' that covered the composite samples. The salivary pellicle is a thin acellular organic film that can form on any type of surface (*e.g.* enamel) in the presence of saliva [24]. This finding may thus also be relevant for clinical circumstances, and warrants further research.

The monomers that were detected in the three different extraction solutions included BisEMA3, BisEMA6, BisEMA10, BisGMA, CQ, HEMA, TCD-DI-HEA, TEGDMA, and UDMA.

Among the detected compounds, the release of HEMA was the highest. This can be explained by the small dimension and the low molecular weight of HEMA [6]. Despite of its relative hydrophilic behavior, a higher elution of HEMA was observed in the organic solution compared to the water-based solutions. Surprisingly, HEMA was not listed as an ingredient in the composite materials by the manufacturers. However, even if a certain compound was not mentioned in the Material Safety Data Sheets (MSDS), this is in agreement with the rule that the manufacturers are only obliged to provide information about the main ingredients of the composite material ($\geq$1%), and as a consequence, the MSDS of the composites are incomplete [25,26]. Another possible explanation for the presence of HEMA in the samples is that this small monomer may be a degradation product of UDMA [27]. This explanation can be justified by the fact that the highest elution of both UDMA and HEMA was observed for the composites G-aenial Anterior and G-aenial Posterior. The presence of HEMA in the compomer Dyract was also observed in the study of Geurtsen et al. [28].

The second highest elution was observed for the monomer TEGDMA. This is in agreement with the hypothesis that com-

pounds with low molecular weight are released faster and more than high molecular weight compounds [6]. Moreover, TEGDMA is hydrophilic, promoting elution in aqueous solutions.

The lowest elution was observed for BisGMA. Even though BisGMA is a hydrophobic monomer and is not likely to elute from the materials in water-based solutions in high quantities, its release in aqueous solutions can be explained by its low double-bond conversion [29]. In the water-based solvents, only very small quantities were detected. This is in line with previous studies [7].

For BisEMA3, BisEMA6 and BisEMA10, the reference compounds were not pure and contained a mixture of the different BisEMA variants, resulting in non-specific transitions [26]. As a consequence, these results should be taken with caution. Only very small quantities of BisEMA were detected in the aqueous solvents. This is in line with previous studies where no BisEMA was detected in the samples [30].

The monomer TCD-DI-HEA, a low-shrinkage methacrylate monomer with increased mechanical performance and improved biocompatibility [31], could only be observed in the samples with Venus Pearl and Venus Diamond, as this proprietary monomer is only used in the composites of this manufacturer (Heraeus Kulzer).

Since CQ is commonly used as the photo-initiator in dental composites, CQ was present in both the water-based and the ethanol samples of all eight materials, however, only until week one and week two of incubation, respectively. The fact that release was already complete after such short period, may be explained by the small amounts of photoinitiator used in composites together with its small dimensions.

Data on recommended limits of intake of monomers is rather limited. All dental materials are subject to regulations concerning 'medical devices', but unlike therapeutic drugs that need to be clinically tested, the regulations for commercializing medical devices are less stringent. Some information is, however, available from the studies by Moilanen et al. [32,33]. Acute toxicity (after ingestion) of BisGMA, TEGDMA and UDMA was assessed in rats and resulted in LD50 values (the lethal dose for 50% of subjects) of 2 mg/kg, 10.837 mg/kg and 5 mg/kg for BisGMA, TEGDMA and UDMA, respectively. It has also been shown that the no observed adverse effect level (NOAEL) for reproductive toxicity in mice after ingestion is 0.8 mg/kg bw/day and 1 mg/kg bw/day for BisGMA and TEGDMA, respectively [32,33]. In these studies, the animals were daily exposed to the monomers for more than one month. Converting this NOAEL in mice to the human equivalent dose (HED) by dividing the animal dose by 12.3 (conversion factor based on the body surface area of mice versus human adults) and subsequently applying a safety factor of 10 [34], resulted in safety limits of 6504 ng BisGMA/kg bw/day and 8130 ng TEGDMA/kg bw/day. In general, due to their lower body weight, children are more vulnerable to compounds leaching from composite materials. The EDI of BisGMA and TEGDMA was below this estimated safety limit (calculated from the NOAEL values) for both adults and children after acute and chronic exposure after replacement of single crowns of the different teeth or all crown in four quadrants. Nonetheless, the conditions in the mouth should be taken into account and the fact that, due to the continuous saliva flow in the mouth, the ingestion of monomers is not comparable to the single dose administration as used in the above mentioned studies. Still, long-term elution and subsequent chronic exposure to monomers from resin-based dental materials should not be neglected when assessing the overall human health risks.

5. Conclusion

As a conclusion, it can be stated that the tested composite materials continued to release certain monomers after an incubation period of 52 weeks. Further research is needed to determine the effect of the measured quantities of monomers on the oral cavity. However, several physiological conditions such as saliva and its flow rate, intestinal absorption, and metabolic clearance must be taken into account when evaluating the potential toxicity of the eluted compounds. Moreover, even if monomer elution may not lead to a risk at a short term, our findings warrant further research on potential long-term toxicity.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.dental.2019.01.005.

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