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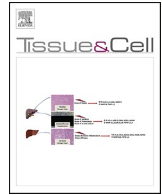

# In vitro biocompatibility of ammonia-free silver fluoride products on human dental pulp stem cells

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ABSTRACT

*Objectives:* Silver fluoride (SF) is a preventive and therapeutic option for dental pathological processes involving structural alterations of the hard tissues, either during their formation or those caused by caries or other pathological reasons. This study aimed to compare the biological properties of two commercial SF products, one of them with ammonium (Riva Star; SDF) and the other ammonium-free (Riva Star Aqua; AgF), both with or without potassium iodide (KI), by the assessment of the cytotoxicity of the materials' eluates.

*Methods:* Human dental pulp stem cells (hDPSCs) were obtained from healthy 18–23-year-old donors. Three dilutions were prepared for the tested materials (0.005%, 0.0005%, and 0.0001%). The following groups were assessed: (AgF, AgF+KI, SDF, SDF+KI, KI, negative control). A series of cytocompatibility assays were performed: MTT assay, IC50 assay, wound healing (migration) assay, cell cytoskeleton staining, analysis of cell apoptosis and necrosis, and reactive oxygen species production. The normality in the distribution of the data was previously confirmed via a Q-Q plot. Statistical significance was tested using one way ANOVA and Tukey's post hoc test.

*Results:* The incorporation of KI improved the cytocompatibility of both SF products in terms of viability, migration, morphology, apoptosis, and reactive oxygen species production. This difference was higher in the AgF group. The lowest dilutions of SF+KI and AgF+KI showed a similar cytocompatibility to that of the control group (MTT assay ($p > 0.05$ after 24, 48, and 72 h of culture); migration assay ($p > 0.05$ after 24, 48, and 72 h of culture); reactive oxygen species production ($p > 0.05$ after 72 h of culture).

*Significance:* Riva Star Aqua shows lower cytotoxicity than Riva Star on hDPSCs. It can be considered as a good alternative in the conservative treatment of dental caries and in the preservation and remineralisation of viable dentine tissue.

## 1. Introduction

Silver fluoride (SF) is a preventive and therapeutic option for dental pathological processes involving structural alterations of the hard tissues, either during their formation or those caused by caries or other pathological reasons. It is cost-effective and easily available. SDF formulation (Silver Diamine Fluoride) has been introduced as a non-invasive method as a sole alternative or in combination with other materials (Chibinski et al., 2017). The use of products with biological properties that allow maximum preservation of tooth structure and recovery of viable lost tissue, in order to preserve maximum pulp vitality, is the basis of restorative procedures in minimally invasive dentistry(Melo et al., 2022).

SDF is the most used formulation. The 38% (w/v) concentration of silver fluoride, which has proved to be the most effective, contains three main components: silver as an antimicrobial agent, fluoride to promote remineralization, and ammonia to stabilize the concentration in the solution. SDF has been proposed as a bioactive material for the management of dental caries in children(Gluzman et al., 2013), root caries in geriatric patients with chronic diseases(Zhang et al., 2013), and special

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needs patients; due to its ability to induce the remission of over 80% of active lesions(Hu et al., 2022a). SF solutions can be applied to exposed dentine to react with hydroxyapatite and generate calcium fluoride, silver phosphate, and silver precipitates. In this way, they can block dentinal tubules and act as desensitizers(Crystal and Niederman, 2019).

Recently, a new formulation of SF in an aqueous solution (AgF) has been marketed: Riva Star Aqua (SDI, Victoriar, Australia). This product is ammonia-free and has a lower concentration of silver diamine (20–30%) than his predecessor Riva Star (SDI, Victoria, Australia), which is composed of 35–40% of silver fluoride and 15–20% of ammonia (SDF); as stated by their manufacturer. Both products have shown similar efficacy in caries lesion remineralization in primary teeth (Turton et al., 2021).

After their placement, both products can stain the tooth structure, due to the oxidation of silver. For this reason, the placement of potassium iodide (KI) or saturated solution of KI (SSKI) immediately after SDF coating have been proposed to decrease the staining(Asghar et al., 2022; Lee et al., 2022; Luong et al., 2022). In their commercial form, both Riva Star and Riva Star Aqua incorporate a second bottle of KI to minimize the staining.

The effect of SDF on pulp tissue has been evaluated in in vitro and in vivo studies. Direct SDF application on vital pulp caused pulp necrosis. Indirect SDF application elicits an inflammatory response of varying intensity, even in cases were remaining dentine was 0,25 to 0,50 mm. When SDF is applied in indirect pulp capping the odontoblasts showed increased cellular activity, tertiary dentine was formed in the pulp side of the cavity, and silver ions were found to penetrate along the dentinal tubules but were not detected inside the pulp (Zaeneldin et al., 2022).

Although SDF is an alkaline solution with high amounts of silver, ammonia, and fluoride that could increase its effectiveness in arresting dental caries, concerns were raised about its undesirable effects(Shabbir et al., 2022). Previous reports have evidenced that silver ions at low concentrations such as 0.5 ppm can be toxic to undifferentiated cells and fluoride ions can be toxic to human pulp cells at 200 ppm (Greulich et al., 2012). Also, in vitro studies have shown that the alkalinity of SDF may affect dental pulp cell viability and severely decrease their proliferation, whereas in rat pulps SDF inhibits their mineralization and proliferation ability at 0.0038% (Hu et al., 2022a; Kim et al., 2021). Due to the recent introduction of the new ammonia-free silver fluoride (AgF) formulation into clinical practice, to the authors' knowledge, there are no previous studies on the biological effects on dental pulp of Riva Star Aqua.

The mechanisms behind the repair of the dentin-pulp complex requires the involvement of dental pulp stem cells (DPSCs) (Gallorini et al., 2021). DPSCs were the first to be isolated from human teeth in 2000 and remain the most common source of dental stem cells. By regulating the extracellular matrix proteins and growth factors of the microenvironment in which hDPSCs are located, hDPSCs can differentiate into odontoblasts and osteoblasts (Gronthos et al., 2000). This is why hDPSCs were used in the present study.

Within this framework, this study aimed to compare the biological properties of two commercial SF products, one of them with ammonium (SDF) and the other ammonium-free (AgF), both with or without KI, by the assessment of the cytotoxicity of the materials' eluates. The null hypothesis was that the components eluted from these materials would show no differences in its cytotoxicity.

## 2. Material and methods

### 2.1. Cell culture

This protocol to obtain human DPSCs (hDPSCs) was approved by the Ethical committee of University of Murcia (Murcia, Spain; IRB number 3686/2021). Informed consent was obtained from ten patients (18–23-years old), who provided molars from which to isolate hDPSCs and agreed to their use in this study. Patient information and images that could be used to identify the study participants are not included in this work. Dental pulp tissues were aseptically removed and immersed for incubation in 0.25% trypsin in 4 ml of EDTA (Life Technologies, USA) for 30 min at 37 °C. After neutralizing them with 4 ml of medium, the cells were detached by forcibly pipetting the solution, and then they were filtered via a strainer (70 µm, Corning, USA). The hDPSCs were cultured in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum, penicillin (100 U/ml), and streptomycin (100 µg/ml) (all from Gibco, Life Technologies) supplemented with 100 mM ascorbic acid (Sigma) in a 37 °C incubator with 5% $CO_2$ (Thermo Forma 3110, Thermo Fisher, USA). hDPSCs of passages 3–5 were used in this study and were characterized as mesenchymal stem cells on previous studies performed by our research group (Tomas-Catala et al., 2017; Tomas-Catala et al., 2018).

### 2.2. Material extracts and pH

Two commercial SF materials were evaluated in this study: Riva Star (SDF) (SDI, Victoria, Australia). And Riva Star Aqua (AgF) (SDI, Victoria, Australia). The first one possesses an ammonia-based formula, which gives it an alkaline character, and the second one with an aqueous excipient. In their clinical presentation, both products are accompanied by a second bottle whose component is KI, which is applied after treatment with SF to minimize the staining caused by SF. The material compositions, manufacturers, lot numbers, and study groups are presented in Table 1. Based on a previous study from our research group and due to the format of tested materials(Garcia-Bernal et al., 2022), which have two separate bottles (SF and KI), it was decided to analyze them separately or mixed at a 1:2 ratio, as discussed below, although the manufacturer's recommendation is to perform the application in two separate steps. Eluates of these materials were prepared following ISO 10993–5 recommendations. To prepare a 10% concentration, 1 ml of each solution was blended with 9 ml of DMEM culture medium (Gibco, Thermo Fisher Scientific, Carlsbad, CA, USA) and filtered through a 0.22-µm syringe filter. Then, eluates were diluted with DMEM culture medium to generate different dilutions (0.005%, 0.0005%, and 0.0001%). Furthermore, the pH of the different extracts was determined using a twin pH meter (GLP21 +, Crison, Barcelona, Spain). Results are represented as the mean ± standard deviation.

### 2.3. IC50 and MTT assays

Half maximal inhibitory concentration (IC50) and cytotoxicity of the tested SF and KI solutions were evaluated as previously reported by similar studies (Ahmed et al., 2011; Garcia-Bernal et al., 2022). IC50 was graphically assessed by plotting the metabolic activity percentage on the Y-axis and the concentration percentage of each SF product on the X-axis. In addition, IC50 values were analyzed by non-linear regression using GraphPad Prism software version 8.1.0 (GraphPad Software Inc, San Diego, CA, USA).

**Table 1**
Materials tested.

| Materials | Manufacturer | Composition | Lot Number |
|---|---|---|---|
| Riva Star | SDI, 3–15 Brunsdon Street, Victoria 3153, Bayswater, Australia | Step 1: Silver fluoride (35–40%), ammonia (15–20%) and water (balance)<br>Step 2: saturated potassium iodide solution | **1158162 N** |
| Riva Star Aqua | SDI, 3–15 Brunsdon Street, Victoria 3153, Bayswater, Australia | Step 1: Silver fluoride (20–30%), nitric acid (<2.5%) and water (balance)<br>Step 2: saturated potassium iodide solution | **1167689** |

For the MTT assay, $4 \times 10^3$ cells were seeded into 96-well culture plates. After 1, 2, and 3 days with the tested material eluates (the two SF solutions, KI and SFs+KI), an MTT reagent (Sigma Aldrich) was added for 4 h, following its manufacturer's instructions. When a purple precipitate was detectable, Dimethylsulfoxide (DMSO) (Sigma-Aldrich) was added to each well (100 µl/well), and the plates were covered and kept in dark conditions for 4 h to solubilize the formazan crystals produced by viable cells after reducing the MTT reagent. Finally, absorbance was measured at 570 nm wavelength using a microplate reader (Synergy H1, BioTek, Winooski, VT, USA).

### 2.4. Migration assay

The effect of the two SF solutions, KI, and SFs+KI extracts on promoting cell migration were performed via wound healing assay. Cells were seeded onto 6-well plates ($2 \times 10^5$ cells per well; n = 3 for each experimental condition) and left to proliferate until cell confluency was reached. Then, a 200-µl sterilized pipette tip was used to create a superficial scratch with adhering cells exposed to the material eluates. hDPSC migration was evaluated using an optical microscope (Olympus, Japan), and images were captured at 0, 24, and 48 h post-wounding with respective treatment groups. The percentage of the migrated area was calculated using ImageJ software (v1.48) and compared to untreated cells (control).

### 2.5. Cell cytoskeleton staining assay

Cellular morphology and cytoskeleton staining were performed on day 3 of treatment. After being washed with PBS buffer solution and fixed with 4% paraformaldehyde, phalloidin staining (ThermoFisher Scientific, Carlsbad, CA, USA) was applied to label the cytoskeleton, and 4,6-diamidino-2-phenylindole dihydrochloride (DAPI) (ThermoFisher Scientific) was used to stain the nuclei. The morphology of the cells was observed by inverted fluorescence microscopy (Nikon Eclipse Ti-U, Japan).

### 2.6. Analysis of cell apoptosis and necrosis and reactive oxygen species production

To assess the cytotoxic effect of different concentrations of the tested materials on hDPSCs, cells were treated with 0.005%, 0.0005%, or 0.0001% of the two SF, KI, or SFs+KI for 3 days at 37ºC, or with cell culture medium as control. Then, they were stained with Annexin-V and 7-AAD (BD Biosciences, San Jose, CA, United States) and analyzed by flow cytometry. A dot plot was obtained for each condition, with viable cells appearing in the bottom left quadrant (Annexin-V negative, 7-AAD negative), early apoptotic cells in the bottom right quadrant (Annexin-V positive, 7-AAD negative), and late apoptotic and/or necrotic cells in the top left and top right quadrants (Annexin V negative, 7-AAD positive, and Annexin-V positive, 7-AAD positive), respectively. Data were analyzed using FlowJo software (FlowJo, LLC, Ashland, OR, United States). For the analysis of the production of intracellular reactive oxygen species (ROS), hDPSCs were treated with the same concentrations of the materials as above, then stained with the general oxidative stress probe 5-(and-6)-chloromethyl-20,70-dichlorodihydrofluorescein diacetate (CM-H2DCFDA) (Invitrogen, Carlsbad, CA, United States) at a final concentration of 10 µM for 30 min at 37ºC and analyzed by flow cytometry. All flow cytometry conditions were analyzed in n = 3 independent assays using n = 3 hDPSC samples from different donors.

### 2.7. Statistical analysis

All the presented data result from experiments performed at least 3 times. For quantification, data were calculated as means and standard deviations (SD). The normality in the distribution of the data was previously confirmed via a Q-Q plot. Statistical significance was tested using one way ANOVA and Tukey's post hoc test using Graph-Pad Prism v8.1.0 (GraphPad Software). Each dilution was considered an independent treatment. Here, * indicates a *P*-value below 0.05, * * indicates a *P*-value below 0.01, and * ** indicates a *P*-value below 0.001.

## 3. Results

### 3.1. pH values, IC50 and MTT assay

pH values are represented in Table 2. No significant differences were evidenced in the presence of different extracts of AgF+KI and SDF+KI. The addition of KI did not result in high variations of pH.

IC50 values of the tested SFs and KI samples were the following: SDF: 0.00068%; AgF: 0.00084%; KI: 0.90%; SDF +KI: 0.0016%; and AgF+KI: 0.0044% (Fig. 1). In general, the MTT assay showed non-adequate cell viability from all concentrations of SDF and AgF at all the tested time points (24, 48, and 72 h of culture), compared to that of the control group ($p < 0.001$). In contrast, KI, exhibited similar cell viability rates than the control group. Finally, the combination of SDF+KI, and AgF+KI improved viability compared with the untreated group (control) after all time points ($p < .001$; Fig. 2).

### 3.2. Cell migration

Cell migration was tested at three-time points (24, 48, and 72 h) (Fig. 3). Wound closure was evidenced in the untreated cells (control) at 72 h. No significant differences were found in the hDPSC migration rates of KI compared to the control. Instead, a delayed cell migration was noted in SDF and AgF treated cells hDPSCs. However, in the presence of KI, the percentage of wound area was higher than SDF or AgF alone treated cells.

### 3.3. Cell cytoskeleton staining assays

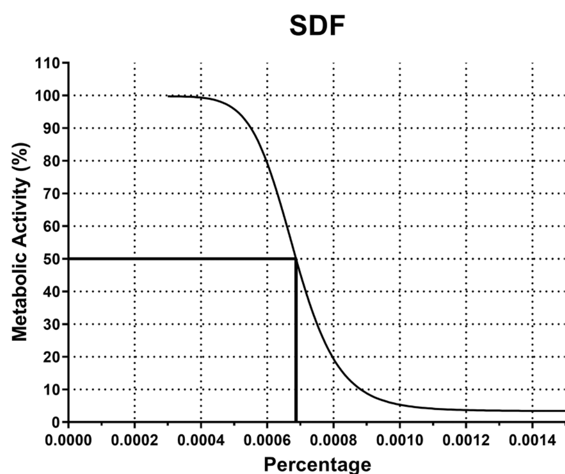
Phalloidin staining was used to stain the cell cytoskeleton in presence of the tested materials(Zhang et al., 2023). The images obtained after 72 h of culture showed a high confluence of cells in presence of KI, similar to that of the control group. On the contrary, no cells were observed in SDF or AgF groups at 0.005% and 0.0005% concentrations. In accordance with the MTT and cell migration assays, the combination of SDF or AgF with KI improved the number and morphology of cells, especially at 0.0001% (Fig. 4).

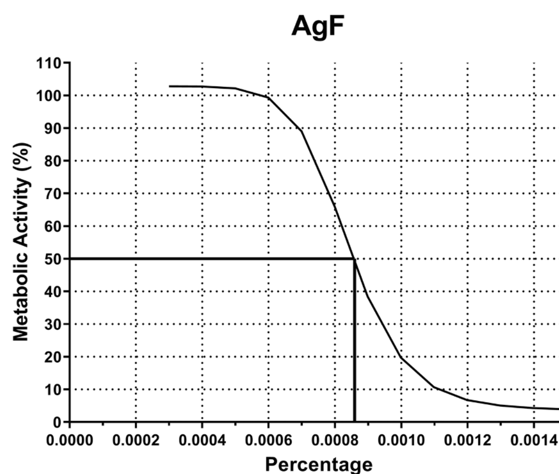
### 3.4. Apoptosis/necrosis and intracellular ROS production assays

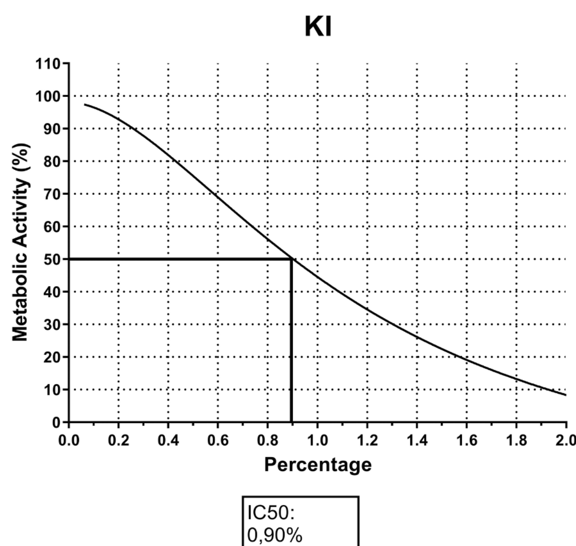
To assess if the concentrations of SDF, AgF or their combinations with KI could affect hDPSC viability, the levels of live, early apoptotic, and late apoptotic/necrotic cells were analyzed after Annexin-V and 7-AAD staining by flow cytometry. Treatment with all the concentrations of KI resulted in a percentage of viable hDPSCs similar to the control group (>93%) (Fig. 5). However, the treatment with increasing concentrations of SDF or AgF caused a significant gradual increase in

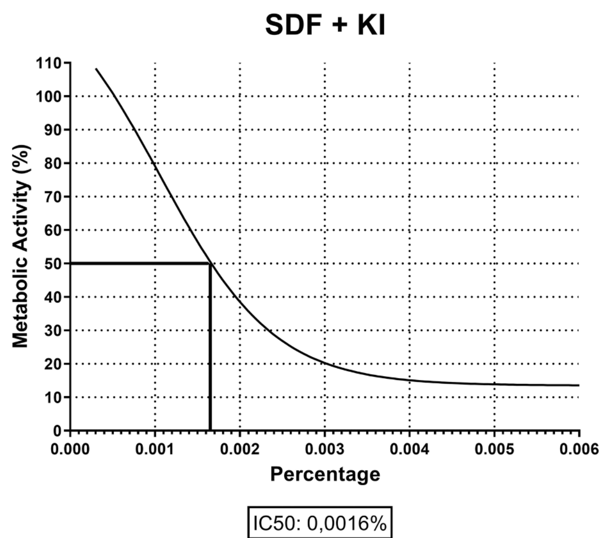
**Table 2**
Mean and standard deviation ( ± SD) of pH values measured in different extracts.

| DMEM | concentration (%) | SDF | SDF + Ki | KI | AgF | AgF + KI |
|---|---|---|---|---|---|---|
| | Non diluted | 10,5 ± 0,05 | 11,2 ± 0.04 | 8.2 ± 0.15 | 7.5 ± 0.1 | 5.33 ± 0.04 |
| 7,75 ± 0,05 | 0005 | 7,76 ± 0,03 | 7,72 ± 0,05 | 7,67 ± 0,05 | 7,72 ± 0,05 | 7,7 ± 0,05 |
| | 0,0005 | 7,66 ± 0,06 | 7,65 ± 0,05 | 7,66 ± 0,05 | 7,63 ± 0,05 | 7,66 ± 0,05 |
| | 0,0001 | 7,7 ± 0,05 | 7,72 ± 0,04 | 7,72 ± 0,05 | 7,71 ± 0,04 | 7,72 ± 0,07 |



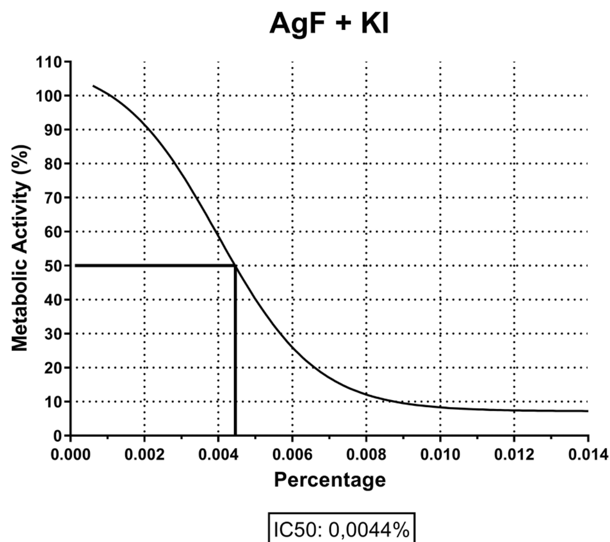




**Fig. 1.** IC50 assay results. Data are illustrated graphically by plotting the percentage of metabolic activity on the y-axis and the concentration percentage of each tested material on the x-axis.

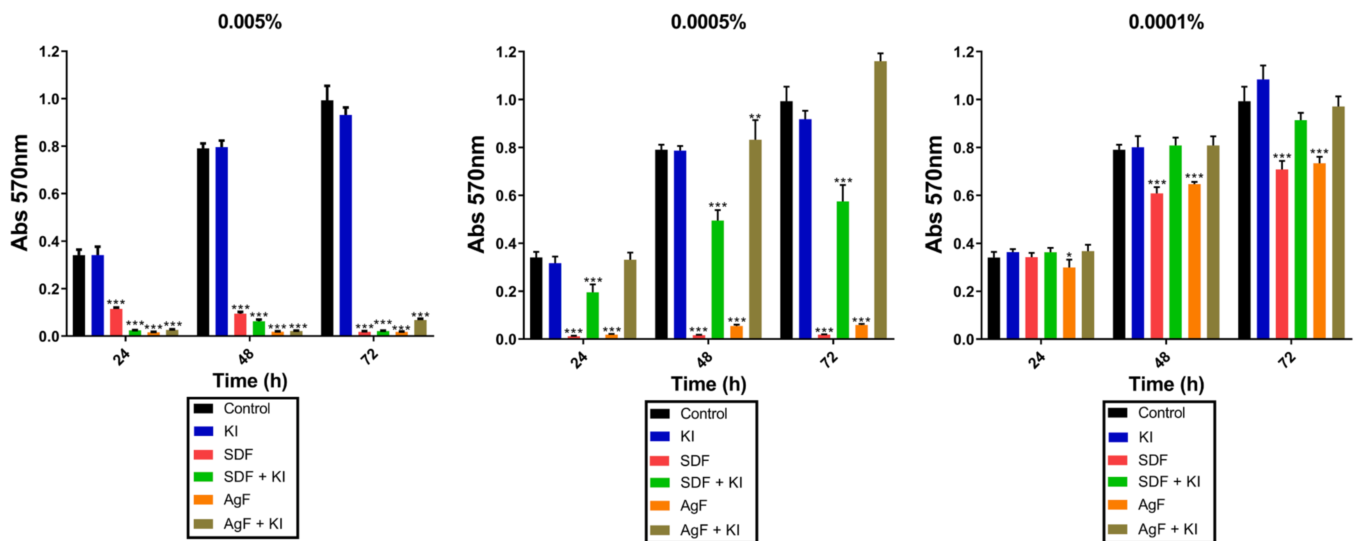



**Fig. 2.** MTT assay results after 24, 48 and 72 h of culture of hDPSCs with the tested groups or the control group. Asterisks represent significant differences with the control group: *p < 0.05; * *p < 0.01; * **p < 0.001.

**Fig. 3.** Cell migration assay results after 24, 48 and 72 h of culture of hDPSCs with the tested groups or the control group. Asterisks represent significant differences with the control group: * *p < 0.01; * **p < 0.001.

early apoptotic cell percentages. Interestingly, when SDF or AgF were combined with KI, a significant reduction of early apoptotic cells was observed up to the 0.005% concentration, mainly with AgF.

To assess the levels of intracellular reactive oxygen species (ROS) production, the percentages of hDPSCs stained with the general oxidative stress indicator CM-H2DCFDA were analyzed by flow cytometry

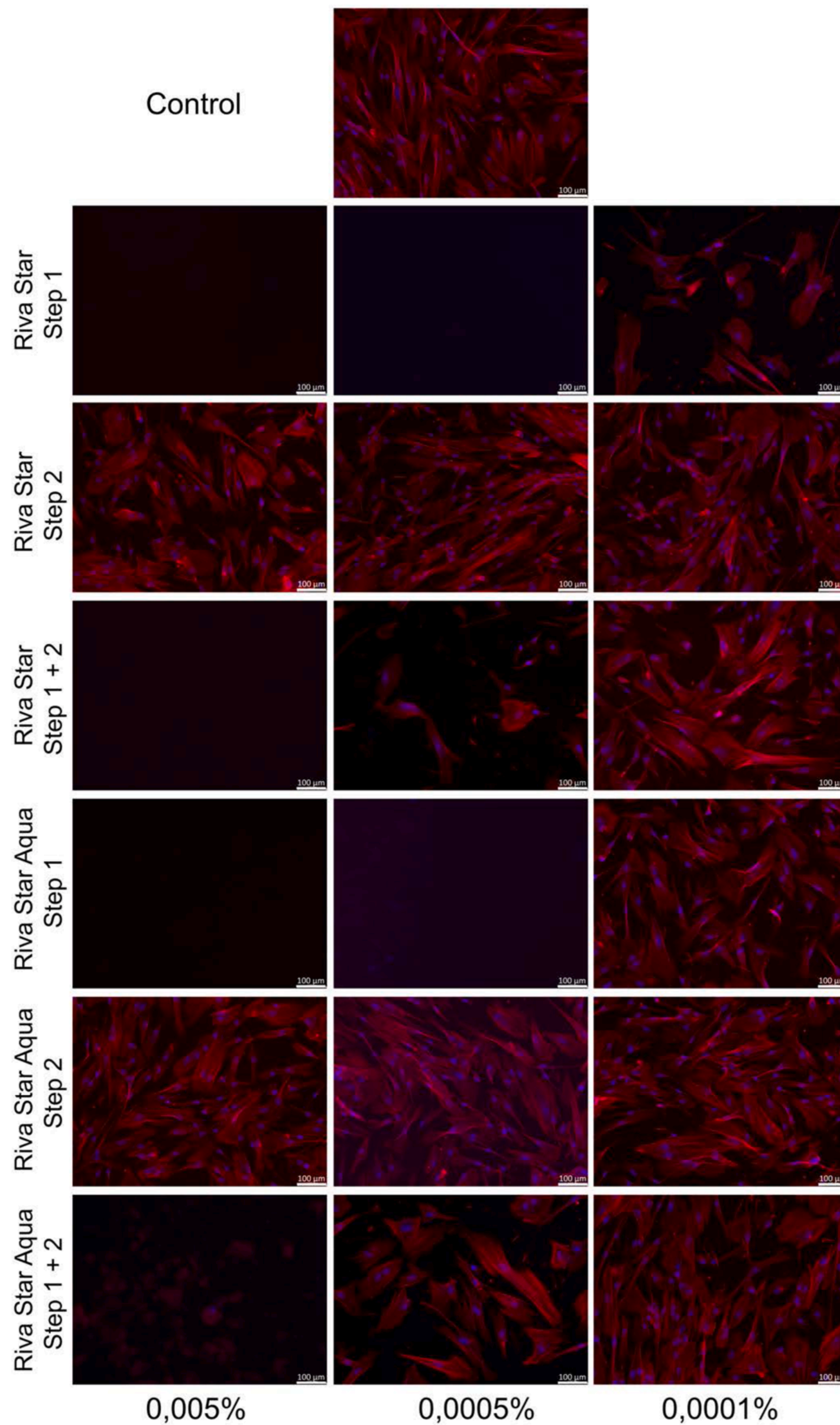



**Fig. 4.** Cell cytoskeleton staining results after 72 h of culture of hDPSCs with the tested groups or the control group.

(Fig. 6). Similar to the results obtained in the Annexin-V/7-AAD experiments, none of the concentrations of KI affected ROS production levels, while SDF or AgF compounds increased percentages of CM-H2DCFDA positive cells in a significant manner. Again, the combined treatment of SDF or AgF with KI decreased ROS production levels compared to the levels obtained with SF products alone, although still at significantly higher levels than those observed in the control cells (Fig. 6).

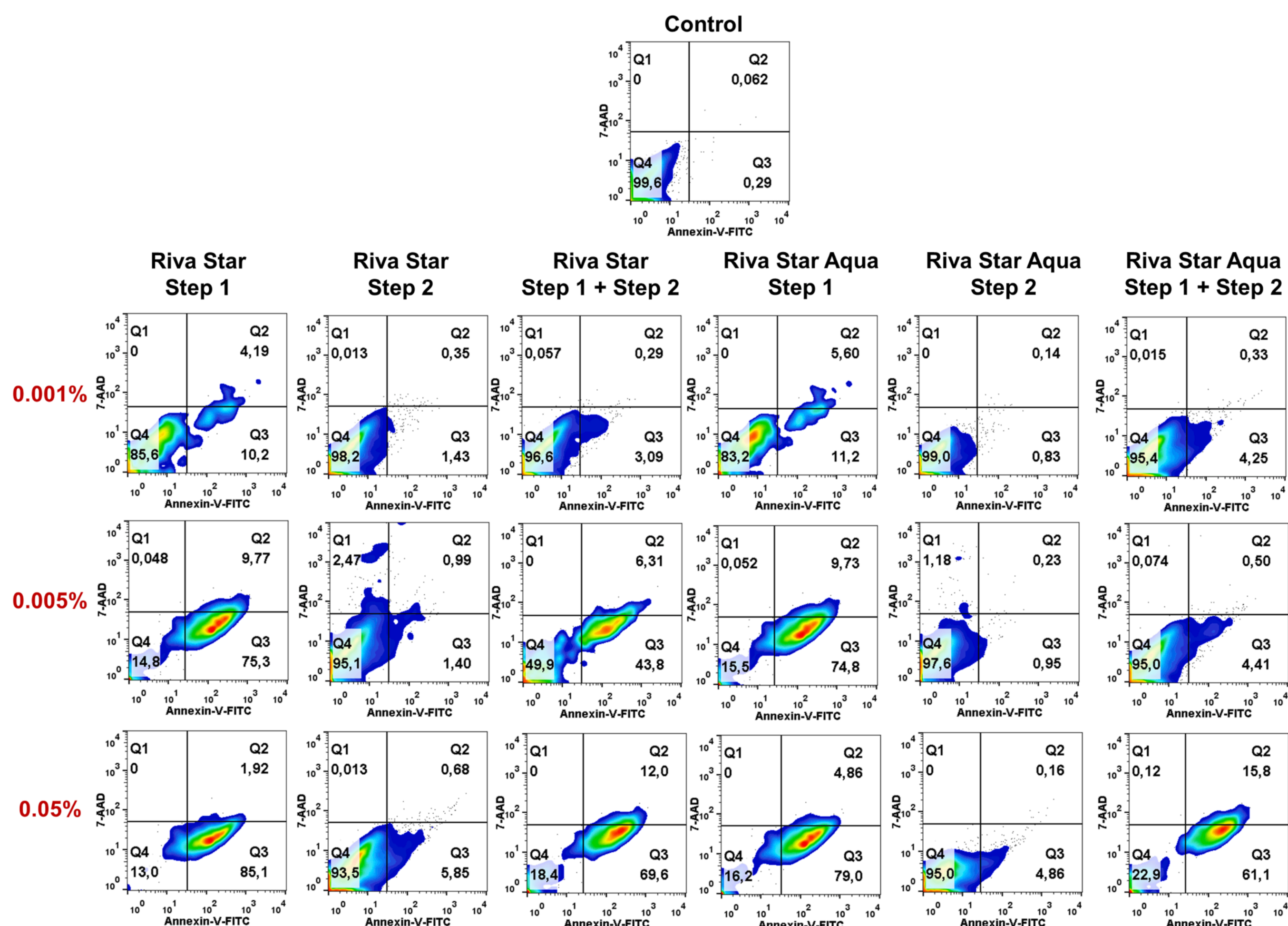



**Fig. 5.** Cell apoptosis assay results. Numbers inside dot plots represent percentages of live (Q4 quadrants), early apoptotic (Q3 quadrants), and late apoptotic and necrotic cells (Q1 and Q2 quadrants). Dot plots shown are representative from n = 3 separate experiments.

## 4. Discussion

The present study evaluated the effects of two SF products on hDPSCs. It was found that Riva Star Aqua was less cytotoxic than its predecessor Riva Star. Thus, the null hypothesis was partially rejected.

Previous studies have proposed the use of SDF as an indirect pulp treatment material because of its remineralizing and antimicrobial abilities and its biocompatibility (Seifo et al., 2020; Mubaraki et al. 2023). In fact, previous reports have proved that SDF enhanced the formation of tertiary dentin during an indirect pulp treatment(Korwar et al., 2015; Rossi et al., 2017). The undifferentiated cells (hDPSCs) in the deep pulp can move to the affected site to replace the denatured cells and differentiate into odontoblast cells. Together with the functional odontoblast cells, they are capable of secreting dentin matrix and then mineralize to form reparative dentin(Spagnuolo et al., 2023). This is also the physiological basis of hDPSCs used for tissue regeneration. hDPSCs demonstrate high proliferation, self-renewal, and multilineage differentiation potential, and have been used in the fields of tissue engineering and regenerative medicine for many years(Li et al., 2023). For this reason, we used hDPSCs as target cells.

For the assessment of biocompatibility, it was decided to carry out an MTT assay, which has been found to be useful for the assessment of cytotoxicity parameters, as already performed by Santos et al.(Santos et al., 2019) or Sauro et al.(Sauro et al., 2018). MTT assay revealed that SDF or AgF exhibited a higher cytotoxicity than KI. These results are in accordance with previous studies which evaluated the cytotoxicity of SDF(Garcia-Bernal et al., 2022; Oropeza et al., 2022). However, to our knowledge, neither the cytotoxicity of AgF nor the combination of AGF or SDF with KI has been previously evaluated. In the present study, when the mix of SDF or AgF + KI was evaluated, the cytocompatibility results improve in all experiments. This could be explained by the composition of the KI bottle which is a saturated potassium iodide solution. The solution is biocompatible, but when added to SDF or AgF, the MTT results were improved, showing a better cytocompatibility. This could suggest that when SDF or AgF products are used, the addition of KI not only improves the staining produced by SF solutions but may also have a protective effect on cells. This effect has not been previously described and more studies are needed to corroborate it. In the same line, cell migration assay showed a slightly better value in AgF-treated cells than SDI-treated cells. The lower concentration of silver diamine fluoride may explain this difference. In vivo animal studies have demonstrated that direct SDF application on pulp tissues resulted in necrosis in almost 100% of the cases. This studies also reported that the materials’ concentration is a key factor in this regard (Hosoya et al., 1990). Also, high pH and ammonia concentrations in SDF solution might irritate the pulp tissue(Luong et al., 2022). As presented in Table 1, AgF is an ammonia-free, which may be related with its biological interactions. In addition, the presence of KI didn’t evidence a substantial variation in pH values (Table 2).

In the current study, a morphological analysis by immunofluorescence was used for the initial analysis of in vitro biocompatibility. It has been reported that cytotoxic components of dental materials induce loss of cell integrity, pyknotic nuclei and alteration of cytoskeleton organization (Rohr et al., 2020). Cytoskeletal alterations, which are an

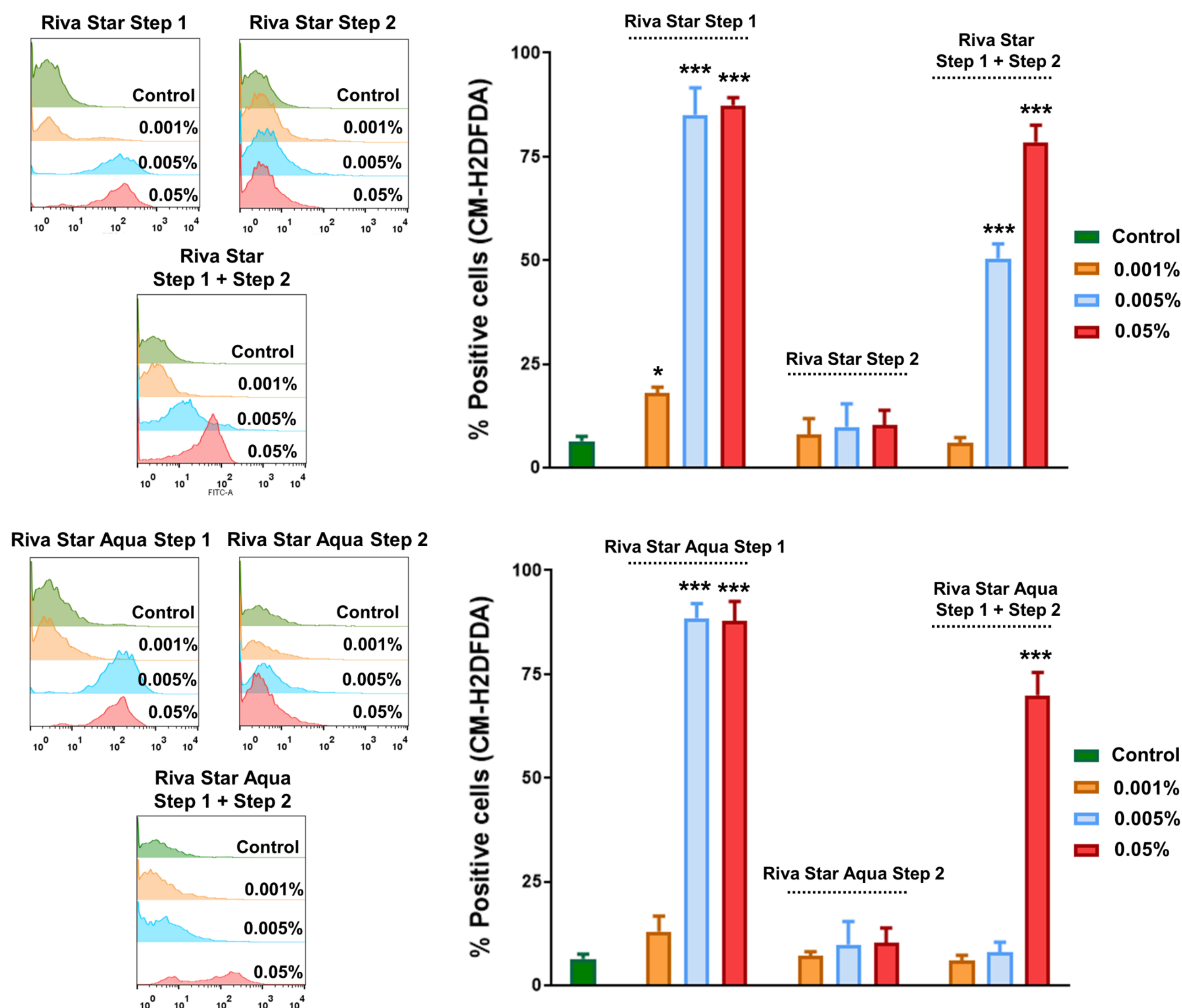



**Fig. 6.** ROS production assay results after 72 h of culture of hDPSCs with the tested groups or the control group. The histograms's y-axis represents the number of cells, whereas the x-axis represents the mean fluorescence intensity (MFI) in logarithmic scale. Asterisks represent significant differences with the control group: *p < 0.05; * **p < 0.001.

important sign of a cytotoxic response, were detected in the presence of SDF or AgF at all concentrations. This phenomenon decreased when SDF or AgF are mixed with KI, especially with AgF+KI treated cells. Again, hDPSCs treated with KI evidenced an adequate attachment and spread morphology, similar to untreated cells (control).

Apoptosis/necrosis assay showed a reduced number of viable cells in SDF or AgF groups. This phenomenon decreased with the mix with KI. The alkaline pH of the SDF solution may be related to biochemical stress, membrane deterioration, and even cell death. In fact, it has been described that the alkaline pH of some bioactive materials induces the formation of a coagulative necrosis layer, with similar effects to those of phosphoric acid when accidentally applied on gingival tissues(Hu et al., 2022a; Kruse et al., 2017). However, the underlying mechanism of the cytotoxicity of SDF is still unknown.

Another commonly used parameter to assess the cytotoxicity and genotoxicity of dental materials is the oxidative stress caused by the contact of these materials with dental pulp cells. Previous studies showed that a high level of ROS production results in telomere shortening in cells, altering its function(Hu et al., 2022b). Excessive ROS production causes DNA damage, accelerates premature senescence, and is associated with cell death(Saengnak et al., 2021). Corroborating the Apoptosis/Necrosis results, the different concentrations of SDF or AgF significantly increased the percentages of CM-H2DCFDA positive cells. Also, the combined treatment of both products with KI decreased ROS production levels compared to levels obtained without KI.

Finally, the use of low concentrations of SDF or AgF (0.005%, 0.0005%, or 0.0001%) evidenced that the application of highly concentrated solution in deep cavities could compromise the vitality of the pulp, although this phenomenon should be confirmed by clinical studies(Hu et al., 2022a).

Among the limitations of the present study, one is that SF in clinical practice is not applied together with KI, but in two separate steps. However, it was decided to introduce two groups in which both compounds (SF/KI) were mixed to standardize the conditions in direct cultures and ease its replication. This was performed because it was considered appropriate to assess the effect of KI on pulp cell viability. On the other hand, SF is also not applied directly on the exposed pulp, but on the dentine, even if it is of minimal thickness or demineralized. In a similar manner, the study of Kim et al. evaluated the viability of pulp cells with SF (Kim et al., 2021). However, the lack of studies on the action of AgF on the viability of pulp cells inspired this study. This may open a line of research in which other factors such as the thicknesses, the degree of demineralization and the source of dentine disks (deciduous or permanent teeth), as well as the age of the donors are considered. The

diffusion rate of SF and KI through the dentine is another factor to be considered in experimental studies, also taking into account the above-mentioned considerations on dentine types and thicknesses. In order to confirm the results obtained in the present in vitro study, further studies with the incorporation of immune cells, dentine barriers and in vivo or animal models are required.

## 5. Conclusions

Within the limitations of the present in vitro study, Riva Star Aqua exhibits a lower cytotoxicity than Riva Star on human dental pulp stem cells.

## Ethical approval

The protocol to obtain human dental pulp stem cells (hDPSCs) was approved by the Ethics Committee of University of Murcia (Murcia, Spain; IRB number 3686/2021).

## Informed Consent

Written informed consent was obtained from healthy donors (n = 10, 18–23-years old), who provided molars (n = 10) from which to isolate hDPSCs and agreed to their use in this study.

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## CRediT authorship contribution statement

**Rodríguez-Lozano Francisco J.:** Conceptualization, Visualization, Writing – original draft, Writing – review & editing. **Llena Carmen:** Funding acquisition, Project administration, Resources, Writing – review & editing. **García-Bernal David:** Conceptualization, Formal analysis, Methodology. **Murcia Laura:** Investigation, Methodology, Validation, Visualization, Writing – original draft. **Oñate Ricardo E:** Conceptualization, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Forner Leopoldo:** Software, Supervision, Validation. **Sanz José L.:** Investigation. **López-García Sergio:** Investigation.

## Declaration of Competing Interest

The authors declare that they have no conflict of interest.

## Data availability

Data will be made available on request.

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