Journal of Dental Sciences

Volume 21 | Issue 2 Article 63

2026

2-Hydroxy ethylmethacrylate activates the extrinsic and intrinsic apoptotic processes through mitogen-activated protein kinase signaling pathways in cementoblasts - An in vitro study

Wei-Ju Lai

Wen-Ying Shen

Chun Chuan Su

Yu-Chao Chang

Available online at https://jds.ads.org.tw/journal/

Digital Commons

journal homepage: https://jds.ads.org.tw/journal/

Original Article

2-hydroxy ethylmethacrylate activates the extrinsic and intrinsic apoptotic processes through mitogen-activated protein kinase signaling pathways in cementoblasts - An *in vitro* study

Wei-Ju Lai [a,b], Wen-Ying Shen [c], Chun Chuan Su [a], Yu-Chao Chang [a,d*]

[a] *School of Dentistry, Chung Shan Medical University, Taichung, Taiwan*
[b] *Yuan Rong Medical Foundation, Yuan Rong Hospital, Changhua, Taiwan*
[c] *Hinew Dental Clinic, Changhua, Taiwan*
[d] *Department of Dentistry, Chung Shan Medical University Hospital, Taichung, Taiwan*

Received 29 January 2026; Final revision received 5 February 2026
Available online 1 April 2026

KEYWORDS
2-hydroxy ethylmethacrylate;
Cementoblast;
Murine;
Apoptosis;
Caspases;
Mitogen-activated protein kinases

Abstract *Background/purpose:* 2-Hydroxy ethylmethacrylate (HEMA) is a hydrophilic monomer that may be released from composite resin after polymerization. Little is known about the adverse effects of HEMA on cementoblasts. Therefore, this study investigated the possible mechanisms underlying the cytotoxicity engendered by HEMA on murine cementoblast cell line (OCCM.30).
Materials and methods: OCCM.30 cells were cultured with HEMA (0, 2, 4, and 8 mM) for 24 h. Cell viability was determined by microculture tetrazolium assay. Flow cytometry with annexin V-FITC/propidium iodide apoptosis staining was conducted to evaluate the cell cycle distribution and the type of cell death. Western blot was employed to investigate the caspase-mediated apoptotic cell death and mitogen-activated protein kinase (MAPK) pathways.
Results: The concentrations of HEMA≧4 mM significantly inhibited cell viability in a concentration-dependent manner ($P < 0.05$). HEMA dose-dependently induced apoptosis by the increase of sub-G1 population, early apoptotic cells, and later apoptotic cells, respectively. HEMA-induced apoptotic mechanisms were found to activate executioner caspase-3, extrinsic caspase-8, and intrinsic caspase-9, respectively ($P < 0.05$). In addition, HEMA increased the phosphorylation of extracellular signal-regulated protein kinases (ERK), c-Jun N-terminal kinases (JNK), and p38, respectively ($P < 0.05$). Using inhibitors of ERK (U0128),

* Corresponding author. School of Dentistry, Chung Shan Medical University, 110, Sec.1, Chien-Kuo N. Rd., Taichung, 40201, Taiwan.
E-mail address: cyc@csmu.edu.tw (Y.-C. Chang).

https://doi.org/10.1016/j.jds.2026.02.003

JNK (JNK-in-8), and p38 (SB203580), HEMA's increases of cleaved caspases-3, -8, and -9 could be expectedly suppressed ($P < 0.05$).
Conclusion: The results demonstrated that HEMA decreased cell viability and induced caspase-mediated apoptosis in cementoblast by activating both extrinsic and intrinsic apoptotic pathways through ERK, JNK, and p38 signaling. These results may provide the preliminary information for the development of less cytotoxic composite resin.

Introduction

Composite resin-based restorative materials that consist of resin matrix, inorganic filler, and coupling agent have widely used in dentistry over past decade.[1] The common monomers used in resin matrix including 2-hydroxyethyl methacrylate (HEMA), triethylene glycol dimethacrylate (TEGDMA), and bisphenol-A-glycidyldimethacrylate (Bis-GMA) act as the bioactive components of denture base materials, bonding agents, and restorative materials.[2] However, residual monomers leached after incomplete polymerization or improper polymerization and degradation have raised the concerns of their toxicity to the adjacent tissues.[2–4]

HEMA, a well-known component of composite resin and adhesive, is a bi-phase resin monomer with high hydrophilicity property. Uncured HEMA could diffuse through dentin more easily than other monomers during restorative procedure via dentinal tubules to cause a negative impact on pulp cells such as the production of oxidative stress, expression of inflammatory mediators, and even cell death.[5–8]

Caspases, a family of cysteine proteases, play the important roles during apoptosis and inflammation.[9] Caspases involved in apoptosis are classified into initiator (caspase-8 and caspase-9) and executioner (caspase-3). Most apoptotic processes divide into extrinsic or intrinsic pathway.[10] Extrinsic apoptosis pathway is activated by binding with a ligand to a death receptor which leads to the recruitment, dimerization, and activation of caspase-8.[9] Intrinsic apoptosis pathway is also known as mitochondrial apoptosis triggered by the activation of cellular stresses. Active caspase-9 then initiates apoptosis by cleaving and thereby activating executioner caspases.[9] Conclusively, caspases play a major role in cell signaling including apoptosis, cell growth, and cell proliferation.[9,10]

MAPK (mitogen-activated protein kinase) signaling transduction pathways including extracellular signal-regulated kinases (ERKs), c-Jun amino-terminal kinases (JNKs), and p38 MAPK play a pivotal role in converting extracellular stimuli into multiple cellular responses such as cell growth, migration, proliferation, and apoptosis.[11,12] Therefore, the role of MAPK signaling transduction pathways involved in the caspase-mediated apoptotic effect is worth to further evaluation.

Composite resin-based restorative materials are popular and also utilized in many applications. Recently, these materials are the superior choice to replace amalgam for the repair of endodontic perforations and as the root end retrograde filling materials. Cementoblasts play an important role in root apex closure with new cementum formation.[13] Therefore, the evaluation of cytotoxicity of HEMA on periapical cells may have highly clinical relevance. In this study, we determined the cytotoxicity of HEMA on murine immortalized cementoblast cell line (OCCM.30) by measuring cell viability, type of cell death, caspase-mediated apoptotic cascade, and MAPK signaling transduction pathways. These data will improve our knowledge concerning the effects of HEMA on periapical tissue.

Materials and methods

Chemicals and reagents

Cell culture materials including Dulbecco's modified Eagle medium (DMEM) and fetal bovine serum (FBS) were obtained from Gibco-BRL (Gaithersburg, MD, USA) and Hyclone Laboratories, Inc. (Logan, UT, USA), respectively. HEMA, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), dimethyl sulfoxide, and phosphate buffered saline (PBS) were purchased from Sigma—Aldrich (St. Louis, MO, USA). Antibodies specific for p38, phosphorylated p38, β-actin, caspases-3 and -8, and FITC (fluorescein isothiocyanate-labeled) Annexin V Apoptosis Detection Kit I were obtained from BD Biosciences (San Jose, CA, USA). Additionally, antibodies specific for ERK, JNK, phosphorylated ERK, phosphorylated JNK, caspases-9, and cleaved caspases-3, -8 and -9 were obtained from Cell Signaling Technology (Danvers, MA, USA). Unless otherwise specified, all chemicals used in this study were purchased from Sigma—Aldrich.

Cell culture

Murine immortalized cementoblast cell line OCCM.30 cells derived from root surface of mandibular first molar of OC large T-antigen transgenic mice were the generous gift by Professor Tsai (Chung Shan Medical University, Taichung, Taiwan) from Dr. Somerman Laboratory (University of Washington, Seattle, WA, USA).[14] OCCM.30 cells were obtained from subclone 30 passages which express bone sialoprotein and osteopontin mRNAs with the indicative of cementoblasts *in situ*. OCCM.30 cells were grown in DMEM

supplemented with 10 % FBS and 1 % penicillin/streptomycin. Cell cultures were maintained at 37 °C in a humidified atmosphere of a 5 % CO2 incubator.

Cytotoxicity assay

MTT colorimetric assay was developed to monitor mammalian cell survival by the measurement of dehydrogenase activity as described previously.[15] Briefly, 5×10^4 cells/well were seeded to 24-well plate and left overnight to attach. Various concentrations of HEMA (0, 2, 4, 6, 8 mM) were added for 24 h. After treatment, 50 ml of the MTT solution (2 mg/mL in PBS) was added to each well and incubated for another 4 h at 37 °C. To each well, 50 mL of dimethyl sulfoxide was added. Plates were then shaken until the crystals were dissolved. Reduced MTT was then measured spectrophotometrically in a dual beam microtiter plate reader at 570 nm with a 650-nm reference.

Cell cycle distribution and annexin V-FITC apoptosis staining

Propidium iodide (PI) staining is used for fluorescence activated cell sorter analysis by flow cytometry. OCCM.30 cells were seeded in 6 cm dishes and treated with 0, 2, 4, and 8 mM HEMA for 24 h. After treatment, cells were incubated with 0.25 % trypsin–EDTA solution, collected, and fixed in cold 70 % ethanol for 24 h. The fixed cells were suspended with PI buffer at room temperature for 30 min in the dark. The BD flow cytometer system was used to analyze the distribution of the cell cycle and Cell Quest software (BD Biosciences) was used to quantitate the flow cytometry data.[16] In addition, Apoptotic cell death was measured with BD Annexin V-FITC Apoptosis Detection Kit. The early apoptotic and late apoptotic cells were examined through flow cytometry performed using Cell Quest software (BD Biosciences).[17]

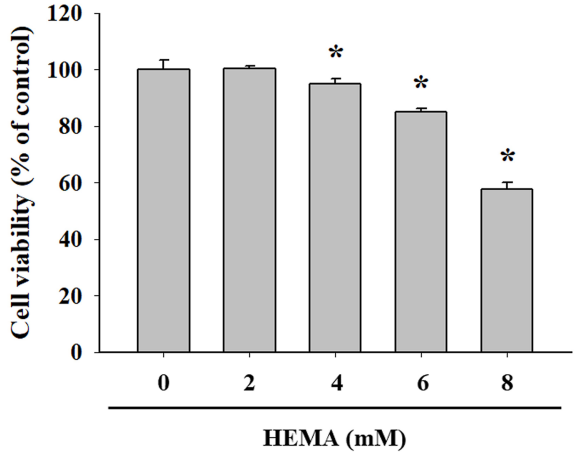

Figure 1 The cytotoxicity of 2-hydroxy ethylmethacrylate (HEMA) on cell viability in OCCM.30 cells for 24 h. Data represent the mean ± standard deviation from three independent experiments. *$P < 0.05$ compared with the control.

Western blot analysis

To investigate the molecular mechanism further, the initiator and executioner caspases and signaling

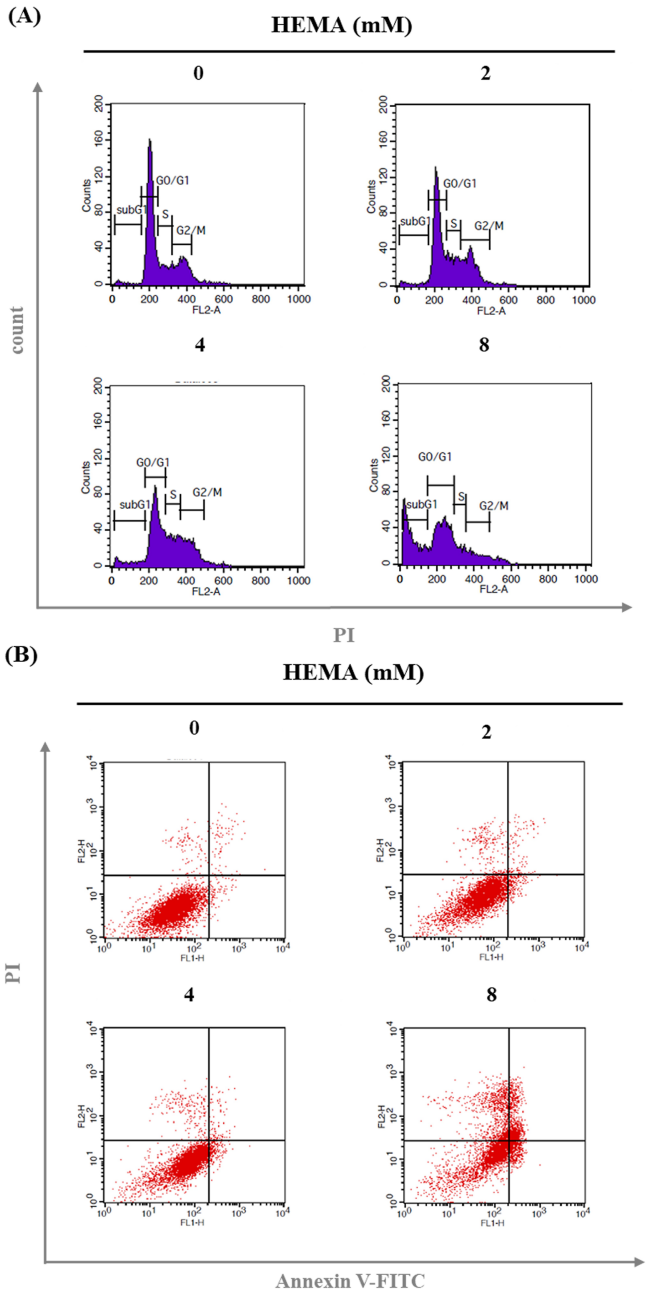

Figure 2 The distribution of cell cycle and type of cell death with 2-hydroxy ethylmethacrylate (HEMA) on OCCM.30 cells for 24 h by using flow cytometry. (A) Propidium iodide (PI) staining was conducted to analyze the distribution of cell cycle phase. The sub-G1 cell fraction represents the apoptotic cell death. (B) Annexin-V/PI double-staining was used to quantify the type of cell death. In this dot plot, lower right quadrant represents early apoptosis. Upper right quadrant represents late apoptosis cells. Upper left quarter represents the necrotic cells. Lower left quarter represents the viable cells.

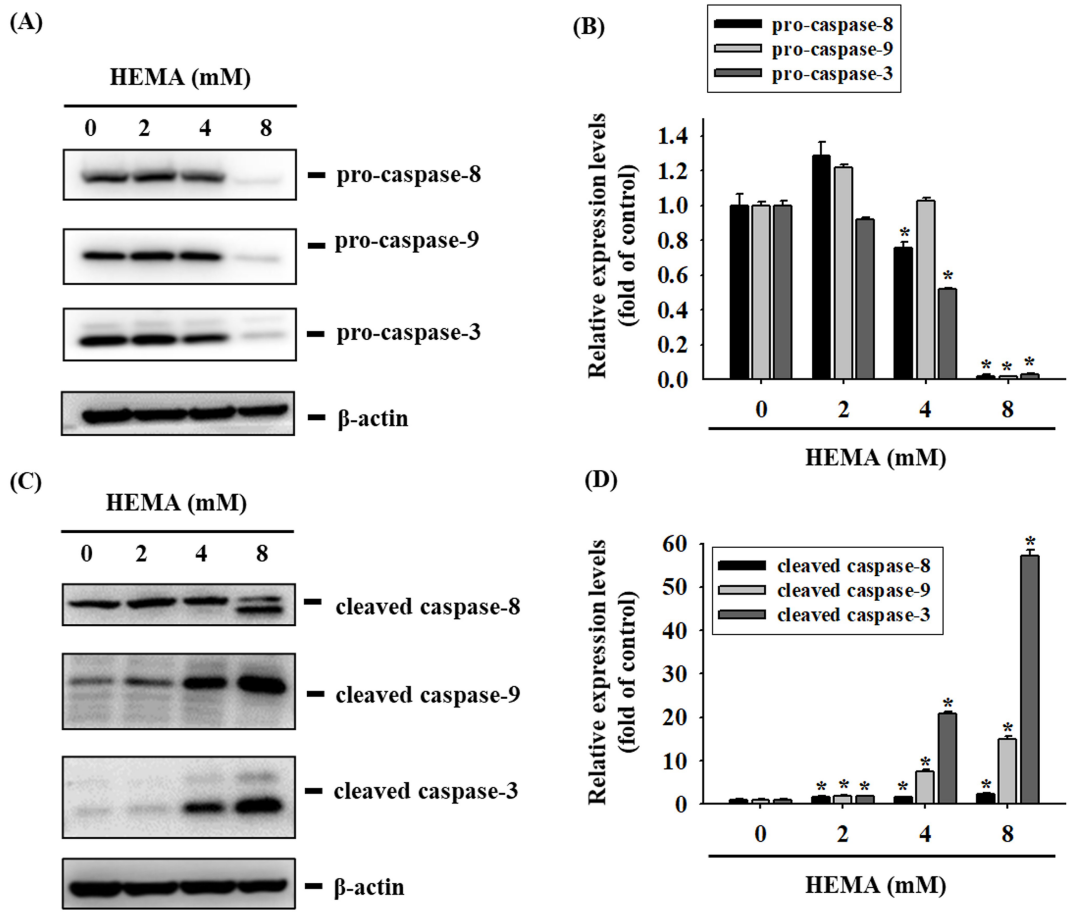

Figure 3 2-hydroxy ethylmethacrylate (HEMA)-induced caspases-mediated apoptosis in OCCM.30 cells. (A) The effects of HEMA (0, 2, 4, and 8 mM) on the inhibition of pro-caspase-8, -9, and -3 in OCCM.30 cells for 24 h were assayed by Western blot. (B) The quantitative results of these protein levels were adjusted by β-actin levels. Values represent the mean ± standard deviation from three independent experiments. *$P < 0.05$ compared with the control. (C) The effects of HEMA (0, 2, 4, and 8 mM) on the activation of cleaved caspase-8, -9, and -3 in OCCM.30 cells for 24 h were evaluated by Western blot. (D) The quantitative results of these protein levels were adjusted by β-actin levels. Values represent the mean ± standard deviation from three independent experiments. *$P < 0.05$ compared with the control.

transduction pathways were detected by Western blot. OCCM.30 cells were cultured in 6 cm plates for 16 h and treated them with 0, 2, 4, and 8 mM HEMA for 24 h. After treatment, total cell lysates of OCCM.30 cells were prepared. Western blot was performed using specific primary antibodies against caspases-3, -8 and -9, cleaved caspases-3, -8 and -9, and the specific antibodies for unphosphorylated or phosphorylated forms of corresponding MAPKs (ERK, JNK, and p38). Blots were then incubated with a horseradish peroxidase goat anti-rabbit or anti-mouse IgG for 1 h, and the intensity of each band was measured via densitometry as described previously.[16–18]

Statistical analysis

Values are revealed as the mean ± standard deviation (SD). Statistical analyses were performed using Sigma Plot (Systat Software Inc., San Jose, CA, USA). Significant differences were considered at $P < 0.05$.

Results

MTT assay was used to evaluate cytotoxicity of HEMA on OCCM.30 cells (Fig. 1). The concentrations of HEMA≧4 mM significantly retarded cell viability in a concentration-dependent ($P < 0.05$). At the treatment of concentrations of 2, 4, 6, and 8 mM HEMA, the rate of viable cells were about 100 %, 94.96 %, 84.83 %, and 57.63 % as compared with control, respectively.

To determine whether the cytotoxicity of HEMA is associated with apoptosis. The impact of HEMA on cell cycle was examined by PI staining using flow cytometry. After 24 h treatment, HEMA significantly increased sub-G1 phase ratio as compared with control in OCCM.30 cells (Fig. 2A). The quantitative analysis of sub-G1 apoptotic fraction was about 0.68 %, 1.44 %, 3.95 %, and 33.04 % in the concentrations of 0, 2, 4, and 8 mM HEMA, respectively.

The type of cell death induced by HEMA was further investigated using annexin V-FITC/PI double-staining

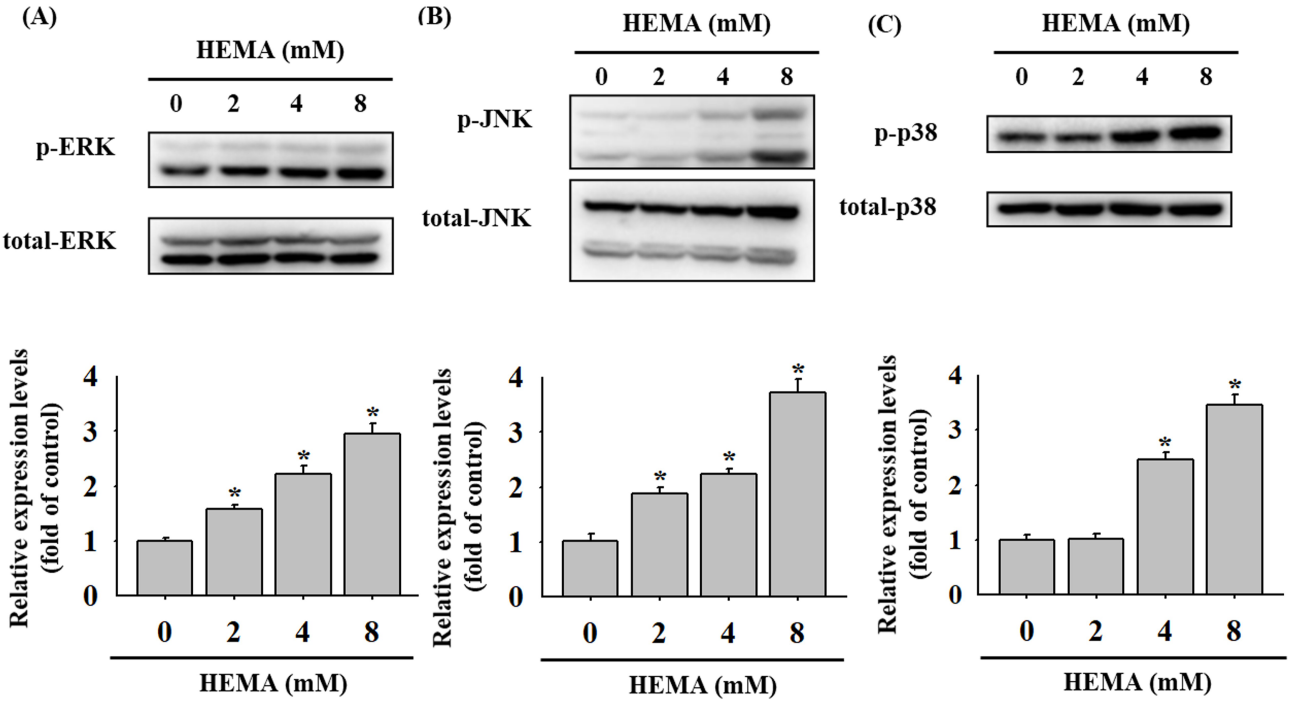

Figure 4 2-hydroxy ethylmethacrylate (HEMA) activates the phosphorylation of ERK, JNK, and p38 in OCCM.30 cells (A–C, upper panel). Cells were treated with different concentrations of HEMA (0, 2, 4, and 8 mM) for 24 h for Western blot. The quantitative results of these protein levels were adjusted by β-actin levels (A–C, lower panel). Values represent the mean ± standard deviation from three independent experiments. *$P < 0.05$ compared with the control.

(Fig. 2B). The percentage of early apoptotic cells in the lower right quadrant and late apoptotic cells in the upper right quadrant was about 3.01 %, 5.82 %, 6.55 %, and 25.77 % after treating OCCM.30 cells in the concentrations of 0, 2, 4, and 8 mM HEMA, respectively. In addition, cell necrosis in the upper left quarter was also noted about 1.74 %, 4.19 %, 4.02 %, and 18.08 % in the treatment of concentrations of 0, 2, 4, and 8 mM HEMA in OCCM.30 cells, respectively.

As illustrated in Fig. 3A, HEMA decreased the expression of pro-caspase-8, -9, and -3 in OCCM.30 cell, respectively. The quantitative results of protein levels were shown in Fig. 3B. The concentration of 8 mM HEMA significantly inhibited pro-caspases-8, -9, and -3 expression down to 0.03, 0.02, and 0.04 fold as compared with control ($P < 0.05$), respectively. Fig. 3C demonstrated that HEMA induced the activation of cleaved caspase-8, -9, and -3 in OCCM.30 cells, respectively. As shown in Figs. 3D and 4 mM HEMA significantly stimulated the levels of cleaved caspase-8, -9, and -3 about 1.54, 7.43, and 20.7 fold as compared with control ($P < 0.05$), respectively. Conclusively, HEMA-induced cell apoptosis was found to activate extrinsic caspase-8 as well as intrinsic caspase-9, and their downstream executioner caspase-3.

MAPKs play a central role in the regulation of cell apoptosis. In a further investigation for the underlying molecular mechanisms, we determined whether MAPKs were activated in HEMA-treated OCCM.30 cells by Western blot. As shown in Fig. 4A–C, data showed that the phosphorylation of ERK, JNK, and p38 were increased in a dose-dependent manner ($P < 0.05$), respectively.

Furthermore, the treatment of 10 μM ERK inhibitor U0126, 1 μM JNK inhibitor JNK-in-8, and 10 μM p38 inhibitor SB203580 with or without 4 mM HEMA were used to identify the regulation of caspases-8, -9, and -3 in OCCM.30 cells (Fig. 5A). In addition, three inhibitors significantly inhibited the HEMA-induced the increase of protein levels of cleaved caspase-8, -9, and -3 as compared with HEMA alone in Fig. 5B ($P < 0.05$).

Discussion

Dental composite resins can release residual monomers into oral environment after polymerization. Therefore, the evaluation of biologic and toxicological properties of monomers is important in the fabrication of composite resin before and after clinical usage. In vitro cytotoxic assays by cell culture are simple, reproducible, cost-effective, relevant, and suitable for the evaluation of basic biological properties of dental materials.[19]

Leached residual HEMA can easily diffuse through dentin due to its hydrophilic properties and low molecular weight leading to the cytotoxicity of dental resin material in human pulp cells.[20] Composite resin as a root end retrograde filling material, residual HEMA leached could cause the adverse interaction with surrounding tissues including cementum. Hence, we performed an *in vitro* study to determine cytotoxicity of HEMA on OCCM.30 cells which may reflect the *in vivo* situation more clinical relevance.

To the best of our knowledge, this is the first study shown that HEMA is a cytotoxic agent to OCCM.30 cells. Similar findings have reported that HEMA exhibited cytotoxicity to human gingival epithelial cells,[5] human pulp derived cells,[5–8] murine macrophage RAW264.7 cells,[21,22] gingival fibroblasts,[23,24] apical papilla stem cells,[25] and

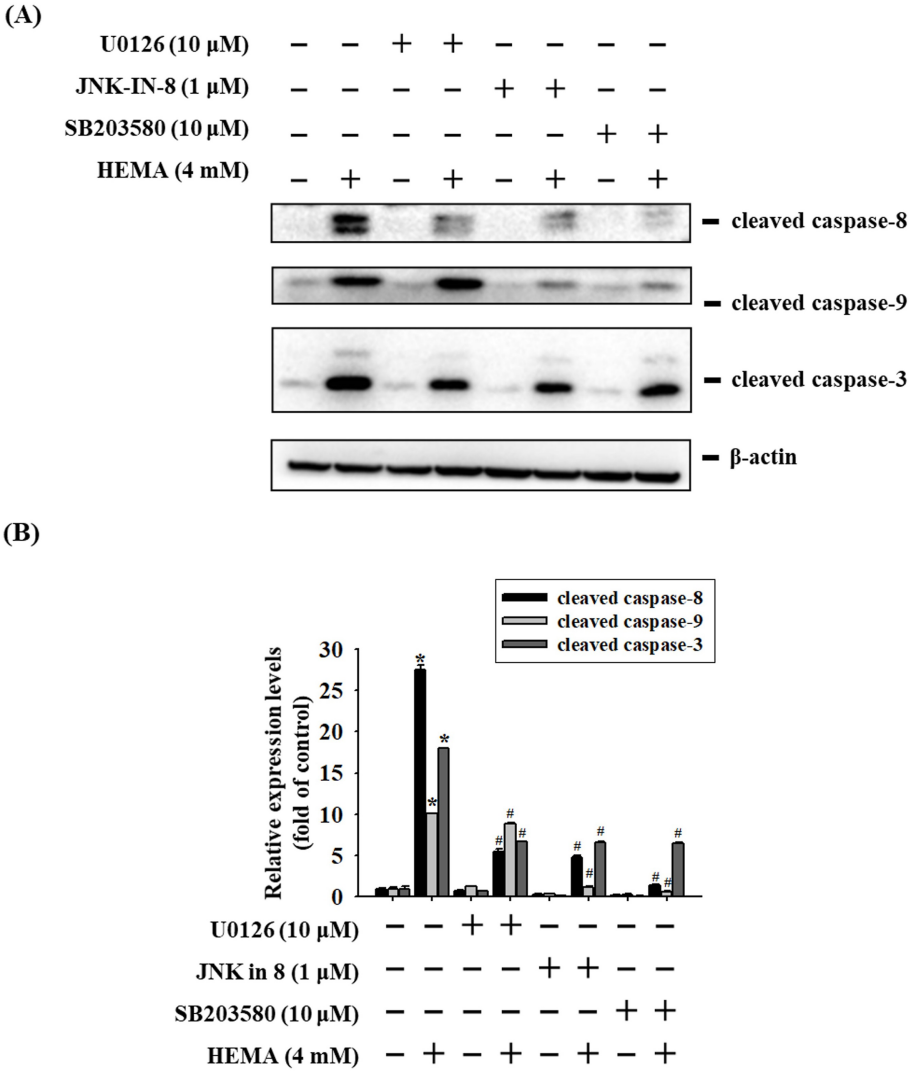

Figure 5 Relationship among the activation of mitogen-activated protein kinases in 4 mM 2-hydroxy ethylmethacrylate (HEMA)-triggered apoptosis of OCCM.30 cells. (A) OCCM.30 cells were pre-treated with U0126, JNK in 8, and SB203580 for 1 h before 4 mM HEMA treatment and untreated cells for 24 h by Western blot. (B) The quantification data are shown in the bar graph adjusted by β-actin levels. Data represent the mean ± standard deviation from three independent experiments. *: $P < 0.05$ compared with the control. #: $P < 0.05$ compared with the 4 mM HEMA treatment group.

human osteoblast-like cells,[26] respectively. Although the cell lines used and culture conditions were different, the cytotoxic nature of HEMA was clearly demonstrated.

The induction of cytotoxicity via apoptosis, programmed cell death, is a major stress response of cell death. The sub-G1 phase plays a hallmark of apoptosis which means the DNA double-strand broken and the generation of pyknotic nuclei.[27] In this study, we first demonstrated HEMA-induced apoptosis in a dose dependent manner through Sub-G1 formation analysis by PI staining as well as annexin V-FITC/PI staining by flow cytometry, respectively. In agreement with our findings, apoptosis induced by HEMA leading to cytotoxicity have been shown in several mammalian cells, including RAW264.7 mouse macrophages,[21,22] dental pulp fibroblasts,[6,8] and gingival epithelial cells.[5] However, one experiment demonstrated without any evidence about the increase of sub-G1 peak by HEMA on human pulp fibroblasts.[5] The reason for this contrary result is not clear. It may result from the different origins of cells or different experimental protocols used in each laboratory. However, further studies are necessary to clarify.

Moreover, the other type of cell death necrosis was also revealed in OCCM.30 cells by the treatment of HEMA. Similar results were found in human pulp derived cells[6,8] and mouse macrophages RAW264.7 cells.[21,22] Taken together, the types of cell death necrosis-induced by HEMA are varied in different cell lines.

The activation of extrinsic pathway or the stimulation of intrinsic pathway is important to trigger apoptosis. The executioner caspase-3 plays a critical role in the underlying programs of apoptosis and relies on the activation of its upstream initiators including extrinsic caspase-8 and intrinsic caspase-9.[9,10] To the best of our knowledge, HEMA was first found to decrease pro-caspase-3, -8, and -9 as well

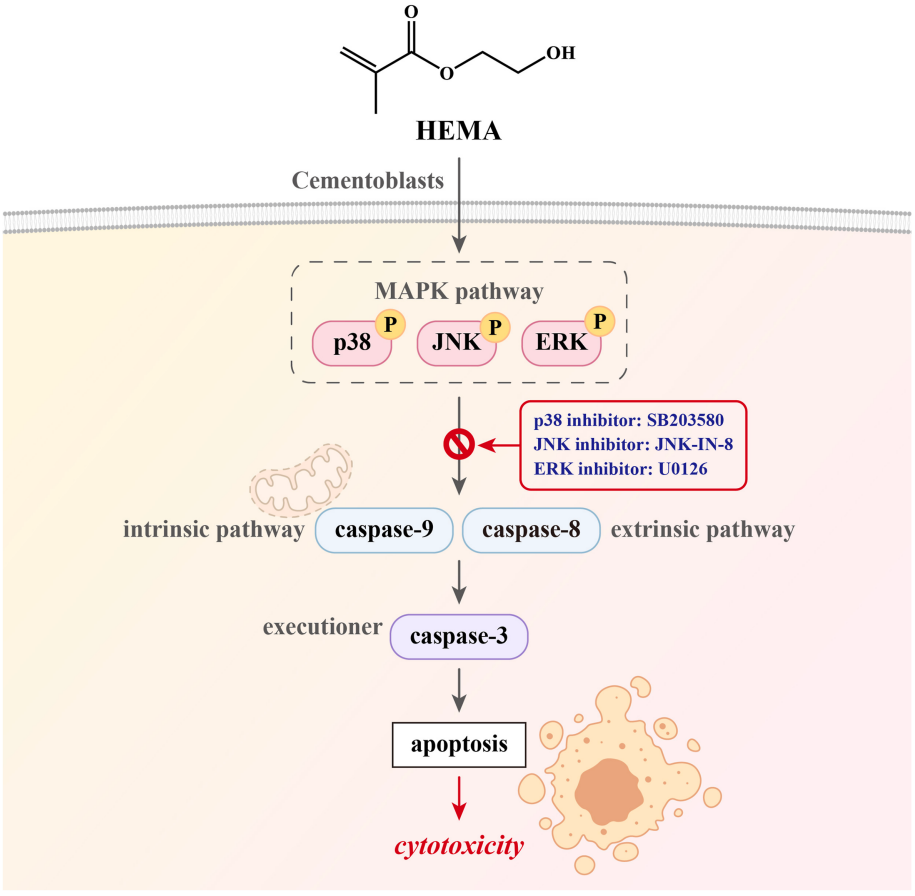

Figure 6 Schematic diagram illustrating the signaling pathways involved in 2-hydroxy ethylmethacrylate (HEMA)-induced cytotoxicity associated with extrinsic and intrinsic apoptotic processes through mitogen-activated protein kinase signaling pathways in cementoblasts.

as stimulate cleaved caspase-3, -8, and -9 expression in OCCM.30 cells. Beside to the executioner caspase 3, HEMA-induced cell apoptosis was found to activate both extrinsic and intrinsic apoptotic processes in OCCM.30 cells. The protein expression of cleaved caspase-3 was the most inducible amount. This finding suggests that caspase-3 may play a major cell apoptotic pathway in OCCM.30 cells. Previously, one experiment showed the activation of caspase–3 and cleaved caspase-3 by the treatment HEMA on human gingival fibroblast.[24] Therefore, caspases-mediated cell apoptotic pathway may play an important signaling transduction pathway in HEMA-induced cell apoptotic death.

MAPK signaling transduction pathways were reported to play a central role in the regulation of cell apoptosis induced by TEGDMA and Bis-GMA with the activation of caspases-3, -8, and -9.[28–31] In this study, the activities of ERK, JNK, and p38 were dominantly increased after HEMA treatment. Therefore, the effects of HEMA on MAPK levels appear to be dependent on the cell types and the concentrations used. Moreover, our study directly found that the induction of caspase-mediated apoptosis by HEMA was retarded by ERK, JNK, and p38 inhibitors, respectively. Therefore, factors that inhibit ERK, JNK, and p38 phosphorylation may appear the useful pharmaceutical agents for the prevention of apoptosis mediated by HEMA. Conclusively, U0126, JNK in 8, and SB203580 might be the valuable therapeutics in HEMA-induced cytotoxic effects.

The possible mechanisms that HEMA-induced caspase-mediated apoptotic cell death in OCCM.30 cells by the regulation of MAPK signalings may be partly explained as followings. The physiological production of reactive oxygen species (ROS) within cells is dramatically enhanced after exposure to external stimuli. Several studies have shown that HEMA clearly shifted the generation of intracellular ROS beyond a stable balance of the cellular redox homeostasis resulted in cytotoxicity via apoptosis.[5,21,22] However, further studies are necessary to investigate the facts in OCCM.30 cells.

As illustrated in Fig. 6, HEMA could induce the phosphorylation of ERK, JNK, and p38 and subsequent stimulate the activation of caspase-3, -8, and -9, which eventually result in the inhibition of growth and the induction of apoptosis in OCCM.30 cells. Moreover, U0126, JNK-in-8, and SB203580 may be the potential pharmaceutical agents against apoptosis from HEMA. These results may provide the preliminary information to develop the less cytotoxic composite resin-based restorative materials. Further animal studies are required to assess the possible mechanisms of HEMA *in vivo*.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

Acknowledgments

This study did not receive any external funding resources.

References

1. Su NY, Wang YH, Chang YC. A registry-based study of tooth-colored restorative materials for decayed teeth in Taiwan. *J Dent Sci* 2023;18:1235–42.
2. Ortengren U, Wellendorf H, Karlsson S, Ruyter IE. Water sorption and solubility of dental composites and identification of monomers released in an aqueous environment. *J Oral Rehabil* 2001;28:1106–15.
3. Durner J, Spahl W, Zaspel J, Schweikl H, Hickel R, Reichl FX. Eluted substances from unpolymerized and polymerized dental restorative materials and their Nernst partition coefficient. *Dent Mater* 2010;26:91–9.
4. Van Landuyt KL, Nawrot T, Geebelen B, et al. How much do resin-based dental materials release? A meta-analytical approach. *Dent Mater* 2011;27:723–47.
5. Chang HH, Guo MK, Kasten FH, et al. Stimulation of glutathione depletion, ROS production and cell cycle arrest of dental pulp cells and gingival epithelial cells by HEMA. *Biomaterials* 2005; 26:745–53.
6. Wanachottrakul N, Chotigeat W, Kedjarune-Leggat U. Translationally controlled tumor protein against apoptosis from 2-hydroxyethyl methacrylate in human dental pulp cells. *J Mater Sci Mater Med* 2011;22:1479–87.
7. Trubiani O, Cataldi A, De Angelis F, D'Arcangelo C, Caputi S. Overexpression of interleukin-6 and -8, cell growth inhibition and morphological changes in 2-hydroxyethyl methacrylate-treated human dental pulp mesenchymal stem cells. *Int Endod J* 2012;45:19–25.
8. Chen RS, Lee MS, Hu YJ, Hu CY, Tseng WY. The effects of low-dose 2-hydroxyethyl methacrylate on apoptosis and survival in human dental pulp cells. *J Formos Med Assoc* 2021;120:1332–9.
9. Sahoo G, Sama lD, Khandayataray P, Murthy MK. A review on caspases: key regulators of biological activities and apoptosis. *Mol Neurobiol* 2023;60:5805–37.
10. Ghorbani N, Yaghubi R, Davoodi J, Pahlavan S. How does caspases regulation play role in cell decisions? Apoptosis and beyond. *Mol Cell Biochem* 2024;479:1599–613.
11. Yue J, López JM. Understanding MAPK signaling pathways in apoptosis. *Int J Mol Sci* 2020;21:2346.
12. Johnson GL, Lapadat R. Mitogen-activated protein kinase pathways mediated by ERK, JNK, and p38 protein kinases. *Science* 2002;298:1911–2.
13. Leonardo MR, da Silva LA, Leonardo RT, Utrilla LS, Assed S. Histological evaluation of therapy using a calcium hydroxide dressing for teeth with incompletely formed apices and periapical lesions. *J Endod* 1993;19:348–52.
14. D'Errico JA, Berry JE, Ouyang H, Strayhorn CL, Windle JJ, Somerman MJ. Employing a transgenic animal model to obtain cementoblasts *in vitro*. *J Periodontol* 2000;71:63–72.
15. Chen YJ, Lee SS, Huang FM, Chang YC. Effects of nicotine on differentiation, prostaglandin E2 and nitric oxide production in cementoblasts. *J Dent Sci* 2015;10:431–6.
16. Chen PN, Lin CW, Yang SF, Chang YC. CLEFMA induces the apoptosis of oral squamous carcinoma cells through the regulation of the p38/HO-1 signaling pathway. *Cancers* 2002;14:5519.
17. Lo SK, Su NY, Su CC, Chang YC. Sodium butyrate activates the extrinsic and intrinsic apoptotic processes in murine cementoblasts. *J Dent Sci* 2025;20:613–9.
18. Yang LC, Chang YC, Chiang CY, Huang FM, Su NY, Kuan YH. Protective effect of wogonin on inflammatory responses in BisGMA-treated macrophages through the inhibition of MAPK and NF-kB pathways. *Environ Toxicol* 2022;37:3007–12.
19. Huang FM, Chang YC. Cytotoxicity of dentine-bonding agents on human pulp cells *in vitro*. *Int Endod J* 2002;35:905–9.
20. Bouillaguet S, Wataha JC, Hanks CT, Ciucchi B, Holz J. In vitro cytotoxicity and dentin permeability of HEMA. *J Endod* 1996; 22:244–8.
21. Krifka S, Hiller KA, Spagnuolo G, Jewett A, Schmalz G, Schweikl H. The influence of glutathione on redox regulation by antioxidant proteins and apoptosis in macrophages exposed to 2-hydroxyethyl methacrylate (HEMA). *Biomaterials* 2012;33:5177–86.
22. Gallorini M, Petzel C, Bolay C, et al. Activation of the Nrf2-regulated antioxidant cell response inhibits HEMA-induced oxidative stress and supports cell viability. *Biomaterials* 2015;56:114–28.
23. Durner J, Wellner P, Hickel R, Reichl FX. Synergistic interaction caused to human gingival fibroblasts from dental monomers. *Dent Mater* 2012;28:818–23.
24. Teti G, Orsini G, Salvatore V, et al. HEMA but not TEGDMA induces autophagy in human gingival fibroblasts. *Front Physiol* 2015;6:275.
25. Bakopoulou A, Leyhausen G, Volk J, Koidis P, Geurtsen W. Effects of resinous monomers on the odontogenic differentiation and mineralization potential of highly proliferative and clonogenic cultured apical papilla stem cells. *Dent Mater* 2012;28: 327–39.
26. Kraus D, Wolfgarten M, Enkling N, et al. In-vitro cytocompatibility of dental resin monomers on osteoblast-like cells. *J Dent* 2017;65:76–82.
27. Allen RT, Hunter III WJ, Agrawal DK. Morphological and biochemical characterization and analysis of apoptosis. *J Pharmacol Toxicol Methods* 1997;37:215–28.
28. Krifka S, Petzel C, Bolay C, et al. Activation of stress-regulated transcription factors by triethylene glycol dimethacrylate monomer. *Biomaterials* 2011;32:1787–95.
29. Krifka S, Hiller KA, Bolay C, et al. Function of MAPK and downstream transcription factors in monomer-induced apoptosis. *Biomaterials* 2012;33:740–50.
30. Kuan YH, Huang FM, Lee SS, Li YC, Chang YC. BisGMA stimulates prostaglandin E2 production in macrophages via cyclooxygenase-2, cytosolic phospholipase A2, and mitogen-activated protein kinases family. *PLoS One* 2013;8:82942.
31. Lai WJ, Shen WY, Yang LC, Huang FM, Chang YC. Triethyleneglycol-dimethacrylate induces caspase-mediated apoptotic cell death in cementoblasts by the regulation of JNK and p38 pathways- an *in vitro* study. *J Dent Sci* 2025;20:1060–7.