Dental Materials Journal 2026; 45(2): 144–151

Effect of solvent pretreatments on trans-dentinal cytotoxicity of bioactive materials

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This study evaluated the effect of solvent-based dentin pretreatments containing dimethyl sulfoxide (DMSO) and/or ethanol on trans-dentinal cell viability of bioactive materials in simulated deep clinical cavities. Dentin discs (300 µm) were prepared, balanced among groups according to dentin permeability and autoclaved. Three-dimensional cultures of odontoblast-like cells were transferred to the pulpal aspect of dentin discs inside perfusion split-chambers designed for trans-dentinal cytotoxicity test following ISO 7405 standards. Dentin pretreatments were composed of ethanol (EtOH), DMSO, their 50% (v/v) aqueous dilutions and 50% (v/v) DMSO/EtOH. Pretreatments (1.5 µL) were applied on cell-seeded dentin discs and a 1-mm increment of one of three bioactive materials: a light-curable methacrylate-based silicate (TheraCal), a mineral trioxide aggregate (MTA) or an experimental pre-reacted glass-ionomer filler (S-PRG). Cell viability was analyzed spectrometrically according to the MTT test and cytotoxicity levels were classified following ISO 7405. TheraCal was considered severely cytotoxic, MTA moderately cytotoxic and S-PRG non-cytotoxic. Assessment of cell damage by solvents was ranked as EtOH=EtOH/H_2O>DMSO>DMSO/EtOH=DMSO/H_2O. The light-curable methacrylate-based silicate was the only material with considerable trans-dentinal cytotoxicity. While ethanol was harmful to odontoblast-like cells, DMSO had a minor role in cytotoxic responses used alone or combined to bioactive materials. Solvents must be considered for safe use during vital-pulp therapy.

Keywords: Cell viability, MTA, DMSO, S-PRG, TheraCal

INTRODUCTION

Selective caries removal techniques are becoming more popular clinical options to delay or even prevent unnecessary root canal treatments. Given the increasing emphasis on preserving the tooth's vitality[1)], the development of new bioactive and biocompatible materials has gained significant attraction. While external factors like caries can harm pulp cells[2)], bioactive materials may also influence pulp cell viability[3)]. Successful vital-pulp therapy hinges on material biocompatibility, the absence of bacterial contamination, and the patient's general health[4)]. Ensuring the compatibility of these materials with pulp cells is crucial for creating a conducive environment for effective tissue repair[5)].

Current methods for assessing the cell viability of bioactive materials *in vitro* typically involve collecting eluates from "fully set" or "freshly mixed" bioactive materials[5)]. This approach, however, overlooks the protective impact of dentin on pulp cells. While substantial progress has been made in understanding the effects of bioactive materials on pulp cell compatibility, translating results from conventional cell culture cytotoxicity tests to real clinical applications can be problematic. The protective dentin-barrier effect plays an important role in determining the compatibility of bioactive materials. Therefore, trans-dentinal cytotoxicity setups help bridge the gap between *in vitro* and *in vivo* studies by simulating more realistic clinical simulations[6)]. Under such testing conditions, bioactive materials initially interact with dentin before releasing ions that diffuse toward pulp cells, mimicking *in vivo* applications more realistically.

Since the introduction of the first hydraulic silicate cement in 1993[7)], various material modifications have aimed to enhance handling and setting properties, reduce discoloration, lower solubility, and improve sealing. Effective interactions with mineralized dental tissues and achieving a good seal are crucial for the long-term success of bioactive materials[4)]. Dimethyl sulfoxide (DMSO; $(CH_3)_2SO$) is a polar aprotic colorless solvent that enhances dentin wettability[8)] and facilitates the penetration of high molecular molecules into dentin[9)]. DMSO's mechanisms that improve interactions between restorative materials and dentin[8,10-13)] may also contribute to better sealing between bioactive materials and dentin. Since the main reason for failure after vital pulp therapy is reinfection of pulp tissue by bacteria[4,14)], lowering the risk of leakage could reduce long-term reinfection risks after material placement. Additionally, more intimate contact between bioactive materials and dentin could favor reparative dentin-bridge formation within shorter times. Exploring such potential benefits of

Received Apr 30, 2025: Accepted Nov 17, 2025
doi:10.4012/dmj.2025-102 JOI JST.JSTAGE/dmj/2025-102

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DMSO pretreatments on biomaterial-dentin interactions would be promising as long as cytocompatibility remains at acceptable levels. While low DMSO concentrations (0.5–1 mM) have been found to have little or no cytotoxicity on pulp-derived cells[15,16], the effect of higher DMSO concentrations (up to 50% v/v) remains unknown. Consequently, this study aims to evaluate the effect of solvent-based dentin pretreatments (DMSO, ethanol, and their aqueous dilutions) along with bioactive materials on trans-dentinal cell viability in simulated deep clinical cavities. The tested null hypotheses were that the composition of (*i*) bioactive materials or (*ii*) their association with dentin pretreatments would have no effect on trans-dentinal cytotoxicity.

MATERIALS AND METHODS

Experimental design

The experimental design was composed of two study factors defined as: (*i*) "dentin pretreatment" and (*ii*) "bioactive material". "Dentin pretreatment" was set at six levels: no pretreatment, ethanol (EtOH), dimethyl sulfoxide (DMSO), 50% (v/v) aqueous ethanol (EtOH/H_2O), 50% (v/v) aqueous dimethyl sulfoxide (DMSO/H_2O), 50% (v/v) ethanolic dimethyl sulfoxide (DMSO/EtOH). "Bioactive material" was set at four levels: no material, a light-curable silicate-based cement (TheraCal, Bisco, Chicago, IL, USA), a mineral trioxide aggregate (MTA; MTA Orbis, Superior Dental materials, Hamburg, Germany) and an experimental ion releasing cement containing surface pre-reacted glass-ionomer (S-PRG; Shofu, Kyoto, Japan). A total of 23 experimental groups were obtained (n=12 discs/group). Figure 1 shows a summary of the experimental design. The composition of test materials and application procedures are shown in Table 1. Bioactive materials were used following manufacturer's instructions as indirect pulp protecting materials. A hydrophobic polyvinylsiloxane material (Imprint 4 super quick ultra-light, 3M ESPE, Neuss, Germany) was employed as a negative control and an experimental glass-ionomer cement as a positive control according to ISO 7405 standards[17].

Preparation of dentin discs

Two-hundred and seventy-six extracted sound third molars collected from anonymous donors and exempt from ethical notification according to local regulations (Tissue Act, section 20) were collected. Teeth were stored in 0.9% NaCl (Sigma-Aldrich, Gillingham, UK) supplemented with 0.02% sodium azide (Sigma-Aldrich) and stored at 4°C. Teeth were rinsed in distilled water and sectioned into dentin discs perpendicularly to the longitudinal axis of the tooth, immediately above pulp horns (500 µm in thickness) using a precision diamond

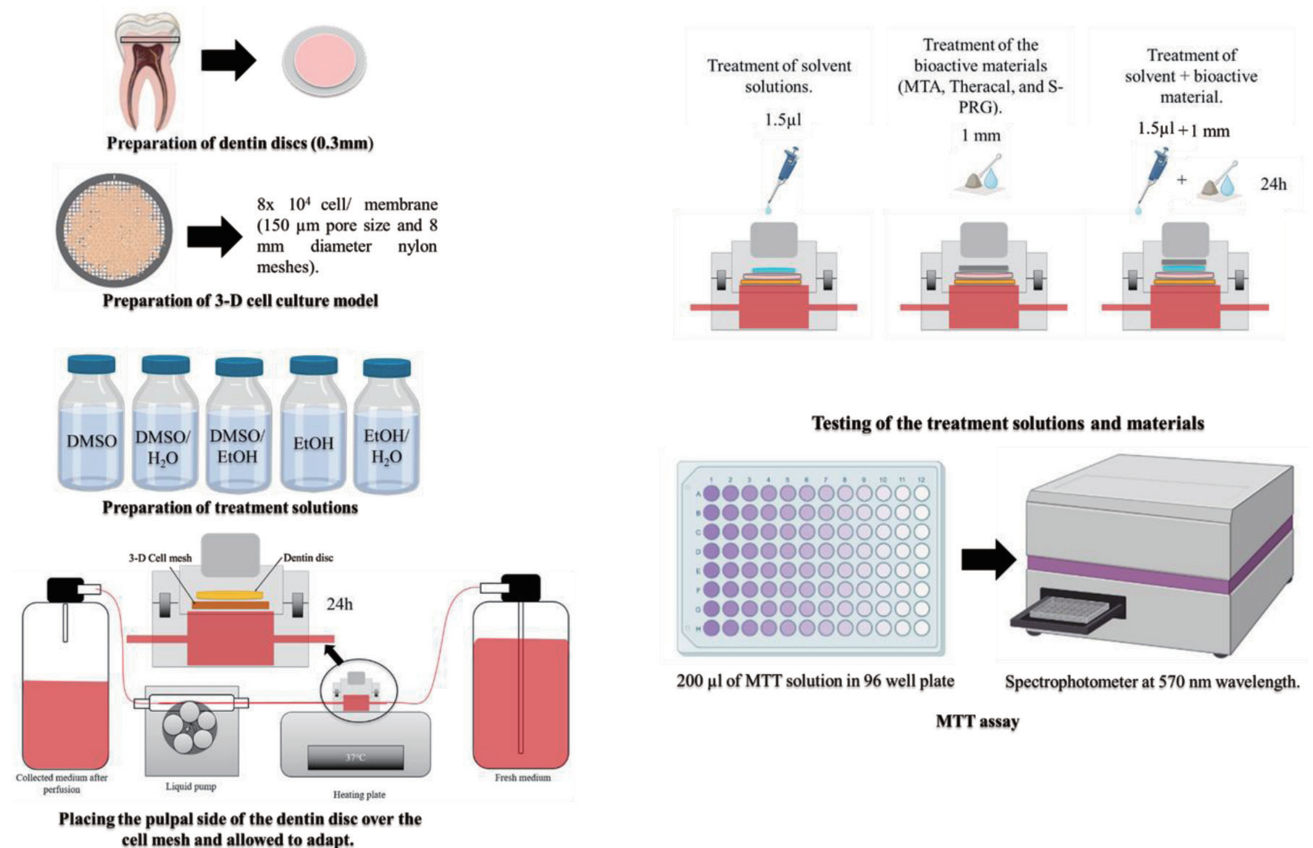

Fig. 1 Flowchart of the experimental design and scheme for the perfusion system used for the dentin barrier cytotoxicity test according to ISO 7405.

Table 1 Composition of bioactive materials and application procedures

	Composition	Application procedures
Resin-modified calcium silicate-based cement (TheraCal LC, Bisco)	Portland cement type III (30–50%), poly(ethylene glycol) dimethacrylate (10–50%), bis-GMA (5–10%), barium zirconate (1–5%), barium sulphate, bismuth oxide, fumed silica	Apply a 1 mm-thick layer over moist dentin and light cure for 20 s.
Mineral Trioxide Aggregate (MTA, Orbis)	Powder: Tricalcium silicate, dicalcium silicate, calcium carbonate filler, zirconium oxide, iron oxide, bismuth oxide, magnesium, calcium phosphate Liquid: Water	Mix 1 full spoon with 2 drops of the liquid. Apply a 1 mm thick layer over moist dentin. Cover the MTA layer with a sterile wet-cotton pellet.
Surface pre-reacted glass (S-PRG, Shofu)	Powder: Zinc oxide-based inorganic compound filler, S-PRG filler, additives. Liquid: Polycarboxylic acid derived solution, water	Mix 2.8 g of powder with 1 g of liquid and apply a 1 mm-thick layer over moist dentin.

saw (Isomet 1000 Precision Saw, Buehler, Lake Bluff, IL, USA) under water-cooling. Discs with perforations, indicating pulpal exposures, were discarded and replaced. The occlusal surface of dentin discs was polished with 320-grit SiC abrasive (CarbiMet, Buehler) to achieve a final thickness of 300 µm (±15).

Dentin permeability measurement

Dentin discs were treated with 50% citric acid (Sigma-Aldrich) for 30 s to eliminate smear layer and smear plugs. Permeability was evaluated using a flow-measurement infiltration apparatus (SLI-1000 Liquid Flow Meter, Sensirion, Zurich, Switzerland) in a modified split-chamber unit connected to a deionized water container to simulate 20 cm of hydrostatic pressure[18]. After maximum permeability measurements, dentin discs were rinsed with distilled water for 15 s and autoclaved in 0.9% sodium chloride (Sigma-Aldrich) at 121°C for 25 min. Samples were then allocated into 23 balanced groups (n=12 discs/group) considering statistical similarities based on dentin disc's permeability.

Pulp-derived three-dimensional cell culture

Clonal large T-antigen bovine pulp cells (SV40) derived from calf dental papilla[17,19] were stored in liquid nitrogen until use. Cells were maintained in Alpha Minimum Essential Media (α-MEM; Gibco, Grand Island, NY, USA) supplemented with 20% Fetal Bovine Serum (FBS; Gibco), 150 IU/mL penicillin, 150 mg/mL streptomycin, 0.125 mg/mL amphotericin B and 0.1 mg/mL geneticin (Sigma-Aldrich). Cell cultures were incubated in 5% CO_2 and 100% humidity at 37°C until use. Polyamide nylon meshes (Merck Sigma-Aldrich) with a pore size of 150 µm and a diameter of 8 mm were prepared and cleaned with 0.1 M acetic acid (Sigma-Aldrich) for 30 min, washed three times with sterile distilled water and coated with 0.03 mg/mL fibronectin (Fibronectin bovine plasma, Sigma-Aldrich). A 6-well tissue culture plate was filled with 1.25 mL of α-MEM supplemented with 20% FBS. Millicel inserts (Merck Sigma-Aldrich) was carefully placed at the bottom of the well plate to support the initial growth of the cells on the meshes. Four meshes were then inserted into each cell culture insert (Greiner bio-one, Nurtigen, Germany), continuously receiving 0.3 mL medium/hour of α-MEM supplemented with fibronectin. The suspension of pulp-derived cells was adjusted to 80,000 cells/20 µL for each mesh and the cultures were incubated for 48 h (5% CO_2 and 100% humidity at 37°C). After incubation, polyamide nylon meshes were separately placed in 24-well tissue plates containing 1 mL of α-MEM and 10% FBS. The medium was changed three times a week for 14 days to produce cells in a three-dimensional form. Calculation of cells on meshes was done with TC20 automated cell counter (Luminex xMAP, BioRad, Hercules, CA, USA) by mixing 15 µL of cell suspension and 15 µL of 0.4% trypan blue (Thermo Fisher Scientific, Waltham, MA, USA), then pipetting 10 µL to the counting slides chamber.

Dentin pretreatment solutions

Five solvent-based pretreatment solutions containing DMSO (Sigma-Aldrich) and/or ethanol (EtOH; Ethanol 99.8%, Sigma-Aldrich) were prepared and stored at room temperature until use. Solvent concentrations (v/v) were set as undiluted EtOH, undiluted DMSO, 50% aqueous ethanol (EtOH/H_2O); 50% aqueous dimethyl sulfoxide (DMSO/H_2O); 50% ethanolic dimethyl sulfoxide (DMSO/EtOH)[8].

Dentin barrier cytotoxicity test

Trans-dentinal cytotoxicity evaluation was carried out according to ISO 7405[17,20] (Fig. 1). After a 14-day incubation period, polyamide nylon meshes containing the three-dimensional cell cultures were placed in the lower compartment of commercially available cell culture perfusion chambers (Minucells and Minutissue, Bad Abbach, Germany) in direct contact with the pulpal side of dentin discs. The pulpal chamber compartment was perfused with assay medium supplemented with 5.96 g/L hydroxyethyl piperazine ethanesulfonic acid (HEPES; Sigma-Aldrich) at a rate of 0.3 mL/h for 24 h using a precision pump (Minucells and Minutissue). Treatment solutions (1.5 µL) were applied over the occlusal surface of dentin slices for 10 s. Bioactive materials (MTA, TheraCal and SPR-G) were then applied following manufacturer's recommendations in a 1 mm-thick layer.

Composition of bioactive active materials and their application modes are summarized in Table 1. MTA was then covered with a wet-cotton pellet embedded in sterile 0.9% NaCl. TheraCal was light-cured for 20 s using a LED light-curing unit (Elipar, 3M ESPE, St Paul, MN, USA) at 1,200mW/cm^2. After closing the split chambers, cells were perfused at a 0.2 mL/h rate for 24 h. Cell meshes were gently sectioned by the metallic inserts into 4 mm^2 circular, and stored for 2 h in a cell incubator (5% CO_2, 100% humidity at 37°C). Each experiment was repeated six times and performed with two replicates. Positive and negative controls were additionally carried out during each test.

Trans-dentinal cell viability (MTT assay)

Cell viability was determined by the MTT assay. Meshes covered with cells were removed from the metallic inserts and placed into 48 well plates containing 500 µL of pre-warmed MTT solution (0.5 mg/mL growth medium) and incubated for 2 h at 37°C. The blue formazan precipitate was extracted from mitochondria using 250 µL of DMSO on a shaker at room temperature for 30 min. After that, 200 µL of this solution was transferred to a 96-well plate and the absorption at 570 nm was determined spectrophotometrically (Synergy HT, BioTek Instruments, Winooski, VT, USA). The percentage of cell viability was calculated based on the optical density of negative control samples.

Statistical analysis

Assessment of cell damage was further categorized as non-cytotoxic, moderately cytotoxic or severely cytotoxic according to ISO 7405[17]. Briefly, if tested bioactive materials, pretreatments or their combination induced comparable or significantly less cell damage to the negative control, it was considered non-cytotoxic. If cell damage was significantly different from negative and positive controls, it was considered moderately cytotoxic. In case of comparable cell damage to the positive control with significant differences from the negative control, it was considered severely cytotoxic. Since trans-dentinal datasets were not normally distributed, data were analyzed by the Kruskal-Wallis test (α=0.05). Calculations were performed with IBM SPSS Statistics for Windows, version 26 (IBM, Armonk, NY, USA).

RESULTS

Assessment of cell damage

Negative controls presented significantly higher cell viabilities compared to positive controls (p<0.05). Bioactive materials, pretreatment solutions or their

Table 2 Assessment of cell damage* (SV40 pulp-derived cells) after different solvent treatments used in combination with bioactive materials

Material	Treatment	Cell damage*
—	DMSO/H_2O	Non cytotoxic
	DMSO	Moderately cytotoxic
	DMSO/EtOH	Non cytotoxic
	EtOH/H_2O	Severely cytotoxic
	EtOH	Severely cytotoxic
Theracal LC	No treatment	Severely cytotoxic
	DMSO/H_2O	Severely cytotoxic
	DMSO	Severely cytotoxic
	DMSO/EtOH	Severely cytotoxic
	EtOH/H_2O	Severely cytotoxic
	EtOH	Severely cytotoxic
MTA	No treatment	Moderately cytotoxic
	DMSO/H_2O	Moderately cytotoxic
	DMSO	Moderately cytotoxic
	DMSO/EtOH	Moderately cytotoxic
	EtOH/H_2O	Severely cytotoxic
	EtOH	Severely cytotoxic
S-PRG	No treatment	Non cytotoxic
	DMSO/H_2O	Non cytotoxic
	DMSO	Moderately cytotoxic
	DMSO/EtOH	Non cytotoxic
	EtOH/H_2O	Severely cytotoxic
	EtOH	Severely cytotoxic

*According to ISO 7405.
DMSO, dimethyl sulfoxide; EtOH, ethanol; MTA, mineral trioxide aggregate; S-PRG, pre-reacted glass ionomer fillers.
DMSO/H_2O, DMSO/EtOH and EtOH/H_2O followed 50% (v/v) dilutions.

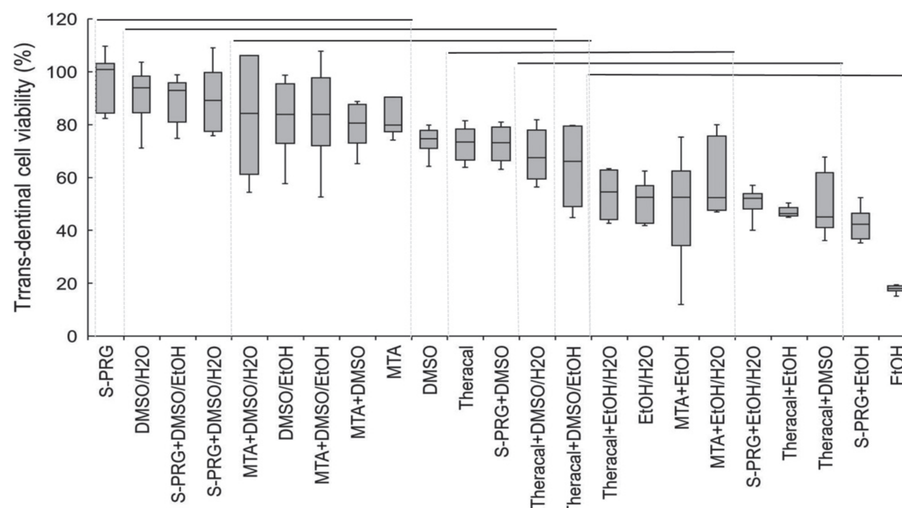

Fig. 2 Box-plot diagram representing the distribution of trans-dentinal viability for pulp-derived cells produced by different bioactive materials used in combination with different solvent pretreatments (n=12).
The line inside each box plot represents the group median. Lines above box plots represent no statistical differences between groups (p>0.05). Statistical comparisons were performed by the Kruskal-Wallis test (α=0.05).

combined use significantly affected cell damage (p<0.05). Grading assessments of cell damage according to ISO 7405[17] are reported in Table 2.

Trans-dentinal cell viability
Trans-dentinal viability of pulp-derived cells after 24 h is shown in Fig. 2. Kruskal-Wallis test revealed that bioactive materials, pretreatment solutions, and their combined use significantly affected cell viability (p<0.001). Negative controls had no effect on cell viability and positive controls reduced cell viability by approximately 50% after 24 h exposure. The highest reduction among pretreatment solutions was observed for EtOH and EtOH/H_2O (p<0.05). No significant differences were observed between DMSO/H_2O, DMSO/EtOH, or DMSO (p>0.05). S-PRG was the least cytotoxic bioactive material followed by MTA, without significant differences from each other (p>0.05). TheraCal presented the highest reduction in cell viability among the tested materials (p<0.05). In general, EtOH and EtOH/H_2O produced the lowest overall cell viabilities, as opposed to DMSO/H_2O and DMSO/EtOH with higher values, irrespective of the bioactive material used (p<0.05). EtOH and DMSO significantly lowered the cell viability produced by TheraCal (p<0.05), while DMSO/H_2O, DMSO/EtOH, and EtOH/H_2O had no significant effect (p>0.05). EtOH and EtOH/H_2O significantly reduced the cell viability produced by MTA (p<0.05), while DMSO/H_2O, DMSO/EtOH, and DMSO had no significant effect (p>0.05). DMSO/H_2O and DMSO/EtOH had no significant effects on the cell viability produced by S-PRG (p>0.05), as opposed to EtOH, EtOH/H_2O, and DMSO, which significantly lowered cell viability (p<0.05).

DISCUSSION

Since the tested bioactive materials affected trans-dentinal viability of pulp-derived cells, the first null hypotheses were rejected. According to ISO 7405 standards, S-PRG was non-cytotoxic while TheraCal and MTA were deemed severely and moderately cytotoxic, respectively. Cells activities and responses can be modulated by certain properties of bioactive materials, including pH, ion release, surface topography, and stiffness. For instance, pH level can affect enzymatic activity and cellular metabolism, whereas ion release is crucial for providing signals necessary for cell differentiation and mineralization[21]. MTA and TheraCal are well known for their calcium release. MTA generally exhibits lower initial calcium release compared to TheraCal[22-24], S-PRG is a surface pre-reacted glass, which does not contain leachable calcium ions in its composition[25]. The composition of materials was a decisive factor, with TheraCal distinguished by its hydrophilic monomer/matrix, exhibiting higher calcium release over 28 days compared to MTA[26].

Silicate-based materials are well known for their alkalinity due to the release of calcium, hydroxyl, and other ions[27]. It has been suggested that the positive effect of MTA cements on the proliferation of human dental pulp cells is enhanced potentially by the continuous and constant release of calcium ions. Calcium ions react with carbonates in the pulp tissue to form calcium carbonate, this process influences pulp cell proliferation and contributes to the progression of mineralization[28-31]. Despite their similar pH profiles[26], resin-containing bioactive materials like TheraCal are generally more cytotoxic[5]. This might be due to the leaching of uncured

hydrophilic monomers, causing cell death[32]. While uncured monomers are cytotoxic to pulp cells, their cured versions present milder effects[33]. The obtained higher cytotoxicity for TheraCal corroborates previous eluate-based cell culture studies. It is important to highlight that the effects experienced by cells differ depending on the type of ion, compound, and concentration. Relatively high concentrations of calcium ions could lead to extensive cell death or reduction of initial proliferation[31,34,35]. The complex structure of the resin-dentin interface likely influences TheraCal's cytotoxicity, on account of different gradients of monomer diffusion and conversion rates in the presence of moisture[11,36]. Uncured methacrylate monomers can increase reactive oxygen species production and oxidative DNA damage[37], impacting cell metabolism and protein expression[38]. The trans-dentinal cytotoxicity setup used in this study allowed a more realistic assessment of the uncured-monomer effect on pulp cells compared to eluate-based cell culture studies[5]. Notably, less efficient light curing conditions might further reduce cell viability, suggesting potential higher cytotoxicity for TheraCal in clinical scenarios[39].

Although MTA was classified as moderately cytotoxic, no significant differences in cell viability between MTA and TheraCal were observed at 24 h. The prolonged hydration reaction of MTA, releases by-products such as calcium hydroxide[40], altering cell processes. Even though the higher pH might be buffered by pulp fluid, leaching of uncured monomers from TheraCal may accumulate over time exhausting the cells' detoxifying glutathione metabolism and other defense mechanisms[41]. Therefore, TheraCal's cytotoxicity, which can be attributed to both high pH and uncured monomers surpasses MTA's moderately cytotoxic nature. Pulp cell viability also depends on exposure times[38]. When cytotoxic eluates are exposed directly to cells, peak reductions in cell viability happen at 7 days[38]. The likely slower diffusion of cytotoxic components did not result in lower cell viability for TheraCal at 24 h, as seen in eluate-based cell cultures[38,42]. Anyhow, TheraCal's cytotoxicity should not be underestimated as clinical applications naturally characterize longer exposure times. Differently, S-PRG demonstrated non-cytotoxic behavior, indicating promising applications for various dental applications[43] due to its ion release and exchange abilities[44,45].

DMSO concentrations were chosen based on a series of studies[8,11,13] showing that high concentrations significantly improve interactions between resin-based materials and dentin and enhance wetting. Low concentrations of DMSO (0.008%) were previously shown to have no impact on pulp-cell cytotoxicity[15]. The safety of higher DMSO concentrations, however, remained uncertain. Since dentin pretreatments significantly affected trans-dentinal cell viability, the second null hypothesis was rejected. This study highlighted DMSO's moderate cytotoxicity, while 50% (v/v) dilutions with water or ethanol resulted in non-cytotoxic binary solutions. Differently, pure ethanol and its dilution in water (50% v/v) were severely cytotoxic according to ISO 7405[17]. The solvent type was a determining factor for pulp cell viability and should be considered carefully depending on clinical applications. This study provides evidence that aqueous or ethanolic dilutions of DMSO (up to 50% v/v) exhibited no measurable signs of trans-dentinal cytotoxicity against pulp-derived cells. Solvent cytotoxicity can be related to their interaction with membranes and the consequent effects of cell permeability and diffusion. Undiluted DMSO proved moderately cytotoxic. Reduction in cytotoxicity responses after DMSO dilutions may be explained by DMSO's impact on cell membranes at concentration-dependent doses[46]. Lower DMSO concentrations produced lower cell membrane damage[46] and consequently lower cytotoxicity. Notably, DMSO presented significantly lower trans-dentinal cytotoxicity than ethanol at comparable concentrations (50% v/v). Interestingly, DMSO showed a protective effect against ethanol's high cytotoxicity, potentially through its ability to hydrogen bond to both dentin and ethanol. This finding warrants further investigation into its underlying mechanisms. The rationale for combining bioactive materials and solvent pretreatments was to benefit from solvents' ability to potentially improve material interaction with dentin. Even though trans-dentinal cytotoxicity findings indicate that DMSO itself would not necessarily harm pulp tissue, the resultant effect of combining DMSO and ethanol to bioactive materials was unknown.

Dentin pretreatments affected the trans-dentinal cytotoxicity of bioactive materials. Severely cytotoxic solutions (EtOH/H_2O and EtOH) significantly reduced cell viability of MTA and S-PRG. However, EtOH/H_2O had no additional effect on the cell viability of TheraCal, while EtOH produced significantly lower values. Since TheraCal itself was severely cytotoxic, only pure ethanol (EtOH) was able to further compromise cell viability. Pure or diluted ethanol pretreatments increased the trans-dentinal cytotoxicity of bioactive materials, which may comprise clinical applications. Differently, the moderately cytotoxic DMSO or its non-cytotoxic aqueous or ethanolic dilutions (50% v/v) presented milder or no effects on the cell viability of tested bioactive materials. DMSO is well established as a tissue penetration enhancer, which may have positive or negative effects on cell viability depending on the carried substances. Due to the cytotoxicity of methacrylate-based monomers[37], DMSO's use in deep cavities along with resin-based materials has raised important concerns about its safety. The effect of DMSO pretreatments on the cell viability of TheraCal was concentration dependent. While pure DMSO significantly reduced the cell viability of TheraCal, aqueous (DMSO/H_2O) or ethanolic (DMSO/EtOH) dilutions had no significant effects. Considering the low dentin barrier thickness employed here (300 μm), it is possible to assume that diluted DMSO pretreatments (up to 50% v/v) would not necessarily bring additional risks to pulp cells when resin-based materials are used *in vivo*. This is the first study to evaluate the interaction between bioactive materials and different solvent-based dentin pretreatments on pulp cell viability. It is worth

mentioning that our findings provide relevant and original insights about the safety of employing DMSO at higher concentrations as dentin pretreatments, a growing trend in adhesive dentistry currently lacking evidence. Nonetheless, future studies should aim to combine DMSO pretreatments to materials containing higher resin content to exclude any possible concentration-dependent effect of methacrylate monomers on trans-dentinal cytotoxicity. Differently, DMSO pretreatments did not affect the cell viability of MTA certainly due to the absence of methacrylate monomers. Only pure DMSO decreased S-PRG's cell viability, while diluted DMSO pretreatments had no effect. Higher DMSO concentrations may facilitate the diffusion of filler ions, such as Al^{3+}, increasing the overall cytotoxicity. It is worth mentioning that DMSO induces osteoblastic differentiation with similar effects on odontoblast-like cells. Therefore, low doses that diffuse through dentin could increase the rate of secondary dentin formation rather than damage the dentin-pulp complex making DMSO not only a safer option, but also likely a more beneficial dentin pretreatment than ethanol.

CONCLUSION

The composition of bioactive materials and their combination to solvent-based dentin pretreatments can affect pulp-cell viability. Attempts to perform such solvent-based pretreatments to potentially improve bioactive material-dentin interactions can substantially increase trans-dentinal cytotoxicity. While pure ethanol or its aqueous dilutions should preferably not be applied in close proximity to the pulp, diluted DMSO at relatively high concentrations has minimal to no effects on pulp cell viability regardless of bioactive materials. Hence, solvents must be carefully selected to avoid unnecessary damage to pulp cells.

ACKNOWLEDGMENTS

This work was supported by grant #296653 from the Academy of Finland to AT-M (PI), and EVO funding of Turku University Hospital to AT-M (PI). The authors declare no potential conflicts of interest concerning the authorship and/or publication of this article. The authors are grateful to Professor Buchalla for the kind donation of the clonal large T-antigen transfected bovine cells that originated from the work of Thonemann and Schmalz (2000) at Regensburg University.

CONFLICTS OF INTEREST

The authors declare no competing interests.

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