Analysis of organic components in resin-modified pulp capping materials: critical considerations

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The purpose of this study was to elucidate the organic composition and eluates of three resin-based pulp-capping materials in relation to their indications and safety data sheets. Uncured samples of Theracal LC, Ultra-Blend Plus, and Calcimol LC were investigated using gas chromatography–mass spectrometry (GC-MS) and ultra-performance liquid chromatography–mass spectrometry (UPLC-MS). Identification/quantification of 7-d leachables of cured samples was performed using GC-MS for 2-hydroxyethyl methacrylate (HEMA), 2-(dimethylamino)ethyl methacrylate (DMAEMA), camphorquinone (CQ), ethylene glycol dimethacrylate (EGDMA), ethyl-4-(dimethylamino)benzoate (DMABEE), and triethylene glycol dimethacrylate (TEGDMA). A similar organic composition was found for Ultra-Blend and Calcimol; however, only Ultra-Blend is indicated for direct pulp-capping. In contrast to the other materials analysed, Theracal contained substances of high molecular weight. The safety data sheets of all materials were incomplete. We detected HEMA, CQ, and TEGDMA in eluates from Ultra-Blend and Calcimol, and it was considered that HEMA might have originated from decomposition of diurethane dimethacrylate (UDMA) in the GC-injector. For Theracal, additives associated with light curing (DMABEE and CQ) were detected in higher amounts (4.11 and 19.95 μg mm^{-2}) than in the other materials. Pores were quantified in all samples by micro-computed tomography (micro-CT) analysis, which could influence leaching. The organic substances in the investigated materials might affect their clinical suitability as capping agents, especially for direct capping procedures.

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Polymer resin-based dental materials (PRMs) represent a widely used group of dental materials with different compositions, properties, and applications. While PRMs in general are used for direct restoration procedures, new materials that utilize the photocuring abilities of PRMs have emerged to simplify procedures such as pulp capping (1). However, the organic additives and methacrylates in PRMs are, in general, associated with negative biological effects in vitro, in animal studies, as well as clinically (2–9). Thus, this warrants a critical evaluation of the organic composition of light-curing materials indicated for pulp capping.

Pulp capping materials are used for two indications. Direct capping materials are placed in direct contact with pulp tissue, while indirect capping materials require a dentin barrier between the pulp and material. Both aim to assist in maintaining pulp vitality. Traditionally, calcium hydroxide has been used for direct capping procedures. However, mineral trioxide aggregate (MTA), a material consisting mainly of calcium silicates, is regarded by some as the new 'gold standard' from results of clinical trials (10–12). Both materials induce hydroxylapatite formation when in contact with

physiological solutions owing to their ability to increase pH and release calcium ions; however, the biological responses to these materials are not similar in vivo and in vitro (13, 14), and MTA has been shown to induce a higher-quality dentin-bridge than calcium hydroxide (13). A beneficial interaction between the constituents of MTA and pulp (e.g. cell adherence to the capping material) is suggested to be the reason for this response (15–18). This indicates the importance of the composition of the material on its biocompatible properties and hence its effectiveness as a capping material (15).

Several light-curing resin-modified pulp-capping materials are available for indirect and/or direct capping. These materials consist of components associated with traditional pulp therapy (e.g. calcium silicate and calcium hydroxide), in addition to methacrylates and additives that enable light curing. Direct pulp capping with polymer resin-based materials are in general associated with negative clinical outcomes (8–10, 19). It is therefore of interest to investigate the organic composition that will make some light-curing materials suitable for direct capping. This can be difficult to extrapolate from safety data sheets, as mass spectrometry (MS)

analyses of PRMs have shown that these data sheets are incomplete (20–23). By analysing the organic composition of, in addition to quantifying organic eluates from, resin-modified pulp-capping materials using MS-based methods, novel insights can be obtained regarding the relationship between indication for use and organic composition. This will provide clinicians and researchers with important information about a group of materials that may seem easy to use compared with other clinically proven materials – but may have a composition that makes their indication for pulp capping dubious (9–11).

The aims of this study were to: (i) analyse the organic composition of three light-curing resin-modified pulp-capping materials; (ii) quantify organic leachables; and (iii) evaluate these findings in light of material composition, safety data sheets, and indication for use.

Material and methods

Overview

Three resin-based pulp-capping materials, Theracal LC (TH), Ultra-Blend Plus (UB), and Calcimol LC (CA), were investigated for organic components and 7-d leachables in water. For identification of organic substances, uncured samples of these materials were dissolved in ethyl acetate and water before analysis with gas chromatography–mass spectrometry (GC-MS) or ultra-performance liquid chromatography–mass spectrometry (UPLC-MS). Subsequently, precured samples of each material were immersed in water for 7 d. The eluates were analysed using GC-MS in full Scan mode and single ion recording (SIR) mode, for identification and quantification of organic substances commonly found in PRMs. A flow chart of the workflow is presented in Fig. 1.

Materials and chemicals

Calcimol LC (VOCO, Cuxhaven, Germany; LOT: 1533389), Theracal LC (BISCO, Schaumburg, USA; LOT: 1500006301), and Ultra-Blend Plus (Ultradent Products,

South Jordan, UT, USA; LOT: BB59V) were bought from dental suppliers in Norway. The safety data sheet for the respective materials was requested from the suppliers. A summary of the safety data sheets provided, and highlights of 'instruction for use' are presented in Table 1. Analytical grade solvents and standards were obtained from Sigma–Aldrich (Oslo, Norway) (Table 2).

Preparation of uncured samples for GC-MS and UPLC-MS

A small amount (~2 mg) of TH, CA, and UB were weighed with an analytical balance in individual polypropylene Eppendorf tubes (Sigma-Aldrich, St. Louis, MO, USA). Two samples of each material were dissolved in either ethyl acetate or water (ISO 3696-Grade II). The tubes were centrifuged at 20,000 g for 5 min to separate the filler and matrix phases. The supernatant of each sample was transferred to a glass vial and diluted before further analysis.

Preparation of cured samples, 7-d leaching in water

Pilot studies were performed to determine appropriate sample thickness as 1-mm-thick samples of TH showed incomplete cure. Accordingly, six samples of TH, CA, and UB (diameter 10 mm; thickness 0.65 ± 0.05 mm) were prepared in a Teflon mould. Uncured material was inserted into the mould, covered with a polyethylene film, and compressed with a glass slide. After removal of the glass slide, light curing was performed using a corded BluePhase G2 light-curing device (Ivoclar/Vivadent, Schaan, Lichtenstein), in accordance with the manufacturer's instructions for each capping material (Table 1). Slight movement of the light curing tip was carried out during curing to compensate for irradiance heterogeneity and discrepancy between the optical tip area and the mould diameter (24, 25). The irradiance of the curing device was assessed before sample preparation using a calibrated laboratory-grade National Institute of Science and Technology (NIST; Gaithersburg, MD, USA)-referenced USB4000 Spectrometer [Managing Accurate Resin Curing (MARC) System; Bluelight Analytics, Halifax, Nova Scotia, Canada] to ensure an irradiance in the range of $1{,}350 \pm 100$ mW cm^{-2}.
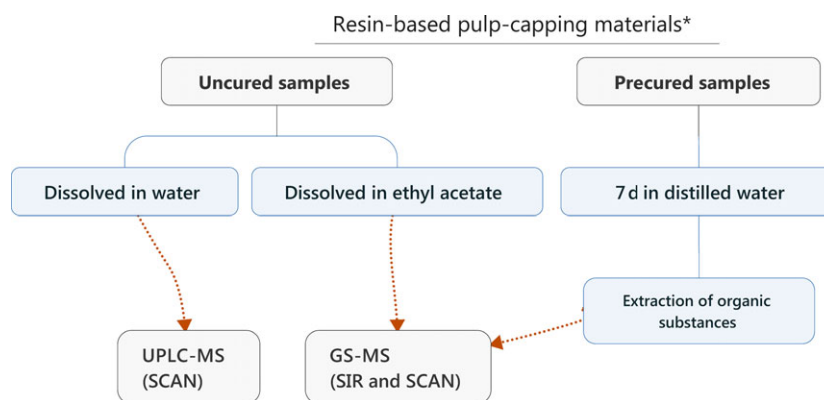

Fig. 1. Workflow of the gas chromatography–mass spectrometry (GC-MS) and ultra-performance liquid chromatography–mass spectrometry (UPLC-MS) analysis. *Theracal LC (TC), Ultra-Blend Plus (UB), and Calcimol LC (CA). SCAN, full Scan mode; SIR, single ion recording.

Table 1

Summary of the safety data sheets and instruction for use of the investigated materials

Material (abbreviation); manufacturer	Safety data sheet summary			Supplier/safety data sheet print-date	Pulp capping indication	Curing time (s)	Maximum curing depth (mm)
	Name	%	CAS				
Calcimol LC (CA); VOCO	Urethane dimethacrylate	25–50	72869-86-4	DENTALSPAR/ 7.04.2015	Indirect	20	1
	Pyrogen silica	10–25					
	Calcium dihydroxide	2.5–5	1305-62/-0				
	2-Dimethylaminoethyl methacrylate	≤2.5	2867-47-2				
Theracal LC (TH); BISCO	Portland cement type III	20–60	65997-15-1	NORSK ORTHOFORM DEPOT/08.29.2011	Indirect and direct*	20	1
	Poly(ethylene glycol) dimethacrylate	10–50	25852-47-5				
	Bis-GMA	5–20	1565-94-2				
	Barium zirconate	1–10	12009-21-1				
Ultra-Blend Plus[†] (UB); Ultradent Products	Calcium dihydroxide	5	1305-62-0	NORSK ORTHOFORM DEPOT/23.04.2015	Indirect and direct[‡]	10	3
	2,2′-Ethylenedioxydiethyl dimethacrylate	<5	109-16-0				
	Tricalcium bis (orthophosphate)	<15	7758-87-4				

*Indicated for carious and mechanical exposures.

[†]The instruction for use of Ultra-Blend Plus states that the resin of the material is diurethane dimethacrylate (UDMA) based. However, UDMA is not mentioned in the safety data sheet.

[‡]Indicated for mechanical exposures.

Bis-GMA, bisphenol A-glycidyl methacrylate; CAS, CAS Registry Number.

Table 2

Chemicals and solvents used in the present study

Abbreviation	Name (vendor, catalogue number)	CAS	Function	Purpose
CQ	Camphorquinone 97% (Sigma-Aldrich, 124893)	10373-78-1	Photo-initiator	Quantification/Identification (GC-MS)
DMABEE	Ethyl 4-(dimethylamino)benzoate ≥99% (Sigma-Aldrich, E24905)	10287-53-3	Co-initiator	Quantification/Identification (GC-MS)
DMAEMA	2-(Dimethylamino)ethyl methacrylate 98% (Sigma-Aldrich, 234907)	2867-47-2	Co-initiator	Quantification/Identification (GC-MS/UPLC-MS)
HEMA	2-Hydroxyethyl methacrylate ≥99%, (Sigma-Aldrich, 477028)	868-77-9	Base monomers	Quantification/Identification (GC-MS/UPLC-MS)
EGDMA	Ethylene glycol dimethacrylate 98% (Sigma-Aldrich, 335681)	97-90-5	Base monomers	Quantification/Identification (GC-MS)
TEGDMA	Triethylene glycol dimethacrylate 99% (Sigma-Aldrich, 759406)	109-16-0	Base monomers	Quantification/Identification (GC-MS/UPLC-MS)
IS	Diethyl phthalate 99.5% (Sigma-Aldrich, 524972)	84-66-2	–	Internal standard
UDMA	Diurethane dimethacrylate, ≥97% (Sigma-Aldrich, 436909)	72869-86-4	Base monomers	Identification (UPLC-MS)
PEGDM	Poly(ethylene glycol) dimethacrylate, average Mn 550 (Sigma-Aldrich, 25852-47-5)	25852-47-5	Base monomers	Identification (UPLC-MS)
Bis-GMA	Bisphenol A-glycidyl methacrylate (Sigma-Aldrich, 494356)	1565-94-2	Base monomers	Identification (UPLC-MS)
EtOAc	Ethyl acetate 99.8% (Sigma-Aldrich, 270989)	141-78-6	–	Solvent
–	Ethanol 96% (Sigma-Aldrich, 24106)	64-17-5	–	Solvent

CAS, CAS Registry Number; GC-MS, gas chromatography–mass spectrometry; UPLC-MS, ultra-performance liquid chromatography–mass spectrometry.

Immediately after curing, the specimens were examined visually for defects, weighed, and thickness was measured using a Heidenhain ND287 micrometer (Heidenhain, Traunreut, Germany). All samples showed visible pores, and it was concluded that pore-free samples could not be produced. The samples were immersed in 4 ml of distilled water (Grade II ISO 3696:1987) in glass vials (Karl Hecht, Sondheim von der Rhön, Germany) and kept at 37 ± 1°C for 7 d. As pH is an important parameter for the claimed therapeutic effect of the materials examined, pH was measured in the immersion medium. Measurements were performed before and after the 7-d immersion using

Universal pH indicator paper (Merck Millipore, Billerica, MA, USA) and a calibrated pH meter (WTW inoLab Multi 9310P; Xylem, Rye Brook, NY, USA).

Extraction of organic substances

After 7 d of immersion, 1 ml of water from all samples was transferred to glass vials for extraction of organic substances. In brief, 0.5 ml of ethyl acetate with 8 μg of diethyl phthalate (internal standard) was added to all samples, and vials were vortexed. After phase separation, the ethyl acetate phase was transferred to liquid chromatography–gas chromatography (LC-GC)-certified vials with glass Pasteur pipettes, before repeating the extraction process twice for all samples (without additional internal standard). Then, the vials were fitted with screw-threaded caps with a Polytetrafluoroethylene/silicone septum and stored at 4 ± 1°C until required for analysis.

Micro-computed tomography analysis of cured samples

A micro-computed tomography (micro-CT) instrument (BRUKER SKYSCAN 1272; Bruker, Kontich, Belgium) was used to assess the extent of internal pores in the cured samples after the immersion period. All samples were stored in a desiccator for 4 wk before scanning. Three random samples of each material were selected and scanned at a resolution of 8.15 μm voxel^{-1}. The projections from the scans were reconstructed with filtered backprojection, using nRecon (Bruker). Quantitative analysis of volume and surface area of voids were performed with CTAn (Bruker). A three-dimensional model of a sample from each material (with voids) was generated using the same software.

GC-MS analysis set up

The GC-MS instrumentation consisted of a 7683B autosampler and a 6890N GC from Agilent (Santa Clara, CA, USA) connected to a QuattroMicro GC from Micromass (Waters, Milford, MA, USA). Instrument control, data sampling, and handling were controlled using MassLynx 4.1 (Waters, Milford, MA, USA). The gas chromatograph was equipped with a capillary column (Restek Rxi 5MS, 30 m; internal diameter = 0.32 mm, film thickness = 0.25 μm; Restek, Benner Circle, PA, USA). Helium (5.0 grade) was used as carrier gas with a flow rate of 1 ml min^{-1}. Splitless injection was used, and the injector temperature was 250°C. The column start temperature was 50°C, which was increased at a rate of 50°C min^{-1} up to 120°C, held at 120°C for 5 min, then increased from 120 to 280°C at a rate of 10°C min^{-1}, then held at 280°C for 1 min.

Identification of substances in the uncured and extracted samples was performed using the mass spectrometer in full scan mode, scanning from 50 to 350 mass-to-charge ratio (m/z). Identification was performed by comparing the retention time and spectra obtained for the samples with reference substances (Table 3). Substances not identified by reference substances were compared with data from the NIST library.

Calibration curves and quantification (GC-MS)

The reference substances 2-hydroxyethyl methacrylate (HEMA), 2-(dimethylamino)ethyl methacrylate (DMAEMA), camphorquinone (CQ), ethylene glycol dimethacrylate (EGDMA), ethyl 4-(dimethylamino)benzoate (DMABEE), and triethylene glycol dimethacrylate (TEGDMA) were weighed out in glassware using a scientific balance, and diluted in ethyl acetate to create a stock solution containing all the reference substances. The stock solution was serially diluted 1:2, 1:4, 1:8, 1:16; 1:32, 1:64, 1:128, and 1:256. Then, 1 ml of each dilution was transferred to a GC vial (Waters) and 0.5 ml of ethyl acetate with 8 μg of diethyl phthalate (internal standard) was added to prepare the calibration curve by plotting the area of the analyte/internal standard against the concentration of the analyte in the eight serial dilutions (30–0.001 μg ml^{-1}).

Quantification of substances in the extracted ethyl acetate was performed by SIR analysis of abundant ions characteristic for each analyte (Table 3). Comparison of area under the peak with the area of the internal standard peak was performed for each analyte. The ratio was used in conjunction with the calibration curve to determine the concentration of substances. The amount of eluate was then calculated and expressed in μg mm^{-2}.

UPLC/Q-TOF mass spectroscopy identification

Analysis of uncured samples of TH, UB, and CA was performed on an Acquity UPLC I-Class System connected to a Xevo G2 quadrupole time-of-flight (Q-TOF) machine (both from Waters, Milfors, MA, USA). Full scan spectra [electron spray ionization (ESI)+ mode] were obtained in the mass range 105–1,200 Daltons with a scan time of 300 ms and interscan time of 14 ms. The column used was an ACQUITY UPLC BEH C18 1.7 μm. Internal diameter was 2.1 mm and length was 150 mm. Mobile phase: water (A) and acetonitrile (B) with 0.1% formic acid mobile phase, gradient 95/5 (A/B) at 0 min and 5/95 at 10 min, flow rate of 0.25 ml min^{-1}, and column temperature 65.0°C.

Identification of substances in the samples was performed by comparing the retention times and mass spectra obtained with the corresponding retention time and spectra of the reference substances of TEGDMA, HEMA, poly(ethylene glycol) dimethacrylate (PEGDM), diurethane dimethacrylate (UDMA), DMAEMA, and bisphenol A-glycidyl methacrylate (Bis-GMA).

Table 3

Molecular and characteristic ions of substances identified and quantified identified and of the internal standard

Substance	Molecular ion (m/z)	Characteristic ions (GC-MS)	Characteristic ions (UPLC)
HEMA	130	69*, 87, 130	130
DMAEMA	193	58*, 71	193
CQ	166	95*, 138, 166	166
EGDMA	192	69*, 113	192
DMABEE	193	148*, 164, 193	193
TEGDMA	286	69*, 113	286
Diethyl phthalate	222	149* 177	222

*Quantifying ion.
CQ, camphorquinone; DMABEE, ethyl-4-(dimethylamino)benzoate; DMAEMA, 2-(dimethylamino)ethyl methacrylate; EGDMA, ethylene-glycol dimethacrylate; GC-MS, gas chromatography–mass spectrometry; HEMA, 2-hydroxyethyl methacrylate; m/z, mass-to-charge ratio; TEGDMA, triethylene-glycol dimethacrylate; UPLC, ultra-performance liquid chromatography.

Validation

Blank samples of equipment (e.g. glassware, polyester films, pipettes, polypropylene tubes, and rubber bulbs) and chemicals (e.g. ethyl acetate, water, and ethanol) used during sample preparation were collected and analysed using GC-MS to identify contaminants that might interfere with the analysis. Carry-over was assessed by analysing blanks of ethyl acetate in between samples in the GC-MS analysis.

Limit of detection and the lowest limit of quantification were set as ≥ 3 and ≥ 10 signal to noise, and were determined by analysing reference substances in concentrations ranging from 0.001 to 30 μg ml^{-1}. The signal to noise was determined visually by inspecting the chromatograms. Precision was assessed by analysing the 2 and 5 μg ml^{-1} samples between repeated measurements within and between days.

Statistical methods

The results are presented as mean ± SD. The quantitative data from the micro-CT analysis were analysed using one-way ANOVA with an alpha value of 0.05. Normality (Shapiro–Wilk) and equal variance (Brown–Forsythe) tests

were performed. Data analysis and plotting data onto graphs were performed in Sigmaplot 13 (Systat, San Jose, CA, USA) and Microsoft Excel 2013 (Microsoft, Redmond, WA, USA).

Results

GC-MS analysis of organic substances in uncured samples

Analysis of the calcium hydroxide-containing capping materials (UB and CA) resulted in similar chromatograms, indicating that these materials contained many of the same substances (Fig. 2). In these materials, HEMA, DMAEMA, CQ, and TEGDMA were identified by use of reference standards. In addition, EGDMA was identified in CA. In the calcium-silicate-containing TH, only substances associated with light curing (CQ and DMABEE) were identified (Table 4).

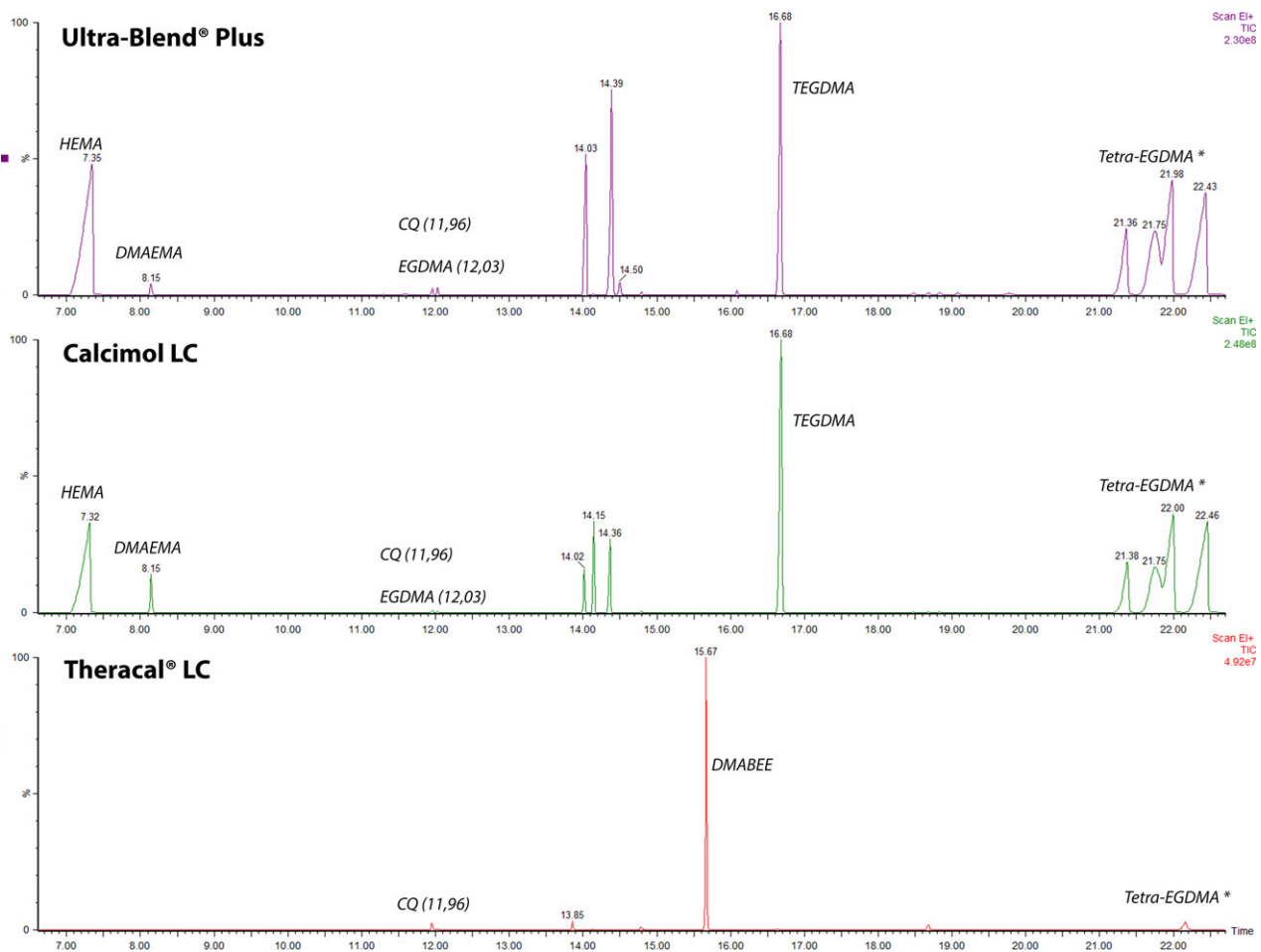The presence of TEGDMA was suggested in all materials when searches in the NIST library were performed

Fig. 2. Gas chromatography–mass spectrometry (GC-MS) chromatograms of uncured samples of Theracal LC (TH), Calcimol LC (CA), and Ultra-Blend Plus (UB) (full scan mode). *As suggested by the National Institute of Science and Technology (NIST) library (Gaithersburg, MD, USA). CQ, camphorquinone; DMABEE, ethyl-4-(dimethylamino)benzoate; DMAEMA, 2-(dimethylamino)ethyl methacrylate; EGDMA, ethylene-glycol dimethacrylate; GC-MS, gas chromatography–mass spectrometry; HEMA, 2-hydroxyethyl methacrylate; TEGDMA, triethylene-glycol dimethacrylate.

Table 4

Summary of substances identified and quantified in the gas chromatography–mass spectrometry (GC-MS) analysis of uncured and cured samples

Resin based pulp-capping material	HEMA*	DMAEMA	CQ	EGDMA	DMABEE	TEGDMA
TH	ND	ND	4.11 ± 0.41 (C)	ND	19.95 ± 10.08 (C)	ND
UB	8.65 ± 3.84 (C)	D	2.05 ± 0.33 (C)	D	ND	0.13[†,‡] (C)
CA	14.14 ± 2.02 (C)	D[†]	0.18 ± 0.02 (C)	D	ND	1.39 ± 0.66 (C)

*HEMA was not detected in uncured samples of Ultrablend Plus and Calcimol LC using ultra-performance liquid chromatography (UPLC).
[†]Reported in the safety data sheet supplied with the material.
[‡]Only one sample had concentrations of TEGDMA above the lower limit of quantification.
Values represent mean ± SD of six samples, and are given as $\mu g\ mm^{-2}$. C, detected from cured samples; CA, Calcimol LC; CQ, camphorquinone; D, detected from uncured samples; DMABEE, ethyl-4-(dimethylamino)benzoate; DMAEMA, 2-(dimethylamino)ethyl methacrylate; EGDMA, ethylene-glycol dimethacrylate; HEMA, 2-hydroxyethyl methacrylate; ND, not detected; TEGDMA, triethylene-glycol dimethacrylate; TH, Theracal LC; UB, Ultra-Blend Plus.

of the spectra obtained from the chromatography peaks with retention time 21.38–22.46 min (probability ~ 10%). The peak eluting at 14.15 min provided a spectrum similar to the NIST library spectrum of butylated hydroxytoluene (~ 30% and 65% probability in TH and CA, respectively). The peaks around 14.02 and 14.39 retention time in the UB and CA chromatograms were suggested to represent 1.3-cyclohexanedione, 2.4.6-trimethyl, and 1.3-cyclohexanedione, 4.5-dimethyl, respectively (~ 30% and 8% probability, respectively).

Validation of GC-MS quantification

The coefficient of determination (r^2) was calculated to be >0.99 for all calibration curves. The limit of detection and limit of quantification were determined to be 0.1 and 1 $\mu g\ ml^{-1}$, respectively, for all substances, except for HEMA, which had a limit of detection and a limit of quantification of 0.1–1 $\mu g\ ml^{-1}$ and 1 $\mu g\ ml^{-1}$, respectively. A summary of precision calculations is presented in Table 5.

GC-MS quantitative analysis of water eluates from cured samples

The GC-MS analysis (SIR mode) of the ethyl acetate fractions revealed that the organic substances detected varied between materials. The only substance detected in all samples was CQ (TH > UB > CA) (Table 4). The amounts of HEMA and TEGDMA detected in the CA samples were two-fold and 10-fold higher, respectively, compared with the amounts found in the UB samples. Co-initiators eluted only from TH samples. Of all the substances investigated, the highest amounts eluted and the highest SD (19.95 ± 10.08 $\mu g\ mm^{-2}$) were found for the co-initiator DMABEE.

UPLC-MS analysis of organic substances in uncured samples

The chromatograms from the UPLC-MS analysis (SCAN-mode) are presented in Fig. 3. As in the GC-MS analysis, similar chromatograms were obtained for UB

and CA. When comparing these chromatograms with analysis of reference substances, UDMA and TEGDMA could be identified in CA and UB. However, no HEMA was detected. In TH, no Bis-GMA was detected despite being declared in the safety data sheet provided by the supplier. The chromatogram obtained of the reference substance of PEGDM differed considerably from that of the PEGDM present in TH, yet they had the same CAS number (Fig. 4).

pH analysis of water media

This analysis showed elevation of pH in the medium of UB (pH 8.42 ± 0.02) and CA (pH 8.41 ± 0.03) compared with the blank (pH 7.32 ± 0.06). The TH samples were considerably more alkaline compared with the other materials tested (pH 9.97 ± 0.04).

Micro-CT

All samples selected for scanning had visible and internal pores. The quantitative analysis of pore volume

Table 5

Within- and between-day precision values for the quantified substances

Substance	Concentration ($\mu g\ ml^{-1}$)*	Within-day		Between-day	
		SD[†]	RSD (%)	SD[†]	RSD (%)
HEMA	5.4	0.002	1.2	0.03	12.7
	2.2	0.002	3.2	0.01	14.7
CQ	4.7	0.010	5.7	0.02	11.2
	1.9	0.004	5.6	0.01	16.3
DMABEE	5.1	0.010	3.6	0.06	14.4
	2.1	0.005	5.6	0.04	23.9
TEGDMA	5.0	0.003	0.9	0.07	17.2
	2.0	0.004	3.7	0.03	26.3

*Concentration of substance in samples used for precision calculations.
[†]$n = 3$.
CQ, camphorquinone; DMABEE, ethyl-4-(dimethylamino)benzoate; HEMA, 2-hydroxyethyl methacrylate; RSD, relative standard deviation; TEGDMA, triethylene-glycol dimethacrylate.
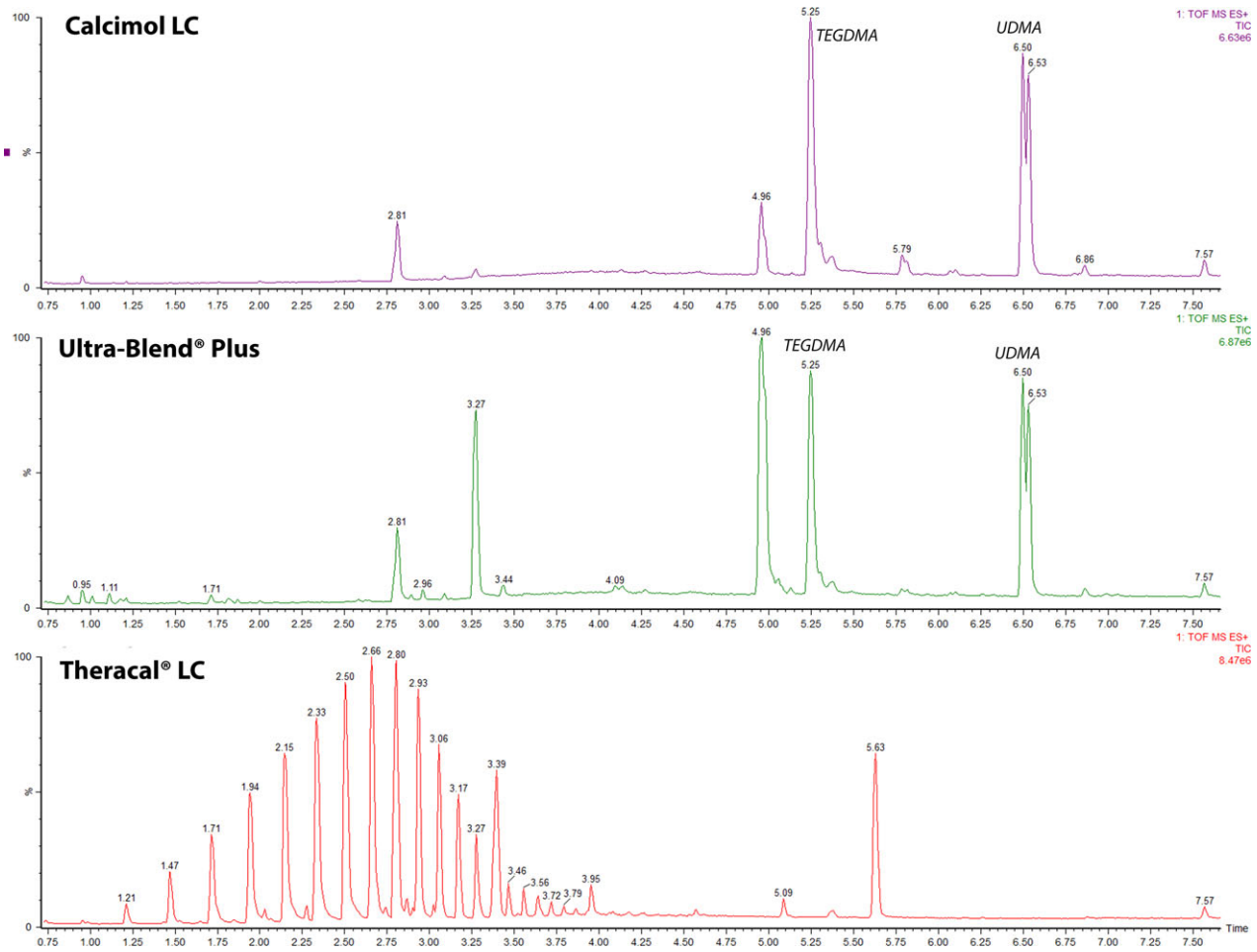
Fig. 3. Ultra-performance liquid chromatography–mass spectrometry (UPLC-MS) chromatograms of uncured samples of Theracal LC (TH), Calcimol LC (CA), and Ultra-Blend Plus (UB). TEGDMA, triethylene-glycol dimethacrylate; UDMA, diurethane dimethacrylate.

revealed that the distribution, amount, and size of pores were heterogeneous between materials and samples (Table 6). The quantitative analysis of pore volume suggested that UB and CA have a higher volume of pores than TH, although this was not statistically significantly different. Yet, the statistical analysis showed that CA had significantly higher surface area of pores compared

Table 6

Results of the quantitative micro-computed tomography (micro-CT) analysis of pores in cured samples

| | Resin based pulp-capping material | | |
Variable	CA	TH	UB
Total volume (mm^3)	42.88 ± 0.08	44.61 ± 2.46	43.1 ± 1.61
Pore volume (mm^3)	0.63 ± 0.10	0.08 ± 0.03	0.42 ± 0.48
% Pore volume of total volume	1.48 ± 0.23	0.19 ± 0.09	0.96 ± 1.10
Pore surface area (mm^2)	27.71 ± 1.35	2.37 ± 0.6	9.94 ± 8.40

Values represent mean ± SD of three samples.
CA, Calcimol LC; TH, Theracal LC; UB, Ultra-Blend Plus.

with UB and TH. A representative picture of the scans – in addition to three-dimensional (3D) models of the cured samples with pores – is displayed in Fig. 5.

Discussion

Analysis of the light-curing resin-modified pulp-capping materials investigated in this study suggests that these materials contain and elute organic substances not declared in the safety data sheet provided by the suppliers. Despite apparently similar organic composition of CA and UB, only UB was indicated for direct pulp capping by the manufacturer. Direct pulp capping with resin-modified capping materials will increase the risk of exposing patients to high doses of organic substances, usually found in PRMs, associated with adverse pulp reactions when used for this purpose (9, 19, 26). The investigated materials contain substances that are usually not found in PRMs (i.e. calcium silicate cement and calcium hydroxide). This will affect their handling compared with other PRMs. Considerations are presented in the following paragraphs.
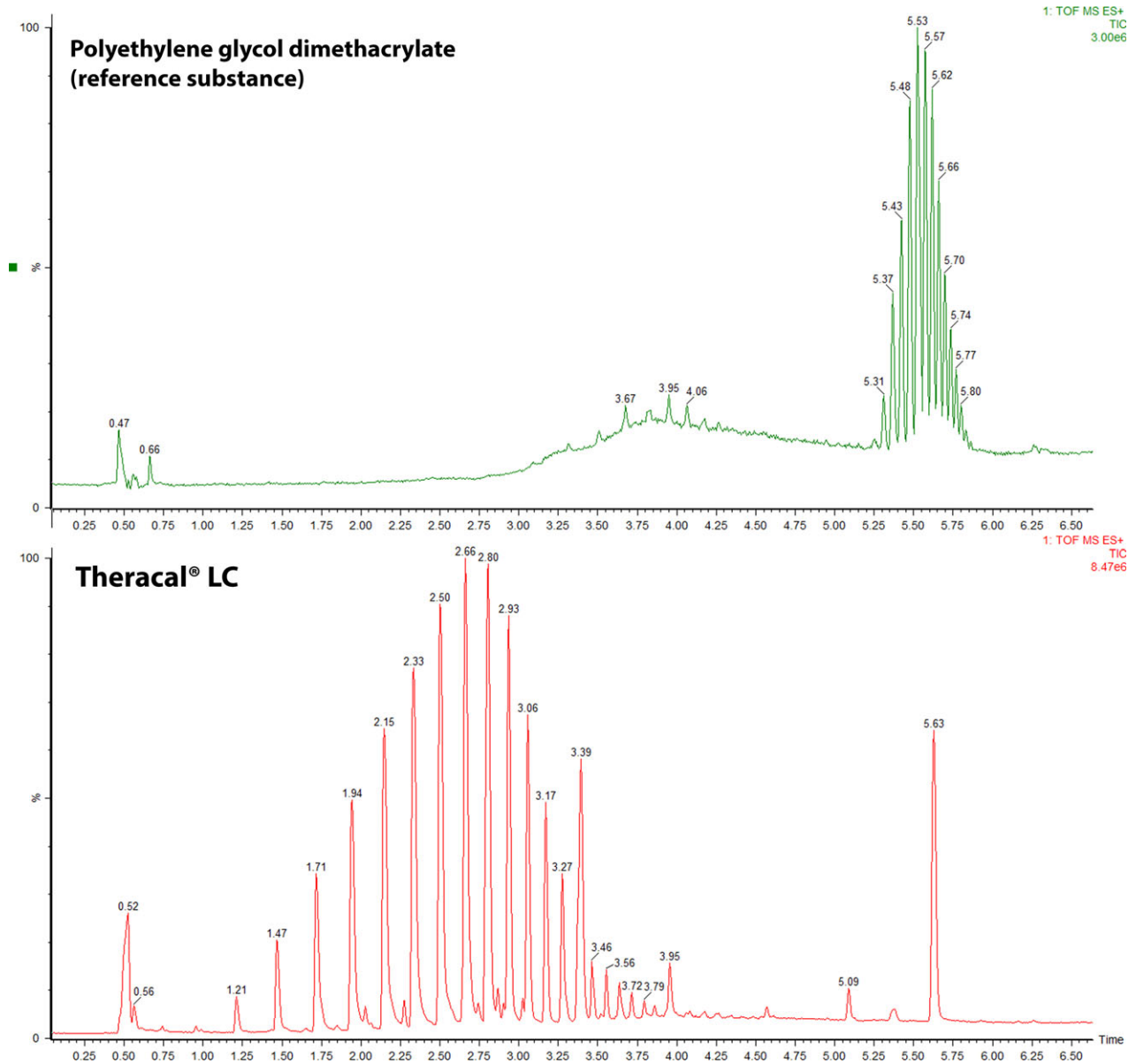
Fig. 4. The chromatograms show the results of ultra-performance liquid chromatography–mass spectrometry (UPLC-MS) analysis of Theracal LC (TH) and the reference substance, polyethylene glycol dimethacrylate.

Pilot studies were performed to determine an appropriate sample preparation. In these studies, a Bluephase-Style (Ivoclar/Vivadent, Schaan, Lichtenstein) device was used for light curing. Interestingly, it was not possible to produce a 1-mm-thick sample of TH that was sufficiently cured with this unit (Fig. 6). As these problems partly persisted with a Bluephase G2 device (Ivoclar/Vivadent) – a device with a more homogeneous light distribution than that of the Bluephase-Style device (24, 27) – it was decided to make samples with a thickness of 0.65 mm in the final experiment. The curing difficulties observed could be the result of poor light transmission in TH because of its high content of calcium silicate cement. In clinical use, this could be detrimental, as clinicians will not be able to detect if the bottom of the material is properly cured.

Additional difficulties encountered during sample preparation were the presence of pores. All cured samples had external and internal pores (Fig. 5). The shape and size of pores varied between the materials and samples. Among the parameters assessed in the statistical analysis of the micro-CT results, only surface area of pores was significantly higher in one of the examined materials (CA). The large surface area of pores in CA suggests a higher number of smaller pores in comparison with the other materials. The origin of pores could be manufacturer/material related (e.g. introduced during mixing of ingredients and/or introduced during insertion of material into the syringes, or introduced during sample preparation). However, great care was taken not to introduce air into the samples. Pores will
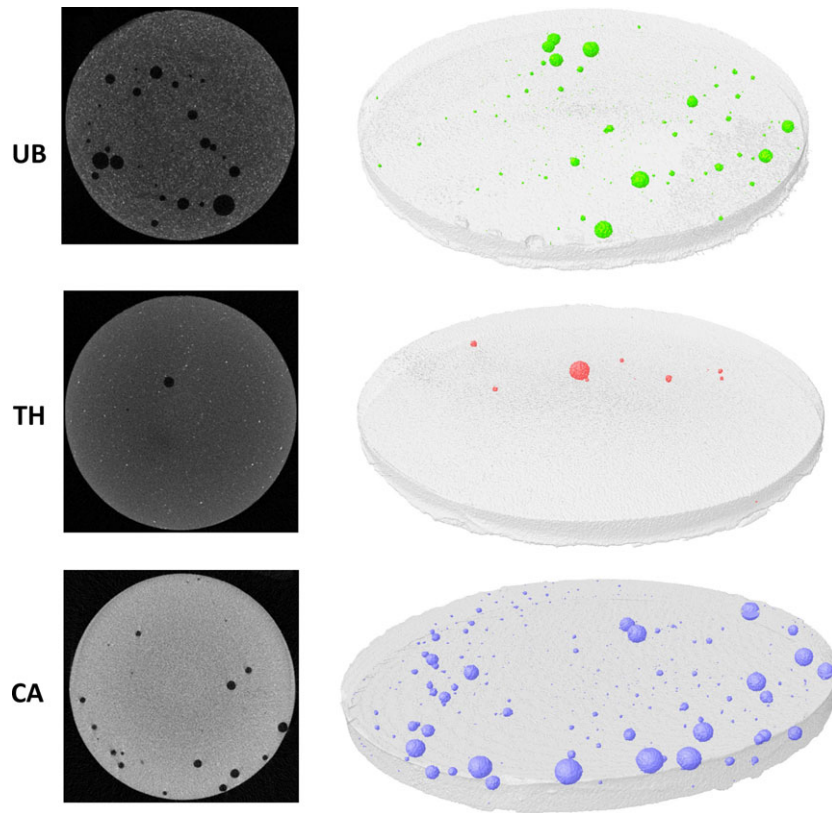
Fig. 5. The micro-computed tomography (micro-CT) analysis revealed internal pores in all samples. Left column, cross-sectional images of reconstructed data. Right column, three-dimensional (3D) models generated using CTAn. Sample diameter = 10 mm. CA, Calcimol LC; TH, Theracal LC; UB, Ultra-Blend Plus.
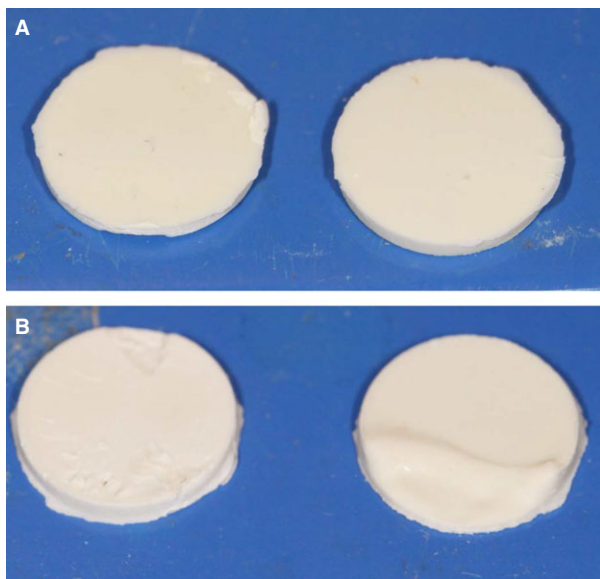

Fig. 6. The photographs show the top (A) and bottom (B) surfaces of 1 mm-thick Theracal LC (TH) samples, cured for 20 s. The top surface was hard, while the bottom surface was sticky and clearly not properly cured.

decrease the sealing ability, as well as increase the internal and external surfaces of the material. The latter could increase the rate of hygroscopic/hydrolytic effects and increase elution (28). In summary, pores will probably have a negative influence on the clinical performance of these materials.

Analysis of the resin-modified calcium hydroxide-containing materials (UB and CA) demonstrated many similarities compared with the resin-modified calcium silicate, TH. They had a similar effect on the pH of the incubation medium, and chromatograms and mass spectra obtained from the analysis of uncured samples of these materials suggested a similar organic composition. Despite this, the manufacturers have different indications for use of these materials. Calcimol LC should only be used as an indirect capping material, whereas Ultra-Blend Plus can also be used directly on the pulp (i.e. following traumatic pulp exposure). Other differences observed in their instructions for use concerns the curing time. For example, UB is stated to need only 10 s of light curing, in contrast to 20 s for CA. Interestingly, smaller amounts of most eluates were detected in samples of UB than in samples of CA. This observation correlated with the higher surface area of pores seen in CA samples. Of the eluates, CQ was the only one detected in smaller amounts in the CA samples compared with the other materials analysed. The higher radiant exposure for the CA samples – with more CQ reacting – could perhaps explain this phenomenon.

2-Hydroxyethyl methacrylate was detected in the GC-MS analysis of the CA and UB samples, but not

when UPLC-MS analysis was performed on the same materials. It can therefore be questioned whether HEMA actually was present. Fragmentation of UDMA to HEMA in the GC-injector has previously been described (23). Accordingly, detection of HEMA in the GC-MS analysis could be cautiously interpreted as an indirect measurement of UDMA elution. Yet, the presence of HEMA in CA and UB cannot be excluded as the concentration of HEMA could be below the limit of detection of the UPLC-MS analysis. Regardless of whether or not HEMA was present in CA and UB, these materials contain and elute several organic substances usually found in PRMs, such as bonding or restorative materials (29).

Theracal LC is a resin-modified calcium silicate that has been described as a light-cured MTA-like material (30, 31). According to its instructions for use, it can be used for direct pulp capping after mechanical and carious exposures. The GC and UPLC-MS analysis of uncured TH suggest that it is composed of some organic substances found in PRMs (i.e. CQ and DMA-BEE) in addition to high-molecular-weight substances that are not widely used in PRMs (i.e. PEGDM). In the cured samples of TH, only substances associated with photopolymerization were detected. Compared with CA and UB, TH eluted two- and 40-fold higher amounts of CQ. In addition, the co-initiator DMABEE was found only in TH and was found in larger amounts than any other eluate. Taking this and the difficulties encountered during the light-curing procedure into consideration, the results could suggest that the composition of TH is not optimal for light curing.

The UPLC-MS analysis of TH revealed that the PEGDM identified in TH did not – despite having a similar CAS number – match the chromatogram of the reference substance. A CAS registry number is a unique numeric identifier that designates only one substance (32). However, a search with the CAS number of PEGDM on Sigma Aldrich's webpage yields five different reference substances for PEGDM [with different number average molecular weight (M_n)] (33). The chromatograms obtained from the reference substance and TH demonstrated that the CAS number of PEGDM symbolizes a range of substances. These substances have shown different biological activities, as the number of repeated units affects cytotoxicity (34). Thus, from a health, safety, and environment point of view, average molecular weight should be reported in the safety data sheet.

No Bis-GMA was detected in the UPLC-MS analysis of TH, despite being listed in the safety data sheet provided by the supplier (dated 2011). Upon further investigation, newer safety data sheets do not list Bis-GMA as an ingredient (35). This implies that clinicians can be provided with outdated safety data sheets. It also suggests that changes in composition of materials could occur without the supplier and/or clinicians being notified. Other studies on TH could, in that case, have been performed with a material of dissimilar composition to the material tested in the present study (30, 31, 36–39). The absence of transparency associated with altering composition of materials is problematic for clinicians and researchers.

Safety data sheets have been reported as incomplete for many products, including PRMs (21, 23, 40–42). The same was evident for the materials investigated in the present study. In addition, manufacturers were not aligned on which substances to include in the safety data sheet. Both CA and UB contained UDMA and TEGDMA; however, UDMA was only listed in the safety sheet of CA. Moreover, TEGDMA was listed in the safety data sheet for UB with the less well-known chemical nomenclature, 2.2′-ethylenedioxydiethyl dimethacrylate. This implies that clinicians will not be able to evaluate the composition of the materials they are responsible for using.

Matrix constituents can affect the availability of therapeutic agents, in contrast to conventional, non-matrix-associated materials. It has been shown that chemically cured materials have a more alkaline effect compared with light-cured capping materials (43). In the present study, all materials – and in particular TH – were able to increase the pH of the medium. Thus, for TH it can be speculated whether the increased pH was partly the result of inadequate curing. The ability of a material to cause pH changes in vitro might also deviate from the performance in vivo. Studies have shown that lower amounts of calcium (Ca) and hydroxyl (OH) ions were released from TH when used in a tooth model than when the material was fully immersed in water (36, 44). In summary, these observations challenge the suitability of resin-modified capping materials as substitutes for traditional, indirect capping-agents.

Having said this, it should be noted that the use of traditional materials for direct capping is perhaps even more precarious. In the case of carious or traumatic exposure, the pulp is a non-epitelized wound surface. If TH or UB is used for direct capping, the wound surface will be exposed to molar concentrations of unpolymerized substances during application (45). In addition, the moist wound surface will interfere with polymerization, thus continuously exposing the pulp to organic substances (9). This can be problematic from a sensitization perspective (46–50). Thermal injury as a result of heat development during light curing may also be hazardous to the pulp and in vitro results indicate an average increase in pulp temperatures of 8.8°C and 7.5°C for UB and TH, respectively, when used for indirect capping (51, 52).

Polymer-resin-based materials have been shown to cause non-symptomatic failures in the pulp–material interface. Although direct capping with resin adhesives caused no clinical symptoms in patients after 30 d, all teeth were diagnosed as subclinical failures when examined by microscopy after extraction (9). Concerning the materials examined, clinicians risk interpreting 'no symptoms' as evidence of clinical success. Although the long-term effect of capping is yet to be investigated, a clinical study involving TH is in progress (53).

Direct pulp capping is a popular treatment modality in Europe (54). In this regard, UB and TH might be very attractive for clinicians because of their ease of

handling. For example, recent research suggests that TH is used more often than MTA for direct capping among dentists in Norway (55). However, based on the results of the present study and of other in vitro studies, the use of these materials may be questioned (38, 39, 56). Patient safety and evidence-based dentistry should govern clinical decision-making. Several meta-analytical studies of randomized clinical trials have proven the safety and efficacy of calcium hydroxide and calcium silicate cements (10, 11). As a consequence, the use of undocumented materials – with seemingly only one benefit (easier application) compared with documented materials – is not recommended.

Our data suggest that the light-curing resin-modified pulp-capping materials investigated in the present study contain and elute several reactive, organic substances that are not declared in the safety data sheets of the respective materials. These materials currently lack clinical and experimental evidence to support their use for pulp-capping procedures over other clinically documented materials.

Conflicts of interest – The authors declare no conflicts of interest.

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