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In Vitro Biocompatibility of CPP-ACP and Fluoride-containing Desensitizers on Human Gingival Cells

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Clinical Relevance

Different commercial desensitizers with the same therapeutic indication exhibit different biological effects and cytotoxicity on human gingival fibroblasts, which could be considered criteria for choosing the optimal treatment of dentin hypersensitivity.

SUMMARY

Objectives: To analyze the biocompatibility of different desensitizers containing casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) and fluoride in their composition: MI Varnish (MV), Clinpro White Varnish (3M Oral Care), Profluorid Varnish (VOCO), Duraphat (Colgate) and Embrace Varnish (Pulpdent) on human gingival fibroblast cells (hGF).

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Methods and Materials: Human gingival fibroblast (hGF) cells were exposed to several desensitizer extracts at different concentrations (0.1%, 1%, and 4% eluates). Then, *in vitro* biocompatibility was studied by analyzing the IC_{50} value, cell proliferation (MTT assay and cell cycle), cell migration (wound healing assay), cell morphology and F-actin content (immunocytofluorescence), and induction of apoptosis/necrosis (flow cytometry). Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey test.

Results: The lowest cell viability and IC_{50} were observed in all concentrations of Embrace Varnish-treated hGFs ($p<0.001$), whereas the highest were exhibited by those treated with Clinpro White Varnish. Similar effects were evidenced when induction of apoptosis/necrosis and cell migration assays were assessed. Finally, MI Varnish, Profluorid Varnish, Duraphat, and Embrace Varnish extracts showed lower numbers of attached cells, some of them with an unusual fibroblastic morphology when cultured with 4% concentration of the varnishes, while Clinpro White Varnish exhibited a similar number of cells with an evident actin cytoskeleton compared to the control group.

Conclusions: The results obtained in this study indicate that hGFs show better *in vitro* biocompatibility after exposure to Clinpro White Varnish, even at the highest concentration employed, making it the most eligible for topical applications. In contrast, Embrace Varnish exhibited a high cytotoxicity towards hGFs that could potentially delay the healing process and regeneration of the oral mucosa, although more studies are needed to confirm this hypothesis.

INTRODUCTION

The average prevalence of dentin hypersensitivity (DH) is 33.5%,[1] affecting the quality of life of a high number of patients by making it difficult for them to perform simple tasks such as drinking cold water, eating ice cream, or even brushing their teeth.[2,3]

The pathogenesis of DH has been explained by different theories throughout history until 1964, when the hydrodynamic theory proposed by Brännström was established, which to date is the most widely accepted theory.[4] Previous reports showed that the DH process is complex and multifactorial, requiring the presence of gingival recession and/or cementum loss from root surfaces that is usually caused by periodontal disease, although it can also be favored by erosion, attrition, or abrasion.[5,6] Once this happens, it is easier for a stimulus, either thermal, tactile, osmotic, or chemical dehydration, to produce an acute and brief pain sensation.[7,8]

Some researchers have shown it is necessary to target pulpal nerves directly to reduce DH or to occlude the dentinal tubes with a precipitating substance or a sealing agent.[9,10] Thus, various treatments for DH include the use of lasers, biocrystals, fluorides, adhesives, oxalates, arginine, or potassium salts.[5,9]

Among all these eligible treatments, one of the most frequently employed in clinical practice, due to their low cost, are the fluoride varnishes.[10-12] These varnishes are applied onto the dental surface, and the chemical components that form are released throughout the oral cavity. They can be detected in the saliva or adhered to the tooth surface (which will cause it to continue releasing over time), and also penetrate into dentinal tubules.[13] For these reasons it is important to assess the toxicity these products may produce in the oral resident fibroblasts with which they come into contact when they diffuse through the gingival tissue. Although there are studies that assess the toxicity of toothpastes for tooth sensitivity,[14] or other products commonly used for DH treatment,[15] there are not many studies about toxicity of fluoride varnishes indicated for DH.[15,16]

Recently, a dental varnish (MI Varnish, GC, Tokyo, Japan) containing fluoride and casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) was introduced in the market,[17] and Clinpro White Varnish (3M Oral Care, North Ryde, Australia) contains tricalcium phosphate. These varnishes include bioactive materials that have a desensitizing action when applied onto the tooth surface.[18] These bioactive agents are popular due to their biocompatibility and similar crystal structure to the dental tissues.[19-21] Other fluoride varnishes such as Profluorid (VOCO, Cuxhaven, Germany) and Duraphat (Colgate Palmolive, Hamburg, Germany) do not have new formulas in their composition, being more traditional varnishes, with the exception of Embrace Varnish (Pulpdent, Watertown, MA) that contains xylitol—a polyalcohol that also acts as caries preventive agent.[22] However, the number of published articles evaluating the biocompatibility of these products is scarce, and only one study on Clinpro White Varnish biocompatibility has been previously reported, which concluded it presents less cytotoxicity than the other products for DH.[15] In particular, the tests with fluoride- or calcium phosphate-based desensitizers are practically null in the literature, which was another reason for this study.

In vitro toxicity tests are useful tools to evaluate the biocompatibility of different materials and are very

frequently used in a wide variety of studies.[23-26] Thus, different oral cell culture models are commonly used to elucidate the mechanisms involved in different biological responses and to investigate oral cell behavior in specific situations.[27]

The aim of this study was to compare the biocompatibility and cytotoxicity of several desensitizers employed in daily clinical practice for the treatment of dental hypersensitivity such as MI Varnish, Clinpro White Varnish, Profluorid Varnish, Duraphat, and Embrace Varnish on human gingival fibroblasts (hGF). The null hypothesis tested was that there are no significant differences among the different desensitizers in terms of their cytocompatibility.

METHODS AND MATERIALS

Preparation of Desensitizer Eluates

The tested materials included five commercially available desensitizers: MI Varnish, Clinpro White Varnish, Profluorid Varnish, Duraphat, and Embrace Varnish. Manufactureŕs data, composition, and lot number of each of the tested materials are shown in Table 1. Eluates of these materials were prepared following the recommendations of ISO 10993-5.[28] To obtain a 10% concentration, 1 ml of each fluoride varnish was mixed gently with 9 ml of Dulbecco's Modified Eagle's Medium (DMEM) culture medium (Gibco, Thermo Fisher Scientific, Carlsbad, CA, United States) and filtered through a 0.22-μm syringe filter. Then, eluates were subsequently diluted with culture medium to obtain different dilutions (0.1%, 1%, and 4%) and sterilized by exposure to ultraviolet light for 2 hours. DMEM culture medium without any eluates served as a negative control.

Isolation and Culture of Human Gingival Fibroblasts

Human gingival fibroblasts (hGFs) were obtained from impacted wisdom teeth (n=8). All participating donors previously signed an informed consent form according to the Helsinki Declaration guidelines.

After extraction, gingival tissues were extensively rinsed with phosphate-buffered saline (PBS) and cut into 1-mm^3 tissue blocks after removing blood vessels. Then, the tissue blocks were placed on polystyrene tissue culture flasks and suspended in PBS containing 1% penicillin/streptomycin (Invitrogen, Paisley, Scotland) to avoid oral bacterial contamination, and digested with serum-free DMEM culture medium containing 0.1% collagenase A (Roche Diagnostics, Basel, Switzerland) and 0.2% dispase II (Sigma-Aldrich, St. Louis, MO, United States) for 2 hours at 37°C. Then, isolated hGFs were washed with PBS, filtered through 40-mm nylon cell strainers (BD Biosciences, San Jose, CA, United States) and cultured in DMEM culture medium supplemented with 10% fetal calf serum (Lonza, Basel, Switzerland), 1% GlutaMAX (ThermoFisher Scientific), and 1% penicillin/streptomycin (ie, complete growth medium) at 37°C and 5% CO_2. For subsequent experiments, hGFs were used from culture passage 2 up to 6.

MTT and IC_{50} Assays

For cytotoxicity evaluation, 1×10^4 hGFs were cultured in the presence of the five studied desensitizer eluates or in DMEM culture medium alone (negative control) prepared as before. Cell metabolic activity was measured 24 hours, 48 hours, and 72 hours after the beginning of the cultures by MTT assay. According to the manufacturer's instructions, the MTT reagent (Sigma-Aldrich) was added to the wells for 4 hours. When the purple precipitate was obviously noticeable, dimethylsulfoxide

Table 1: *Tested Materials*

Material	Manufacturer	Composition	Lot Number
MI Varnish	GC Corporation	5% NaF (22.6 mg) + Casein Phosphopeptide-Amorphous Calcium Phosphate	1810041
Clinpro White Varnish	3M Oral Care	5% NaF (22.6 mg) + Tricalcium Phosphate	N994659
Profluorid Varnish	VOCO	5% NaF (22.6 mg), ethyl acetate-isoamylpropionate, polyurethane, difluorosilane	1931557
Duraphat	Colgate Palmolive	5% NaF (22.6 mg)	213549
Embrace Varnish	Pulpdent	5% fluoride (22.6 mg) + Xylitol-coated Calcium and Phosphate (CXP)	190314

(DMSO) (Sigma-Aldrich) (100 µl/well) was added to solubilize the formazan dye. Covered plates were kept in the dark for 2-4 hours. Finally, absorbance at 570 nm wavelength in each well was measured in a microplate reader (ELx800; Bio-Tek Instruments, Winooski, VT, United States). Each experimental condition was performed in quintuplicate for each desensitizer and analyzed in three independent experiments.

Complementarily, the dose of each of the tested materials that could decrease cell viability by 50% after 72 hours of culture (ie, IC_{50}) was analyzed graphically by plotting the percentage of the metabolic activity obtained in previous MTT assays on the Y axis and the percentage concentration of each fluoride varnish on the X axis. IC_{50} data were analyzed by nonlinear regression using GraphPad Prism software (version 8.1.0, GraphPad Software Inc, San Diego, CA, USA).

Cell Cycle Analysis

Cell cycle analysis by measuring the DNA content is a method that most frequently employs flow cytometry to distinguish cells in different phases of the cell cycle. Cells were cultured in 25 cm^2 culture flasks at 3×10^5 cells/cm^2 in presence of the five studied desensitizers' extracts prepared at different concentrations (0.1%, 1%, and 4%) as described above for 24 hours. Then, 1×10^5 cells were collected, fixed in 70% ethanol, and incubated with 40 µg/ml of propidium iodide and 200 µg/ml RNase for DNA content analysis. Propidium iodide fluorescence was measured with a FACSCanto II Flow Cytometer (Becton Dickinson, San Jose, CA) excitation wavelength: 488 nm; emission wavelength: 617 nm) and the percentage of cells in G_0/G_1, S and G_2/M phases was analyzed using CellQuest and Modfit LT programs (Becton Dickinson). Each experimental condition was performed in triplicate for each desensitizer and analyzed in three independent experiments.

Cell Migration

A wound healing assay was used to determine hGF migratory ability in presence of the five studied desensitizers' eluates. 2×10^5 hGFs/well were plated in 6-well plates to create a confluent monolayer. Then, the monolayer was scraped in a straight line to create a scratch or wound with a 100 ml pipette tip, washed twice with PBS to remove detached cells, cultured in complete growth medium alone (control) or in complete growth medium containing 0.1%, 1%, or 4% of the five tested desensitizers prepared as before and imaged using a phase-contrast microscope at 0, 24, 48, and 72 hours. The open wound area between both fronts of cell migration was quantified using Image J software (National Institutes of Health, Bethesda, MD, United States). Data are represented as percentages of migrated cells related to open wound areas just after scratching (100% of open wound area) and expressed as the mean ± standard deviation (SD) from three independent experiments performed in triplicate for each desensitizer.

Cell Cytoskeleton Staining

Phalloidin staining was used to analyze possible changes in cell morphology, and in the actin cytoskeleton structure and organization of hGFs cultured with the five studied eluates or DMEM culture medium alone as a negative control. Briefly, 3×10^4 hGFs were seeded on glass coverslips, allowed to adhere, and cultured in complete growth medium alone (control) or in complete growth medium containing 0.1%, 1%, or 4% of the five studied desensitizers' eluates for 72 hours from cell seeding at 37°C. Then, hGFs were rinsed twice with prewarmed PBS at 37°C, fixed in 4% formaldehyde solution (Merck Millipore, Darmstadt, Germany) for 10 minutes, permeabilized with 0.25% Triton X-100 solution (Sigma-Aldrich) for 5 minutes, and rinsed thrice with PBS. Cell cytoskeleton and nuclei were then stained at room temperature in the dark for 30 minutes with Invitrogen AlexaFluor594-conjugated phalloidin (ThermoFisher Scientific) and 4,6-diamidino-2-phenylindole dihydrochloride (DAPI) (ThermoFisher Scientific), respectively. Finally, immunofluorescence images were observed in a Leica TCS SP2 confocal microscope (Leica, Wetzlar, Germany). Each experimental condition was carried out in triplicate for each desensitizer and analyzed in three independent experiments.

Apoptosis/Necrosis Assay

To evaluate hGF viability after exposure to the different desensitizers, 1×10^5 hGFs per experimental condition were cultured in complete growth medium alone (control) or in complete growth medium containing 0.1%, 1%, or 4% of the different eluates for 72 hours from cell seeding at 37°C. Cell viability was assessed by incubation with 5 ml of Annexin-V-FITC and 5 ml of 7-AAD staining (BD Biosciences) in 100 ml 1× Annexin-V buffer per experimental condition in the dark for 15 minutes at room temperature. Samples were analyzed in an LSR Fortessa X-20 flow cytometer (Becton Dickinson) within 1 hour of staining. Finally, the percentages of viable (double negative), early apoptotic (Annexin-V-FITC positive, 7AAD negative), and late apoptotic and necrotic (double positive and Annexin-V-FITC negative/7-AAD+, respectively) cells were determined. Each experimental condition

was performed in triplicate for each desensitizer and analyzed in three independent experiments.

Statistical Analysis

Migration and MTT data were represented as the mean ± standard deviation (SD). One-way analysis of variance (ANOVA) or Student's *t*-test followed by Tukey post hoc comparison test was used for statistical comparison among groups using GraphPad Prism software version 8.1.0 (GraphPad Software, Inc). *p*-values < 0.05 were considered significant.

RESULTS

IC_{50} and MTT Assays

Viability of hGF in contact with the desensitizer extracts varied depending on the culture time, concentration, and material used (Figure 1). At 0.1% concentration, only Embrace showed a slight but significant decreased cell viability at all times studied compared to the control group ($p<0.001$), whereas at 1% concentration the comparison of cell viability among the different desensitizers and control group was: Control > Clinpro White Varnish > MI Varnish > Profluorid Varnish > Duraphat > Embrace ($p<0.001$). Finally, at 4% concentration, cell viability was more compromised at all times than that observed at 1% and ordered from highest to lowest as: Control > Clinpro White Varnish > Profluorid Varnish = Duraphat = MI Varnish = Embrace ($p<0.001$). Given these results, the IC_{50} values at 72 hours of culture (ie, percentage concentration of each desensitizer to inhibit 50% of hGF viability) were: Clinpro White Varnish = 4.4%; Profluorid Varnish = 1.6%; Duraphat = 1.4%; MI Varnish = 1.1%, and Embrace = 0.2% (Figure 2).

Cell Cycle Analysis

Cell cycle phase distributions are shown in Figure 3. At 4% concentration, in the Clinpro White Varnish group, the majority of the cells were found in G_0/G_1 phase (73.40%) with very few cells in S phase (15.88%) and G_2/M phase (10.72%). Conversely, MI Varnish, Duraphat, and Profluorid showed that the percentage of cells in phase G_0/G_1, S and G_2/M was 44.79%-50.59%, 26.75%-39.09%, and 16.77%-22.66%, respectively. At 1% concentration, only Embrace exhibited few cells in G_0/G_1 phase (50.24%); whereas, at 0.1% concentration, all groups showed abundant cells in G_0/G_1 phase (74%-76%).

Cell Migration

Open wound areas of migrating hGFs in presence of different concentrations of the analyzed desensitizers were measured after 24, 48, and 72 hours after wound infliction of confluent hGF monolayers (Figure 4). In general, except with Clinpro White, migration rates exhibited by hGFs cultured with any of the tested fluoride varnishes at 4% concentration were significantly lower at 24, 48, and 72 hours compared to the control group ($p<0.001$). At 1% concentration, Embrace displayed a statistically significant decreased cell migration after 24,

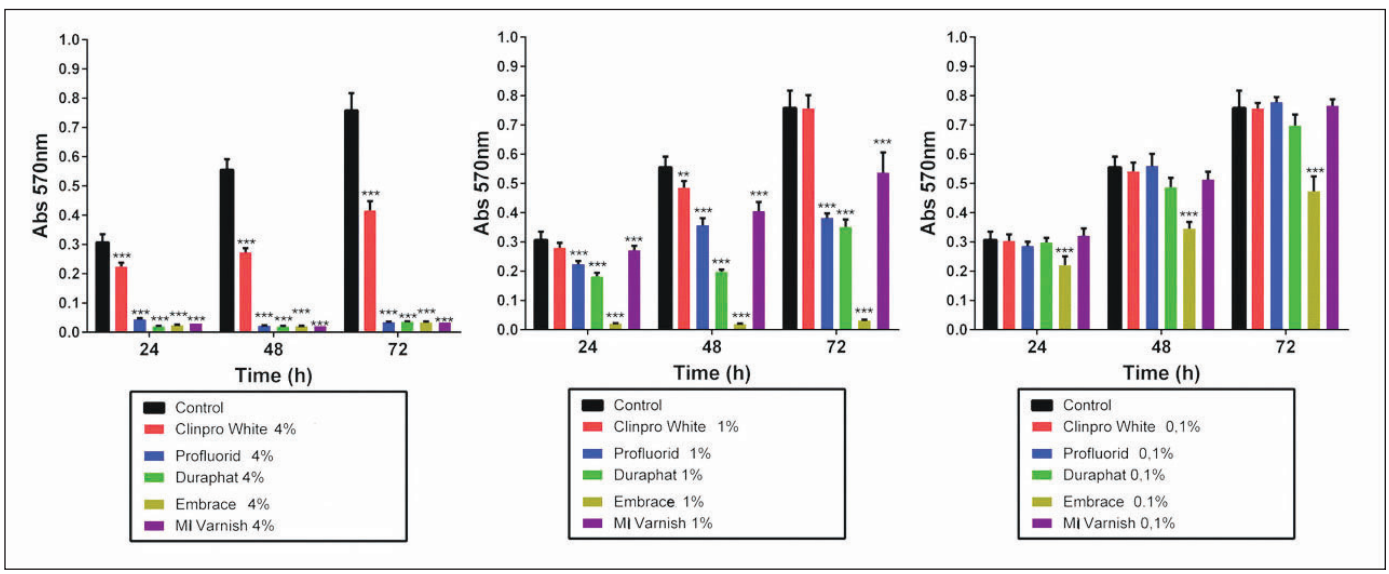

Figure 1. *Analysis of the metabolic activity of human gingival fibroblasts (hGFs) after exposure to different concentrations of desensitizer varnishes by MTT assays. Absorbance at 570 nm was significantly lower compared to the control conditions (**p<0.01; ***p<0.001, respectively) by one-way analysis ANOVA followed by Tukey post hoc test. Data are expressed as mean ± SD from n = 3 separate experiments.*

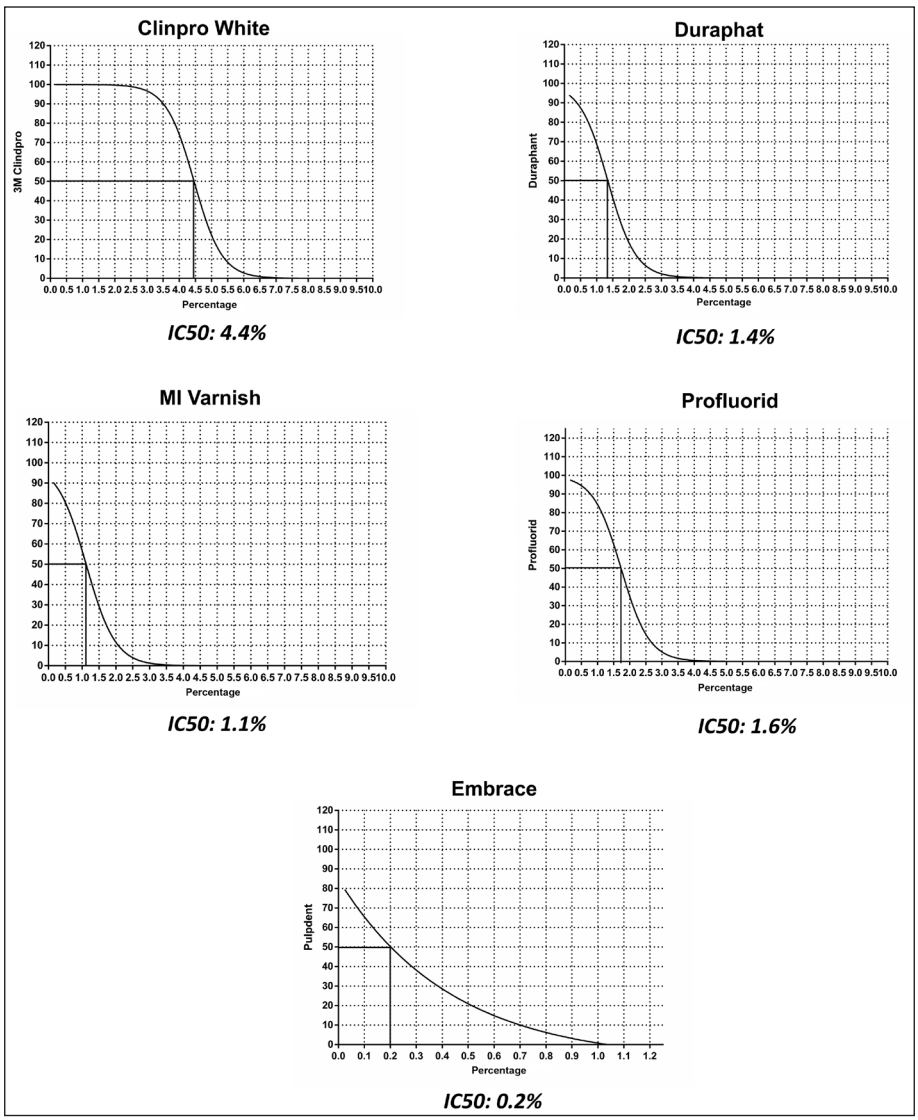

Figure 2. *Calculation of IC50 values of the different desensitizers. The percentage concentration of each varnish in the extract required for a 50% inhibition of metabolic activity of hGFs after 72 hours of culture was calculated. Data were analyzed by nonlinear regression by plotting the percentage of the metabolic activity on the Y axis and the percentage of each varnish on the X axis. Curves shown are representative from n = 3 separate experiments.*

48, and 72 hours of culture ($p<0.001$), while no significant differences were found among the other sensitizer groups. Finally, at 0.1% concentration, neither Clinpro White, Duraphat, MI Varnish, Profluorid, nor Embrace showed a significant decrease in the hGF migration compared to the control at any of the times studied.

Cell Cytoskeleton Staining

After 72 hours of exposure of hGF cultures to the five sensitizer extracts at 0.1% concentration, many well-attached and spread cells with a fibroblastic spindle-shaped morphology and high F-actin content were observed, similar to the control cells. However, Embrace at 1% concentration showed a considerably smaller number of attached cells with an aberrant morphology, whereas the other sensitizer groups exhibited a high number of well-adhered cells. Finally, Duraphat, MI Varnish, Profluorid, and Embrace extracts showed smaller numbers of attached cells, some of them with an aberrant morphology at 4% concentration, while Clinpro White exhibited a similar number of cells with well evident actin cytoskeleton compared to the control group (Figure 5).

Apoptosis/Necrosis Assay

The apoptosis/necrosis rate in each group was calculated by flow cytometry. As shown in Figure 6, at 4% of the concentration the percentage of viable cells was: Clinpro White (99.1%) > MI Varnish (98.03%) > Profluorid (90.9%) > Duraphat (11.7%) > Embrace (5.79%), whereas early/late apoptotic and necrotic cell percentages were: Clinpro White (0.9%) < MI Varnish (1.97%) < Profluorid (9.1%) < Duraphat (88.3%) < Embrace (94.21%). At 1% concentration, all materials

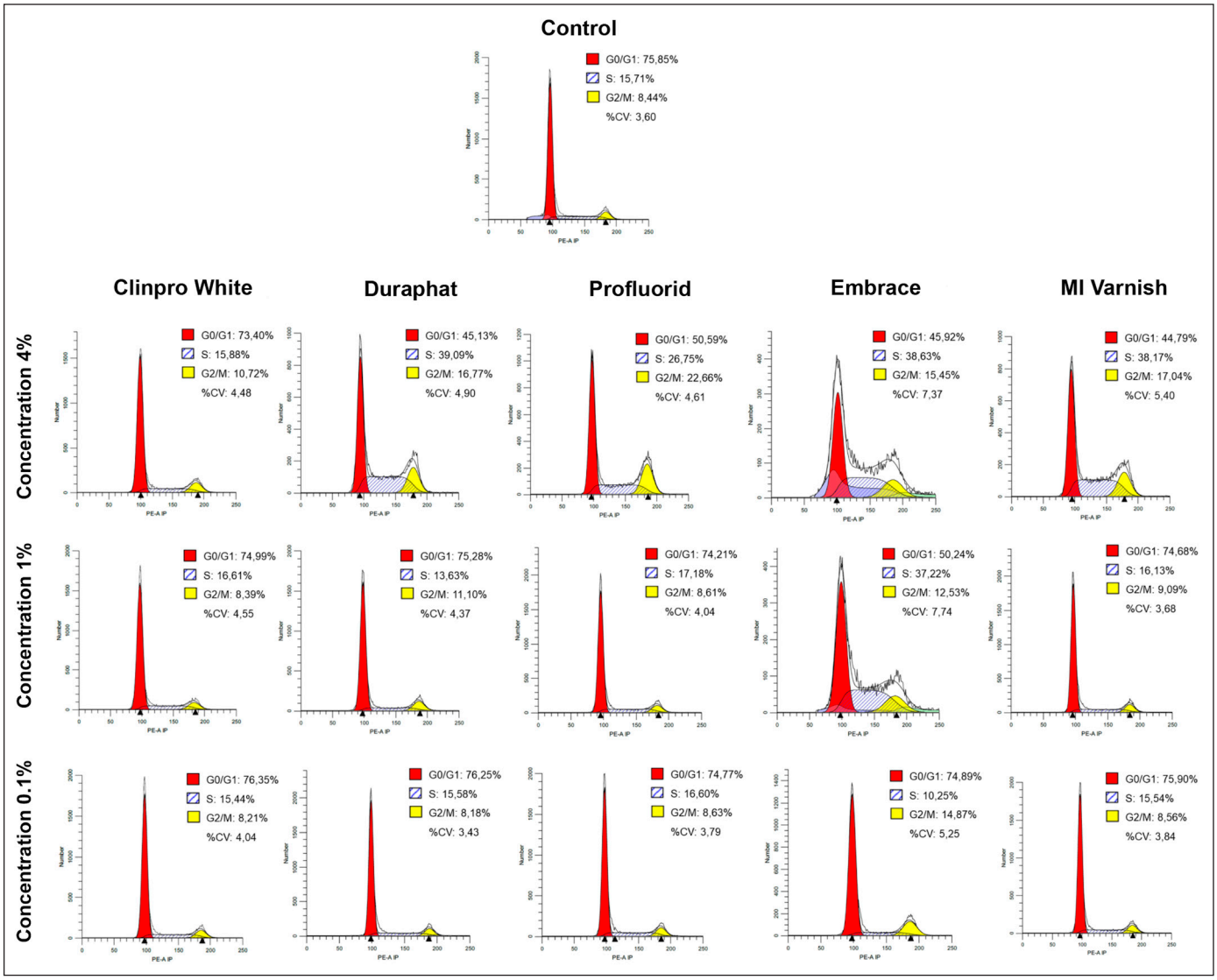

Figure 3. *Cell cycle analysis of the human gingival fibroblasts (hGFs) after exposure to the desensitizer concentrations (0.1%, 1%, and 4%). Dot-plots shown are representative from n = 3 separate experiments.*

displayed a high percentage of viable cells (>95%), except with Embrace (7.83%). Similarly, at 0.1% concentration, all desensitizers displayed a similar biocompatibility compared to the control medium, exhibiting more than 95% of viable cells, except with Embrace (88.3%).

DISCUSSION

Bioactive dental products were recently introduced and developed to optimize properties such as ion release, the promotion of remineralization, or the stimulation of hydroxyapatite formation.[29,30] Thus, varnish desensitizers containing fluoride (F), calcium (C), phosphate (P), or CPP-ACP such as MI Varnish, Clinpro White [fluoride (F), calcium (Ca2+), phosphate PO_4^3], or Embrace, have been commercially released.

The results of this study showed that there are statistically significant differences among the different desensitizers in terms of their cytocompatibility. Hence, the null hypothesis was rejected. There is a lack of studies that assesses the toxicity of varnish desensitizers among the available literature. These products have been used for years, when biocompatibility was not yet taken into account, so it has been assumed that there are no biocompatibility problems with them; but, as we have shown in this study, there are differences between some products with regard to biocompatibility, so it is an important issue to investigate before continuing with their clinical use. Eyüboglu and others[15] evaluated the cytotoxicity of dental cells after contact with dentin-desensitizing products (not only varnish desensitizers)

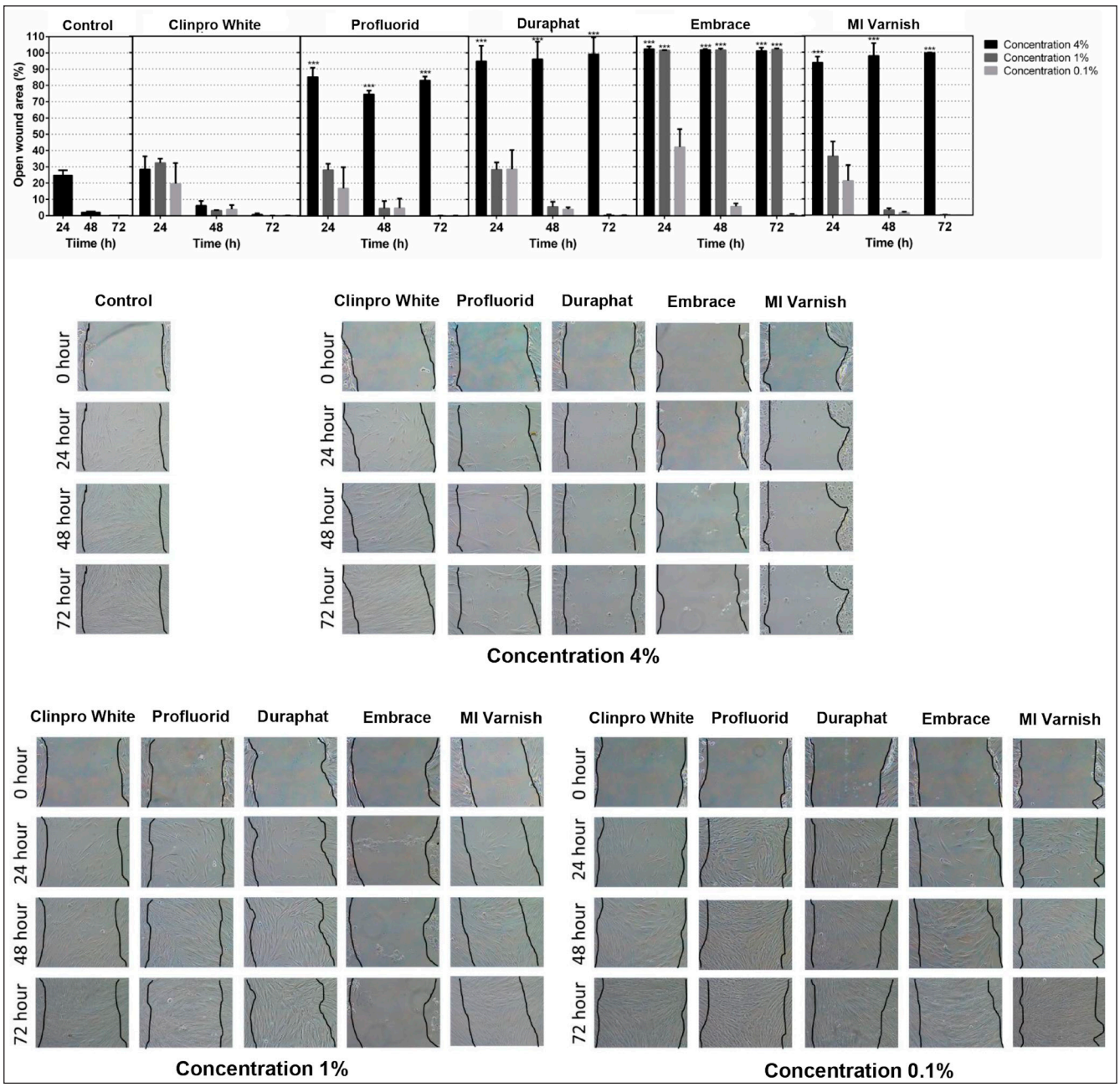

Figure 4. *Cell migration was evaluated using wound healing assays. Cells were cultured at different concentrations of the different desensitizers. The control condition consisted of cells cultured in culture growth medium without any desensitizer. Open wound areas were measured at 24, 48, and 72 hours and compared to the initial scratched wound area at 0 hour in the same well (100% migration). Cell migration was significantly lower compared to the control conditions (*p<0.05; ***p<0.001, respectively) by one-way analysis of variance (ANOVA) followed by Tukey post hoc test. Data are expressed as mean ± SD from n = 3 separate experiments.*

and noticed that some of them were cytotoxic towards human gingival cells and pulp fibroblasts. In this study we used human gingival cells, since this is the only population of cells exposed to the application of the varnish. Other authors have also previously tested fluoride varnishes using hGFs or other fibroblast cell lines to evaluate DH and cytotoxicity.[16,31,32]

The use of MTT, IC_{50}, cell cycle analysis, cell migration, cell cytoskeleton staining, and apoptosis/necrosis assays helps us to assess the biological behavior of human fibroblasts when they are exposed to these commercial desensitizers, showing an altered cell morphology or adhesion, a greater level of apoptotic and necrotic cells, and lower proliferation with some of these products.

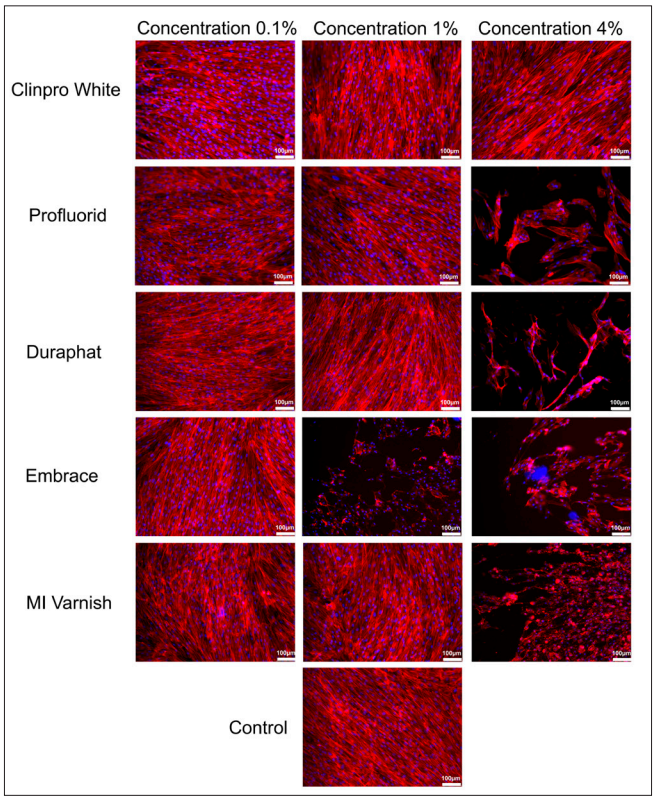

Figure 5. *Analysis of cell morphology changes in the actin cytoskeleton structure and organization on hGFs after treatment with the different desensitizers by confocal fluorescence microscopy. F-actin fibers were stained with AlexaFluor 594-conjugated phalloidin (red), whereas cell nuclei were counterstained with DAPI (blue). Confocal fluorescence microscopy images shown are representative from n = 3 separate experiments. Scale bar: 100 micron.*

In the present study, the MTT assays revealed that Embrace negatively affected human gingival cell viability at all extraction medium concentrations, while Clinpro White and MI Varnish showed cytotoxicity, which decreased with further dilutions. At the same time, these products exhibited IC_{50} values of 4.4% and 1.1.%, respectively. In a similar manner, a previous study reported that Clinpro White showed less cytotoxicity on gingival and dental pulp cells than other desensitizing products.[15] In addition, it has been reported that Duraphat in intimate contact with the oral tissues is not potentially harmful to host cells.[31] In the present study, we observed cytotoxicity at 4% and 1% but not at 0.1% concentrations.

It is well known that desensitizers release substances that could potentially delay or improve healing.[33] For this reason, we decided to use wound healing assays in order to preliminarily predict how the coordinated migration of hGFs would occur during inflammation or after injury. The marked decrease in cell migration in the Embrace-treated group could be due to the effect of these materials on cell viability. Our cell migration results suggest a correlation with our cell attachment and spreading results. In general, cells need to attach and spread on a surface for subsequent migration. Low cell attachment and F-actin fiber content and an aberrant morphology were evidenced in the 4% and 1% Embrace-treated groups, as observed by phalloidin staining; hence, their migration would also be affected. Although all materials studied presented 5% sodium fluoride in their composition, other components could be responsible for their biological effects on hGFs. Among the limitations of the present study could be the lack of experiments to assess the ions released by the different desensitizers to determine what composition can alter their biocompatibility. In fact, previous studies reported that low levels of sodium fluoride promote cell proliferation, cell migration, and accelerates wound closure, in turn.[34,35]

Apoptosis/necrosis assay evidenced a reduced number of viable cells in presence of Embrace varnish. A previous study reported that high concentrations of sodium fluoride (5000 ppm) promoted apoptotic morphological changes and DNA fragmentation on cementoblasts.[36] However, our observations suggest that sodium fluoride may not be solely responsible for the biological effects of these materials. The lack of information about these materials acts as the main limitation of this study. Thus, the components of these desensitizers should be further evaluated to better understand the basic mechanism/s of human gingival cell biological responses.

CONCLUSIONS

Within the limitations of this study, the results obtained indicate that hGFs show better *in vitro* biocompatibility after exposure to Clinpro White Varnish, even at the highest concentration employed, making it more eligible for topical applications. In contrast, Embrace Varnish exhibited a high cytotoxicity towards hGFs that could potentially delay the healing process and regeneration of the oral mucosa; more studies are needed to confirm this hypothesis.

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Regulatory Statement

The study protocol was approved by the Ethics Committee (Institutional Review Board for Human Subjects Research) of the University of Murcia (protocol ID: 2199/2018).

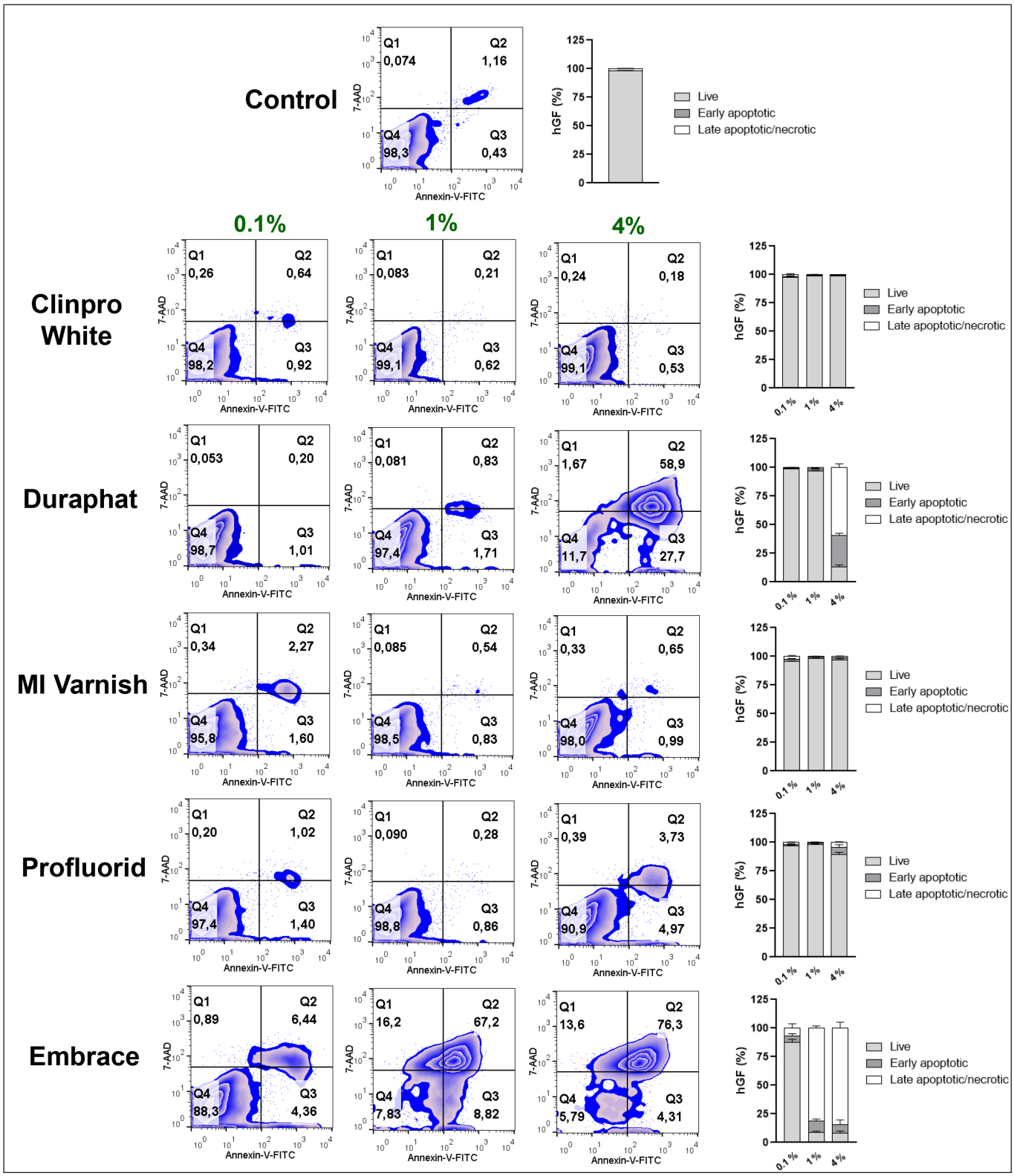

Figure 6. *Apoptosis/necrosis assays. Human gingival fibroblasts (hGFs) were cultured in MI Varnish, Clinpro White Varnish, Profluorid Varnish, Duraphat, and Embrace Varnish eluates at different concentrations (0.1%, 1%, and 4%) for 72 hours at 37°C. Numbers within the different quadrants represent the percentages of live (Q4; Annexin-V-/7-AAD-), early apoptotic (Q3; Annexin-V+/7-AAD-), or late apoptotic and necrotic cells (Q2 and Q1; Annexin-V+/7-AAD+ and Annexin-V-/7-AAD+, respectively). Dot plots show representative flow cytometry results obtained from three independent experiments, and graphs show mean ± SD from n = 3 separate experiments.*

Conflict of Interest

The authors of the present study certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in the present article.

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