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In-vitro-cytotoxicity of self-adhesive dental restorative materials

Ella Ohlsson [a], Carola Bolay [b], Sevgi Arabulan [c], Kerstin M. Galler [a], Wolfgang Buchalla [b], Gottfried Schmalz [b,d], Matthias Widbiller [b,*]

[a] Department of Operative Dentistry and Periodontology, Friedrich-Alexander-University Erlangen-Nürnberg, Glückstraβe 11, 91054 Erlangen, Germany
[b] Department of Conservative Dentistry and Periodontology, University Hospital Regensburg, Franz-Josef-Strauβ-Allee 11, 93053 Regensburg, Germany
[c] Department of Pedodontics, Ege University, Ege University Campus, 35040 Izmir, Turkey
[d] Department of Periodontology, University of Bern, 3012 Bern, Switzerland

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ABSTRACT

Objectives: Although the introduction of self-adhesive composites in restorative dentistry is very promising, the innovation of new materials also presents challenges and unknowns. Therefore, the aim of this study was to investigate the cytotoxicity of four different self-adhesive composites (SAC) in vitro and to compare them with resin-modified glass ionomer cements (RM-GIC), a more established group of materials.
Methods: Samples of the following materials were prepared according to ISO 7405/10993–12 and eluted in cell culture medium for 24 h at 37 °C: Vertise Flow, Fusio Liquid Dentin, Constic, Surefil One, Photac Fil and Fuji II LC. Primary human pulp cells were obtained from extracted wisdom teeth and cultured for 24 h with the extracts in serial dilutions. Cell viability was evaluated by MTT assay, membrane disruption was quantified by LDH assay and apoptosis was assessed by flow cytometry after annexin/PI staining.
Results: Two SAC (Constic and Vertise Flow) and one RM-GIC (Photac Fil) significantly reduced cell viability by more than 30% compared to the untreated control ($p < 0.001$). Disruptive cell morphological changes were observed and the cells showed signs of late apoptosis and necrosis in flow cytometry. Membrane disruption was not observed with any of the investigated materials.
Conclusion: Toxic effects occurred independently of the substance group and need to be considered in the development of materials with regard to clinical implications.
Clinical Significance: SAC have many beneficial qualities, however, the cytotoxic effects of certain products should be considered when applied in close proximity to the dental pulp, as is often required.

1. Introduction

Adhesive technology in dentistry has developed rapidly in recent years, with the establishment of universal adhesives aimed at simplifying multistep systems [1]. Self-adhesive composites (SAC) now represent a further simplification of the restorative procedure. As neither separate etching nor conditioning is required, these flowable composites promise a time-saving application and a reduced susceptibility to errors [2,3]. This property is particularly useful in areas where it is difficult to isolate from moisture for a longer time period. Indications for this group of materials include cervical fillings, applications in pediatric dentistry, temporary fillings, and the fixation of slow or non-curing pulp capping materials [4–6]. As filled, flowable composites, SAC are similar to conventional composites in terms of polishability and aesthetics, and show promising clinical results [7].

SAC, due to their specific requirements, contain functional monomers commonly found in dentin bonding agents, such as glycerol phosphate dimethacrylate (GPDM) or 4-methacryloyloxyethyl trimellitate anhydride (4-META) [2,8,9]. These monomers are acid reactive and therefore capable of modifying the smear layer and to achieve adhesion to dentin [10–13]. In contrast to conventional etching and bonding techniques, acidic components are not rinsed away and solvents cannot evaporate, but remain part of the whole composite filling [12].

Extensive evidence suggests that unpolymerized compounds can leach from adhesives or composites, diffuse through dentinal tubules, and reach the dental pulp [14]. Furthermore, substances can be eluted from restorations by dentin fluid even after polymerization [15]. Hydroxyethyl methacrylate (HEMA) and triethyleneglycol

* Correspondence to: Department for Conservative Dentistry and Periodontology, University Hospital Regensburg, Franz-Josef-Strauß-Allee 11, D-93053 Regensburg, Germany.
E-mail address: matthias.widbiller@ukr.de (M. Widbiller).

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dimethacrylate (TEGDMA) are among the most frequently detected monomers in extracts of resin-based dental materials [16]. These monomers are also present in large quantities in SAC to allow better penetration of the dentin's collagen network and improve compatibility with the hydrophobic dimethacrylate comonomers [10,17].

Eluted monomers, especially those with low viscosity and hydrophilic properties, have been reported to cause cytotoxic reactions and pose a significant risk to the dental pulp [18,19]. In particular, monomers possess genotoxic and mutagenic properties [19,20] and have been shown to induce oxidative stress, affect lipid metabolism, and lead to cell cycle arrest or apoptosis in pulp cells [19,21,22]. The repair mechanisms of the dental pulp and its ability to form tertiary dentin may also be affected [23,24]. Furthermore, TEGDMA increases the release of inflammatory markers such as IL-6 and IL-8 in vitro [25], and exposure to HEMA reduces TNF-α secretion, which can interfere with immune processes [21]. In vivo, pulp damage often occurs without clinical symptoms, but can be detected histologically [15].

In the clinical context, self-adhesive dental restorative materials compete with classical resin-modified glass ionomer cements (RM-GIC) due to their material qualities and field of application. RM-GIC are mainly utilized for primary dentition or as temporary fillings, but they also find application in class V restorations, as liners, bases, fissure sealants or as bonding agents for orthodontic brackets [26]. They are mainly placed in bulk and they have rather poor mechanical properties and are difficult to polish [10,27,28]. However, the main components and the curing method differ between RM-GIC and SAC. In traditional GIC, the interaction between polyacrylic acids and ion-leaching glass fillers are responsible for hardening. This base-acid reaction is accompanied by the polymerization of added resin monomers in RM-GIC [29]. Therefore, they contain both acidic components and hydrophilic monomers such as HEMA or TEGDMA. These components can potentially lead to increased cytotoxicity compared to conventional GIC [30].

Given the recent technical developments and advantages in the use of SAC, it would be of great interest to know whether SAC are superior to RM-GICs in terms of biocompatibility. However, due to their novelty, there are only few studies on the cytotoxicity of SAC [2,31]. Thus, the aim of this study was to investigate the cytotoxicity of four different SAC in vitro and to compare them to three RM-GIC. The null hypothesis was that the material group of the SAC is not different from that of the RM-GIC regarding cytotoxicity.

2. Material and methods

2.1. Dental materials

Direct cytotoxicity testing was performed according to ISO 7405 and ISO 10993. As shown in detail in Table 1, four flowable SAC (VF, FLD, CON, SF) and three RM-GIC (FUJ, PF, SFO) were included. Shade A3 was used for all materials to ensure uniformity. The light-curing GIC formulation described in ISO 7405 B.3 [32], which is known to cause toxic effects, was used as positive control material (see Table 2 for details).

Table 2
Composition of positive control material.

Component	Weight concentration
2-Hydroxyethyl methacrylate (stabilized with hydroquinone monomethyl ether; Sigma-Aldrich, St. Louis, MO, USA)	15%
Diphenyliodonium chloride (<98,0%; Lot: 43088-5 G; Sigma-Aldrich, St. Louis, MO, USA)	2%
Camphorquinone (97%; Lot: 09003AQV; Sigma-Aldrich, St. Louis, MO, USA)	0.05%
Ethyl 4-dimethyl-aminobenzoat (for synthesis, Mat #: 8.41086.0100; Sigma-Aldrich, St. Louis, MO, USA)	0.05%
Schott Dental Glass (Mat #: GM35429; Schott, Mainz, Germany)	66.3%
Poly(acrylic acid) (Mat #: 323667-100 G; Sigma-Aldrich, St. Louis, MO, USA)	11.7%
Water	4.9%

2.2. Extract preparation

The specimens were prepared by filling the materials into Teflon molds (PTFE rings; 5 mm inner diameter and 2 mm height; IBG Monoforts, Mönchengladbach, Germany). The samples were then light-cured between transparent matrix stripes (Frasaco, Tettnang, Germany) and glass slides (Marienfeld Superior, Marienfeld, Lauda-Königshofen, Germany) to prevent overfilling and the formation of an oxygen inhibition layer. The mono-wave LED polymerization light (Bluephase C8; Ivoclar Vivadent; Schaan, Liechtenstein) was positioned directly on the slide and the specimens were cured in a standardized manner for 40 s from each side to ensure adequate polymerization. The light intensity was tested to exceed 700 mW/cm^2 in this configuration (Bluephase Meter II; Ivoclar Vivadent; Schaan, Lichtenstein).

The single components of the positive control material were mixed, injected into the molds using a syringe and light-cured in the same way as the investigated materials, but, after preliminary experiments, allowed to cure further in a humid environment at 37 °C and 5% CO_2 for 24 h in order to reduce the cytotoxicity and optimize methodological performance as a positive control.

All samples were removed from the molds and stored in cell culture medium (MEM Alpha, Gibco, Billings, USA) containing 1% PenStrep (Sigma-Aldrich, St. Louis, USA) and 5% FBS (Fetal Bovine Serum, Gibco, Billings, USA) in sterile borosilicate tubes (Pyrex Disposable Screw Cap Culture Tubes, Corning, Corning, USA) at 37 °C and 5% CO_2 for 24 h. In accordance with ISO 7405, the ratio between the surface area of a specimen and the volume of the eluent was maintained at 0.33 ml/cm [32]. Extracts of the positive control material are subsequently referred to as positive control (PC).

As the investigated materials contain acidic compounds, the pH of the extracts was recorded using a pH meter (InoLab pH 7110, WTW, Xylem Analytics, Weilheim, Germany) after 30 min of incubation at 5% CO_2 and 37 °C (n = 9).

2.3. Cell exposure

Primary pulp cells were isolated from patients aged 15 to 20 years

Table 1
Investigated dental materials.

Name	Abbreviation	Manufacturer	Lot number	Material class	Shade
Vertise Flow	VF	Kerr, Scafati, Italy	8515505	Self-adhesive flowable composite	A3
Fusio Liquid Dentin	FLD	Pentron, Orange, CA, USA	8361156	Self-adhesive flowable composite	A3
Constic	CON	DMG, Hamburg, Germany	8751813	Self-adhesive flowable composite	A3
Super Flow	SF	Imicryl, Konya, Turkey	21E014	Self-adhesive flowable composite	A3
Surefil One	SFO	Dentsply, Milford, USA	2202000937	Resin-modified glass ionomer	A3
Fuji II LC	FUJ	GC, Tokyo, Japan	210717B	Resin-modified glass ionomer	A3
Photac Fil	PF	3M, Neuss, Germany	8673510	Resin-modified glass ionomer	A3
Positive control	PC	-	see Table 2	Resin-modified glass ionomer	-

with informed consent approved by the Ethics Committee (16-101-0022; Faculty of Medicine, University of Regensburg, Regensburg, Germany) using a previously established method [33]. They were used up to passage 3 and characterized by determining their doubling rate. For this purpose, cells were seeded in culture flasks and counted every 24 h using a Neubauer improved cell counter (Marienfeld, Lauda-Königshofen, Germany). The experiment was performed in six replicates and repeated three times (n = 24).

For cytotoxicity testing, 20,000 pulp cells/well were seeded in 96-well plates (651160, Greiner Bio-One, Kremsmünster, Austria) to establish a subconfluent layer. After 48 h, they were exposed to extracts (200 µl/well) at serial dilutions (1:1 to 1:16) with cell culture medium used as extraction vehicle and cultured for 24 h.

2.4. Cytotoxicity testing

Cytotoxicity was assessed at 24 h using three different endpoints: (i) impact on cell metabolism was measured by the 3-(4,5-dimethylthiazol-2-yl)– 2,5-diphenyl-2 H-tetrazolium bromide (MTT) test (ii) apoptosis was detected by flow cytometry after annexin/propidium iodide (PI) staining, and (iii) cell membrane damage was quantified by a lactate dehydrogenase (LDH) assay. In addition, images of cell cultures were taken by light microscopy (Axio Vert.A1, Carl Zeiss Microscopy, Jena, Germany).

2.5. MTT test

An MTT test was performed to verify the effects of the extracts on cell metabolism. The supernatant was replaced with 100 µl MTT solution (Sigma-Aldrich, St. Louis, MO, USA; 0.5 mg/ml diluted with PBS). During an incubation period of 80 min, cells converted MTT to purple formazan, which was then dissolved by DMSO (dimethyl sulfoxide, Merck, Darmstadt, Germany) and measured photometrically at 540 nm. Cell viability was normalized to the untreated control. The experiments were carried out in six replicates and conducted four times (n = 24).

2.6. Flow cytometry

For the measurement of apoptosis by flow cytometry, 100,000 cells/well were seeded into 6-well plates. After 48 h of attachment, cells were exposed to extracts at two dilutions (1:1 and 1:2) for 24 h. Cells were washed and adherent cells were detached with accutase (A6964, Sigma-Aldrich, St. Louis, USA), washed in PBS with 2% BSA (Sigma-Aldrich, St. Louis, MO, USA) and resuspended in 100 µl annexin binding buffer (Invitrogen, Waltham, USA) with 1% annexin (TACS Annexin V-FITC, R&D Systems, Minneapolis USA) and stained for 15 min at room temperature in the dark. This was followed by the addition of 250 µl of binding buffer. Subsequently, 5 µl of propidium iodide (10 ×, R&D Systems, Minneapolis USA) was added to each sample and flow cytometry was performed (FACSCanto, BD Biosciences, San Diego, USA). At least 10,000 events were collected and analyzed using FlowJo software (v10, Treestar, Ashland, Oregon, USA). The experiments were performed in duplicates and repeated three times (n = 8).

2.7. LDH assay

Membrane integrity was evaluated using an LDH assay (CytoTox 96 Non-Radioactive Cytotoxicity Assay, Promega, Madison, USA). The enzymatic colorimetric reaction was quantified by measuring the absorbance at a wavelength of 450 nm on a photometer (Tecan Infinite F200, Männedorf, Switzerland). According to the manufacturer's instructions, cytotoxicity was calculated as a percentage of the expected maximum concentration of LDH from lysed cells. Experiments were performed in six replicates and repeated three times (n = 24).

2.8. Statistical analysis

Data were tested for normal distribution (D′Agostino-Pearson test) and then analyzed using nonparametric procedures (Kruskal-Wallis-test) at a significance level of $\alpha = 0.05$. All statistical calculations were performed with GraphPad Prism 9 (GraphPad Software, La Jolla, CA, USA).

Results from all replicates were summarized and presented as medians with interquartile range. Statistically significant differences are marked with an asterisk, as explained in the captions.

3. Results

3.1. Cell characterization and pH value

The doubling time for the untreated dental pulp cells was calculated to be 30.63 h (Supplementary Fig. 1A).

Analysis of the extracts with which the cells were treated showed a lower pH for RM-GIC (pH 7.0 to 7.1) compared to SAC (pH 7.8 to 7.9). However, all extracts, except for the PC (pH 5.4), had a neutral pH within the range of the phenol red indicator (pH 6.8 to 8.2) present in the cell culture medium (Supplementary Fig. 1B).

3.2. MTT test

Cell viability was assessed by measuring metabolic activity using a colorimetric MTT test. CON, VF, SF, FLD, PF and SFO showed a significant reduction in metabolic activity in undiluted form compared to the untreated control ($p \leq 0.0119$). Representatives of both material groups, SAC (CON, VF) and RM-GIC (PF), reduced the metabolic activity by more than 30%, respectively 52%, 34% and 47% (Fig. 1). CON had the greatest impact on cell metabolism compared with the untreated control ($p < 0.0001$). As shown in Fig. 2, VF did not affect the cells as much, but both materials still had a statistically significant effect at the 1:2 dilution ($p \leq 0.0161$). Among the RM-GIC, undiluted extracts of PF and SFO significantly reduced the metabolic activity ($p < 0.0001$), however, this effect did not persist at the 1:2 dilution ($p > 0.1582$). Within the dilution series, both FUJ and FLD had no effect on viability compared to the untreated control ($p > 0.2247$).

3.3. Cell morphology

Untreated cells and those exposed to extracts or positive control differed greatly in their morphological appearance. Microscopic images of the untreated control showed a confluent monolayer of spindle-

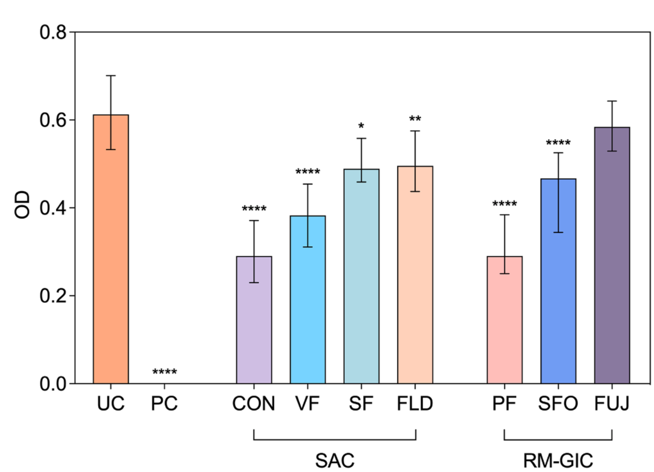

Fig. 1. Cell viability for undiluted samples determined by MTT test. Optical density (OD). Median and interquartile range are shown. Asterisks indicate significant differences compared to the UC (Kruskal-Wallis test, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

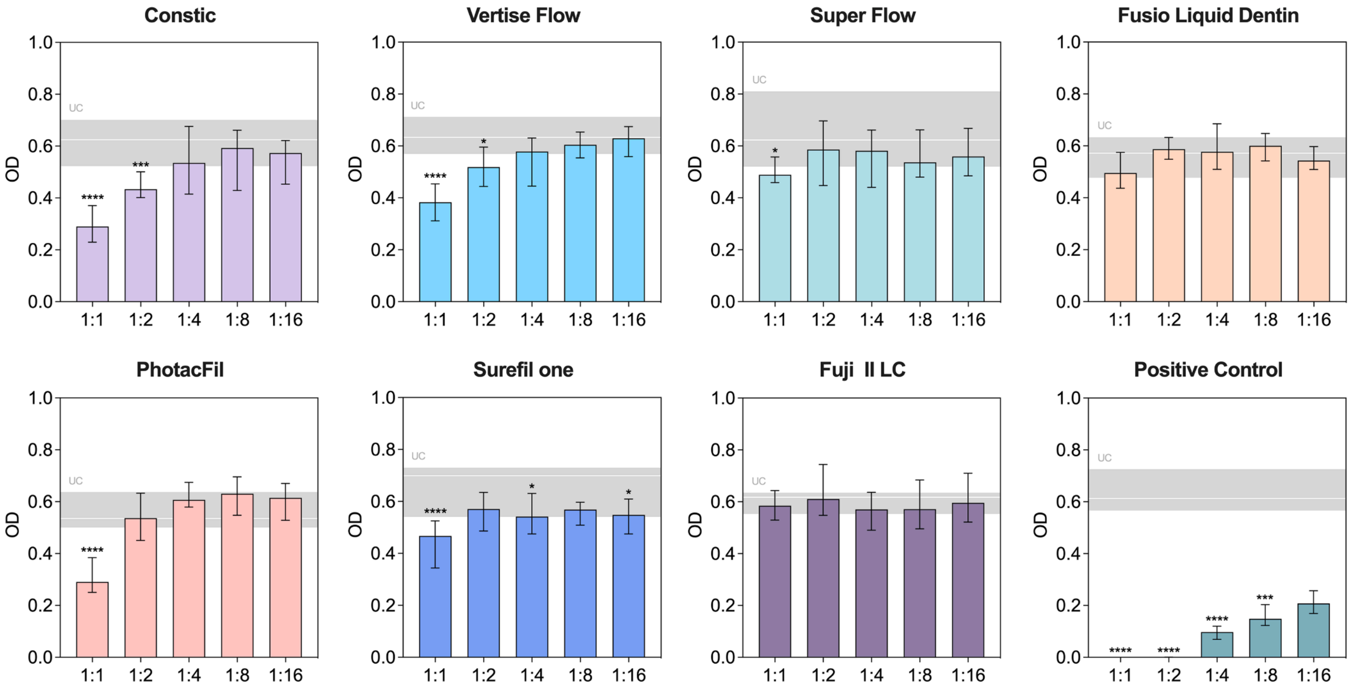

Fig. 2. Cell viability for investigated materials and positive control in serial dilutions as determined by the MTT test. Optical density (OD). Median and interquartile range are shown. Asterisks indicate a significant difference from the UC (Kruskal-Wallis test, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$). Each individual level of untreated control is marked by a white line (median) in a grey area (interquartile range).

shaped fibroblasts with long cell processes and some debris (Fig. 3A). The cells incubated with the positive control extract were enlarged and rounded. Areas where cells had detached, were visible (Fig. 3B). Similarly, almost all cells in exposed to CON extract were swollen and rounded (Fig. 3C). Disaggregated areas were visible, and only few cells appeared unaffected (Fig. 3C). Cells treated with extracts of the SAC, VF and FLD appeared as a single layer of cells interspersed with a few swollen and granular cells (Fig. 3D-F). With PF, a RM-GIC, cells were covered by a thick layer of irregular precipitates, however, the monolayer underneath appeared to be intact (Fig. 3G). In FUJ and SF extracts, the cells were similar to those in the untreated control (Fig. 3E and I).

3.4. Flow cytometry

To further elucidate the cytotoxic stimulation, flow cytometry was performed with annexin/PI staining to differentiate between apoptosis and necrosis. Annexin is used to detect apoptosis, as it conjugates to the phospholipid phosphatidylserine, which is externalized during early apoptosis. PI, on the other hand, can penetrate damaged membranes and therefore stains cells in the late apoptotic or necrotic state. As depicted in Fig. 4, over 89% of the cells in the UC were viable and fewer than 11% showed staining for annexin and/or PI. PC showed a significant increase in annexin/PI positive cells in undiluted form and at 1:2 dilution ($p \leq 0.0024$), leaving almost no unstained cells ($p \leq 0.0194$).

CON, VF and PF show the highest percentage of annexin and PI-stained cells and even SFO produced many necrotic or late apoptotic cells. Early apoptosis, as indicated by annexin staining alone, was only significantly detectable in CON ($p < 0.0001$). SF and FUJ showed no increase in annexin/PI positive cells compared to the UC ($p > 0.9999$).

3.5. LDH assay

The LDH assay detected membrane disruption and subsequent release of LDH into the supernatant in a concentration-dependent manner in the positive control, but severe cytotoxicity was restricted to the 1:1 and 1:2 dilutions of the extract (Fig. 5A). Neither SAC nor RM-GIC caused a statistically significant increase in LDH release compared to the untreated control (Fig. 5B).

4. Discussion

The innovation of better, simpler and more biocompatible materials has been instrumental in the success of modern dentistry. In recent years, developments in the field of self-adhesive restorative materials like SAC have come to the fore and are challenging established materials such as RM-GIC. Thorough and independent cytotoxicity testing of new materials is prudent, especially when the cytotoxicity of components is well documented [19]. Conducting comprehensive biocompatibility assessments and incorporating these biological findings alongside mechanical factors appears to be an important factor in material development. In particular, materials indicated for use in deep cavities, which may directly affect dental pulp cells, deserve further scrutiny. Therefore, this study evaluated the cytotoxic effect of SAC on dental pulp cells in vitro and compared it with that of RM-GIC.

Three endpoint observations monitoring different stages of cytotoxicity showed adverse effects on pulp cells exposed to both SAC and RM-GIC extracts. There were marked differences in the cytotoxicity observed between the materials investigated, however, the effects were individual regardless of whether it was a SAC or RM-GIC. Therefore, the null hypothesis that there is no difference in cytotoxicity between the two classes of materials could not be rejected. Biocompatibility therefore appears to depend less on the material class than on the composition of the individual products.

A reduction of cell metabolism is considered to be an initial cellular cytotoxic reaction and is commonly interpreted as a direct parameter of viability. The impact of extracts on cell viability was significant and concentration-dependent with all investigated materials. Notably, three materials caused a reduction in cell metabolism exceeding 30%, thereby meeting the criteria for cytotoxicity according to ISO 10993 [34]. However, these included two SAC (CON and VF) and one RM-GIC (PF), with CON showing the most severe decrease in cell metabolism. The cytotoxicity of VF has been previously documented [2,35,36], but to

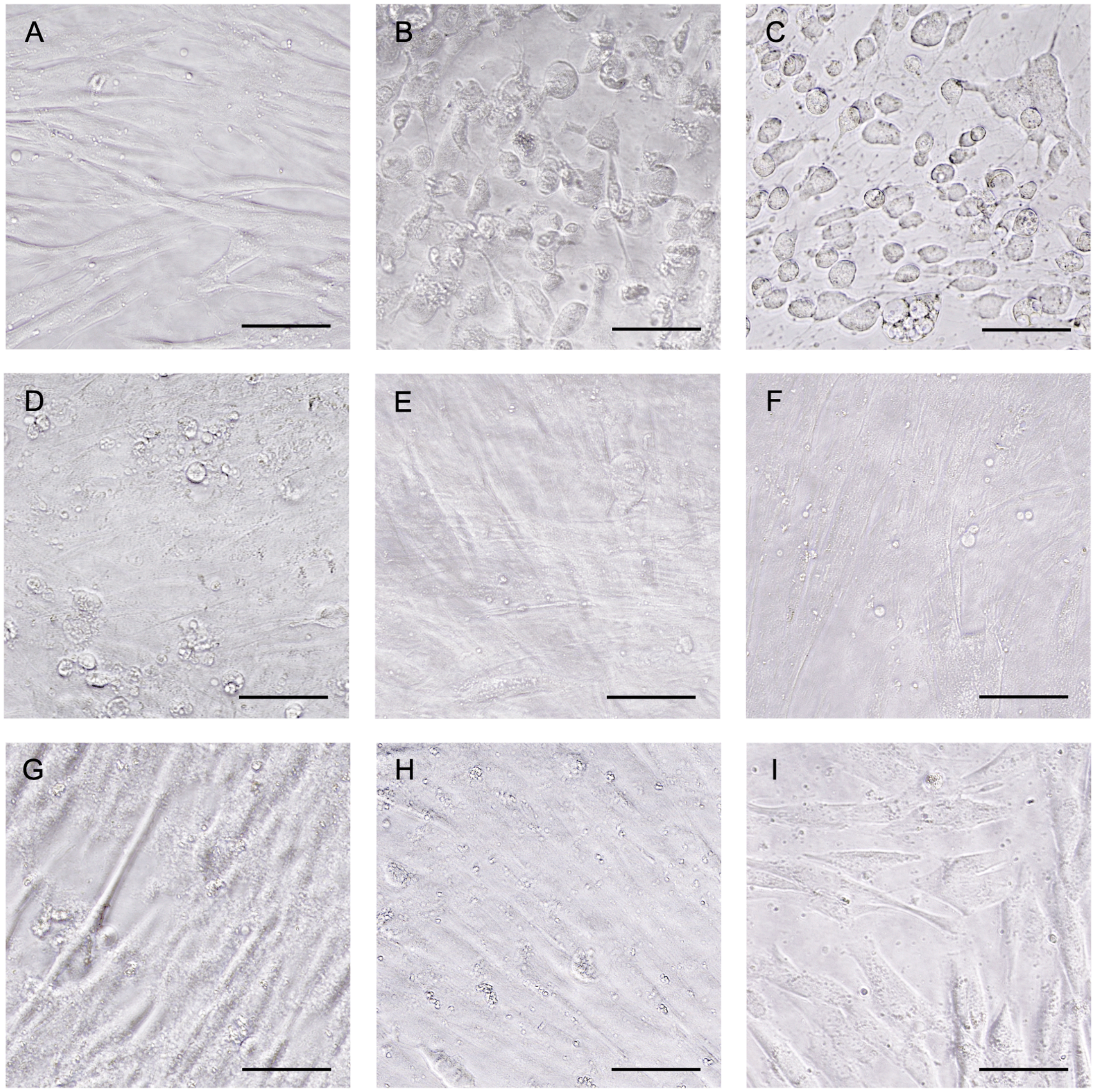

Fig. 3. Light microscopic images of cells exposed to SAC extracts (C-F) and RM-GIC (G-I) for 24 h. (A) UC, (B) PC, (C) CON, (D) VF, (E) SF, (F) FLD, (G) PF, (H) SFO and (I) FUJ. Severe morphological changes were observed in the PC, with CON and with VF extracts. Scale bar = 50 μm.

date there are no published studies on CON. Interestingly, the manufacturer advises against the use of CON on etched dentin, which may allude to the necessity to protect the dental pulp as removal of smear layer increases dentin permeability [37]. In addition, cytotoxic effects of RM-GIC have also been observed [38] and reported to be stronger than those of conventional GIC [30,39]. In addition to a low pH during the setting process, the most likely cause is the release of toxic monomers such as HEMA [39]. However, SFO, a RM-GIC that does not contain HEMA or TEGDMA, still showed a reduction in cell viability. Therefore, monomers do not appear to be the only reason for the cell response. Contrary to the results of this study, PF, the RM-GIC with the greatest reduction in cell metabolism in the MTT test, did not exhibit cytotoxic properties in other studies [39,40]. Remarkably, FUJ stands out as the only material that did not exhibit any cytotoxicity. This is not in line with existing literature where FUJ has been reported to be low cytotoxic [30,41]. These discrepancies can be attributed to the wide variation in experimental set-ups and underline the challenges of achieving standardization despite ISO standards.

Morphological changes are another reflection of cell degeneration. The observed morphological changes of cells exposed to extracts were congruent to the results of the MTT test: VF, CON and PF seem to have severely affected cells. The observed cell swelling and disintegration is consistent with descriptions of necrosis [42].

Flow cytometry and annexin/PI staining is a sensitive tool to detect and differentiate between early and late apoptosis and necrosis. Positive annexin staining registers the exposure of phosphatidylserine which is classified as an early apoptotic event [43]. As monomers such as HEMA are known to cause apoptosis through DNA damage and the intrinsic mitochondrial pathway [44,45], an increase in the occurrence of apoptosis was expected. However, in this study, toxin-induced necrosis appeared to predominate. One hypothesis for this observation is that subtle stress had discernible effects on both metabolism and morphology, as evidenced by MTT assay results and light microscopy, but due to the transient nature of apoptosis, this specific state may not have been captured by flow cytometric analysis. Further research is required to fully understand the mechanism of cytotoxicity and the underlying pathways.

As extracellular stressors increase, plasma membrane damage can occur [46]. Since the cytotoxicity of HEMA and TEGDMA is membrane mediated, we investigated the release of LDH into the supernatant after membrane disruption [47]. The assay quantified LDH present in the supernatant through a colorimetric reaction. Notably, the results revealed no significant increase in LDH for the investigated materials, while the positive control confirmed methodological soundness. The

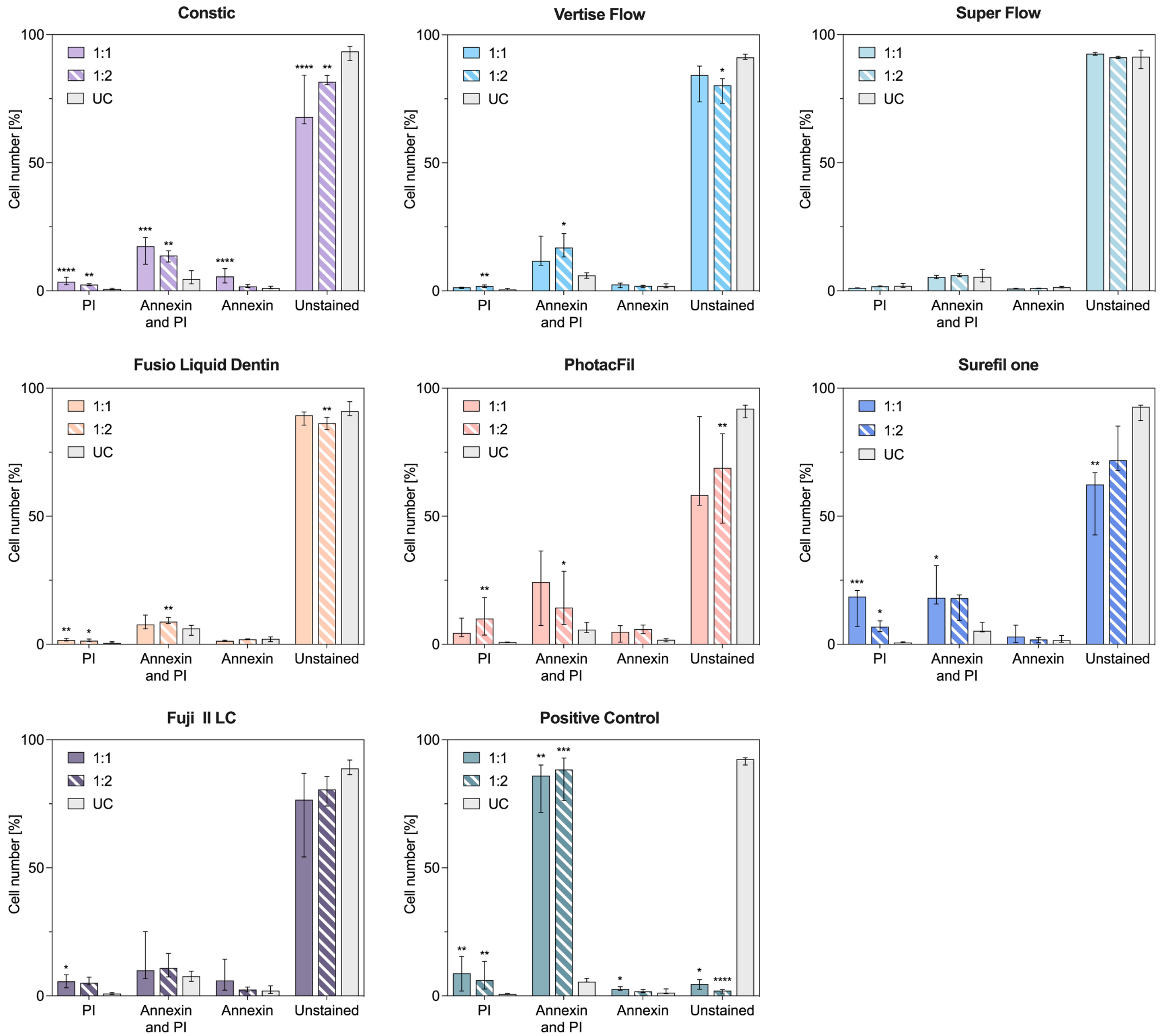

Fig. 4. Results of flow cytometry after annexin/PI staining of cells after 24 h of exposure to material extracts. The number of stained cells is shown as a percentage of the total number of cells. Median and interquartile range are shown. Asterisks indicate significant differences from the UC (Kruskal-Wallis test, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

lack of membrane damage, despite the cytotoxic effects demonstrated by MTT and flow cytometry, is consistent with other studies [40] and may be attributed to the inherent reparative capacity of primary cells [48]. In contrast, a study using immortalized cell lines for cytotoxicity screening showed an increase in LDH release [49], probably due to a lack of self-repair potential, as immortalized cells have reconfigured metabolic pathways and experience a significant upregulation of cell cycle-associated processes [50].

In line with the recommendations of the ISO standard, primary dental pulp cells were used in this study as they better represent the in vivo target cells. Both immortalized and primary cell lines are approved, each offering advantages and disadvantages. While primary cells are, as mentioned, less sensitive to cytotoxicity [48,51], immortalized cell lines are readily available in consistent quality, which supports standardization and reproducibility. Shade A3 was used for each material, as increased cytotoxicity has been observed for dark shades [52].

The experiment was carried out using extracts from sufficiently polymerized samples as insufficient curing may increase cytotoxicity and should therefore be ruled out in this study [53]. This was ensured by an extended two-sided light polymerization of 40 s. Elution time was 24 h, as specified by the ISO 10993–5 regulations, which is much shorter than the lifetime of a dental filling [34,54]. However, with resin-based composites, most substances are released shortly after polymerization [55], and eluted within the first 24 h [56]. As recommended, the extracts were not centrifuged or filtered to avoid removing suspended particles [57], which was evident in the light microscopy images.

Since the binding mechanisms of both material classes are based on an acidic reaction, the pH of the eluates was investigated. The initial pH values ranged from 5.4 (PC) to 7.9 (SF), but were kept neutral by the bicarbonate-containing cell culture medium for all investigated materials. After a short time, the CO_2-rich environment of the incubator adjusted the pH to around 7.4 in all groups, which resembles the in-vivo-situation where buffer systems such as bicarbonate, proteins and phosphates maintain a physiological pH [58]. It has been reported that dental pulp cells exhibit growth arrest or cell death in the range of pH 6.5 to 7.5. However, this may only have occurred in PC at the very beginning.

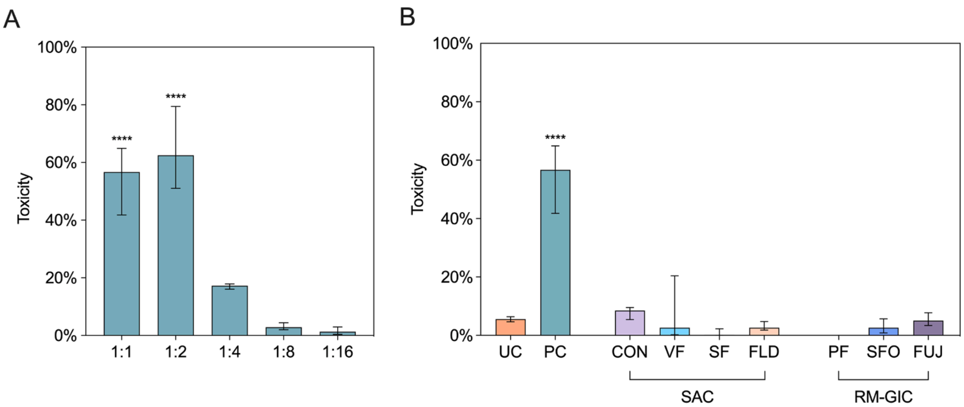

Fig. 5. Cytotoxicity assessed by membrane disruption detected using the LDH assay. (A) Toxic effect of the PC in serial dilutions. (B) Comparison of the cytotoxicity of the different materials. Median and interquartile range are shown.Asterisks indicate significant differences from the UC (Kruskal-Wallis test, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

Complying with ISO 10993–5 standards, the exposure time was also 24 h [34]. Cell reactions to known monomers are difficult to predict and interactions between different components can be relevant. For example, the combination of UDMA and TEGDMA results in a less cytotoxic reaction than the compounds individually [59]. Ratanasathien *et al.* reported three different interactive effects: synergistic, additional, and antagonistic [60]. Antagonistic effects are supposed to be dominant during the first 24 h, which directly falls into our timeframe. In some studies, more severe cytotoxic effects were observed after 72 h of exposure and cytotoxic risks are strongly related to the contact time [61,62]. Since a repetition of this experiment with a different time frame could lead to different results, the ISO-standardized approach is crucial for the comparability of the data and the data quality.

In vitro, dentin provides protection against monomers both as a mechanical barrier [15] and chemically, as collagen can neutralize acids and bind certain monomers [63]. This protective effect is not taken into account in this set-up and a dentin barrier test, as described in the ISO regulations [32], may be a sensible continuation to closer assess the in-vivo-cytotoxicity of SAC. However, permeability of dentin is high, especially in close proximity to the dental pulp [64]. Therefore, protective measures such as indirect pulp capping should be used to protect the dental pulp from cytotoxic compounds in deep cavities [65,66].

5. Conclusion

In summary, cytotoxic effects were observed for both material groups, SAC and RM-GIC, without categorical differences. Individual representatives of SAC as well as the established group of RM-GIC affected human dental pulp cells. In particular, CON and VF, both SAC, and PF, a RM-GIC, impaired cell metabolism to a cytotoxic extent. The study demonstrates the variability of dental materials in terms of biocompatibility and emphasizes the need to address the biological performance of restorative materials from the development stage.

Contributions

Ella Ohlsson: Conceptualization, Methodology, Formal analysis, Writing - original draft, Investigation, Visualization, Funding acquisition. **Carola Bolay:** Investigation. **Sevgi Arabulan:** Writing - Review & Editing. **Kerstin M. Galler:** Writing - Review & Editing. **Wolfgang Buchalla:** Resources. **Gottfried Schmalz:** Writing - Review & Editing. **Matthias Widbiller:** Conceptualization, Methodology, Formal analysis, Resources, Writing - original draft, Visualization, Supervision.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.dental.2024.02.015.

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