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Influence of dual-cure and self-cure abutment cements for crown implants on human gingival fibroblasts biological properties

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ABSTRACT

Aims: To analyze the biological effects of the cements Relyx Unicem 2, Panavia V5, Multilink Hybrid Abutment and SoloCem on human gingival fibroblast cells (HGFs).
Materials and methods: HGFs were exposed to different eluates (n = 40) of the studied resin-based cements. Their cytotoxic effects and influence on cell migration were assessed using MTT and wound-healing assays, respectively. Level of HGF attachment, cell morphology and F-actin cytoskeleton content after exposition to the different eluates were analyzed by scanning electron microscopy (SEM) and confocal microscopy analysis, respectively. The levels of intracellular reactive oxygen species (ROS) produced by the eluates of the different cements were also determined by flow cytometry. Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey´s test.
Results: Eluates of SoloCem significantly reduces the viability of HGFs (69% reduction compared to control at 48 h). Cell migration of HGFs in presence of undiluted SoloCem eluates was significantly lower than in the control (88% open wound area at 24 h). Contrarily, migration speed with Multilynk eluates was similar to that of the control group at all periods of time and all dilutions studied. SEM analysis showed very few cells in SoloCem group, and a moderate cell growth in Multilink, Panavia and Relyx groups were detected. Finally, ROS levels detected in HGFs treated with the more concentrated SoloCem and Relyx dilutions were significantly enhanced compared with that in the control cells or the other groups (44% and 11% ROS positive cells, respectively).
Conclusions: The results obtained in the present work suggest that Multilink hybrid abutment has better biological properties and lower cytotoxicity for cementing implant crowns on abutments.

1. Introduction

Resin-based cements are commonly used by clinicians for cementing implant crowns on abutments (Welander et al., 2008). Abutments connect two pieces that join the crown with implant and represent a barrier for bacterial colonization and a support for the peri-implant soft tissue to the bone. Implant restoration with abutments is a widely used technique due to the increase in computer-aided design and computer-aided manufacturing technologies (i.e., CAD-CAM). When the crown is cemented on the abutment peri-implant soft tissues contact the cement, and since there are always remnants at the margins of the crown-abutment complex, to ensure that all the remnants have been removed is very difficult, especially when the margin is located in the subgingival area (Agar et al., 1997; Gehrke et al., 2019; Linkevicius et al., 2011; Puzio et al., 2020). In this regard, the presence of any residual cement has been described as a risk factor on previous reports, being as it can cause inflammation, bleeding, acute severe bone resorption or even implant loss (Staubli et al., 2017; Taheri et al., 2020; Weber et al., 2006).

Remarkably, cement composition and its related biocompatibility are key aspects to take into consideration since the cement, after being implanted, is in direct contact with the peri-implant area, which could affect the subsequent soft tissue seal around dental implants and crown-abutments (Arslan Malkoc et al., 2015; Kong et al., 2009; Oguz et al., 2020; Soanca et al., 2018).

The gingival tissue is an abundant and quickly source of mesenchymal stem cells (MSCs), an adult progenitor cells with self-

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renewal, clonogenicity and multi-differentiation properties that could be isolated using minimally invasive procedures (Collado-González et al., 2017; Lopez-Garcia et al., 2019a). However, many employed dental materials are in contact with a wide proportion of the total gingival tissue, and gingival-derived fibroblasts, a cell population actively involved in the gingival wound healing and tissue regeneration (López-García et al., 2021; Smith et al., 2019), also represent a well-reported source for testing biomaterials such as the resin-based cements (Fawzy El-Sayed and Dorfer, 2016; Soanca et al., 2018).

The purpose of this study was to evaluate the influence of four different cements for implant crown-abutment (Relyx Unicem 2, Panavia V5, Multilink Hybrid Abutment, and SoloCem) on human gingival fibroblasts biological properties by analyzing their cytotoxicity and effects on cell morphology, adhesion, migration and proliferation. The null hypothesis was that there would be any significant cytotoxic effects between the analyzed resin-based cements.

2. Materials and methods

2.1. Cements

The materials used in this study were two dual cure cements: Relyx Unicem 2 (RU) (3 M, Seefeld, Germany) and Panavia V5 (Kurakay Medical Inc., Sakazu, Kurashiki, Okayama, Japan); and two self-cured cements: Multilink Hybrid Abutment (IvoclarVivadent, Schaan, Liechtenstein) and SoloCem (Coltene/Whaledent, Altstaetten, Switzerland) (Table 1).

2.2. Preparation of biomaterials

Cements were prepared according to the manufacturer´s instructions in terms of working and setting times and placed in cylindrical molds of 6-mm diameter and 2-mm height (n = 40). After, Relyx and Panavia were self-cured 30 s and then light cured by Bluephase G4 (Ivoclar Vivadent) for 20 s each one at 515 nm and 1200 mW/cm^2, whereas Multilink and SoloCem were placed in the cylindrical molds until their complete setting, checking that it was not unctuous or shiny. Following, samples were disinfected by exposition under an ultraviolet light lamp for 20 min and stored in 1.25 cm^2/ml culture medium (DMEM; Gibco, Thermo Fischer Scientific, Carlsbad, CA, United States) for 24 h at 37 °C, 5% CO_2 and humid atmosphere. International Organization for Standardization (ISO) guidelines 10993-12 for biological evaluation of medical devices sample preparation and reference materials were accurately followed (Standardization, 2009). Before being used in cell cultures, the different cement eluates were filtered through a 0.22 μm syringe-filter and tested in different dilutions in the same experiments (undiluted (1:1), 1:2, 1:4), as shown in previous reports (Lopez-Garcia et al., 2019b).

2.3. Cell isolation and culture

Human gingival tissues were collected from impacted tooth after extraction (n = 10) in accordance with the Ethical Committee of the University of Murcia (UM; ID: 2199/2018). All participants provided written informed consent to participate in this study. Gingival tissues were digested with 3 mg/ml collagenase type I (Sigma-Aldrich, St. Louis, MO, United States) for 1 h at 37ºC. After, single-cell suspensions were obtained and cultured in Dulbecco Modified Eagle Medium (DMEM) (Gibco) supplemented with 10% fetal bovine serum (FBS, Gibco), 1% L-glutamine (Lonza, Basel, Switzerland),100 μg/ml penicillin/streptomycin and incubated at 37 ºC and 5% CO_2. Human gingival fibroblasts (HGFs) at passage 3 were cryopreserved in FBS containing 10% DMSO until used.

Table 1
Chemical composition and features of the biomaterials tested.

Material	Abbrev.	Type	MANUFACTURER	COMPOSITION	LOT NUMBER
Relyx Unicem 2®	RU	Dualcure	3 M, Seefeld, Germany	Methacrylated phosphoric esters, methacrylated monomer, DMA fillers, silanated fillers. 72 wt%/54 vol% fillers	5867714
Panavia V5®	PV5	Dualcure	Kuraray Medical Inc., Sakazu, Kurashiki, Okayama, Japan	A paste: Bis-GMA, TEGDMA, hydrophobic aromatic dimethacrylate, hydrophilic aliphatic dimethacrylate, silanated barium glass filler, fluoroalminosilicate glass filler, colloidal silica, accelerator, initiator B paste: Bis-GMA, hydrophobic aromatic dimethacrylate, hydrophilic aliphatic dimethacrylate, silanated barium glass filler, silanated aluminium oxide filler, accelerator, dl-camphorquinone, pigments	450101
Multilink Hybrid Abutment®	MHA	Selfcure	Ivoclar Vivadent, Schaan, Liechtenstein	Monomer matryx: dimethacrylate, HEMA Inorganic fillers (aprox 36%): barium glass, ytterbium trifluoride, spheroid mixed oxide, titanium oxide.	Y31604
SoloCem®	SC	Selfcure	Coltene/Whaledent, Altstaetten, Switzerland	UDMA, TEGDMA, 4-META, 2-HEMA, DBP; BP	J46204

2.4. Cytotoxicity assay

The MTT assay was employed to assess the cytotoxic effects of the different cement eluates on HGF up to 3 days in culture. Eluates were obtained after immersing 3 discs of each material in culture medium. In all experiments, 2.5×10^3 cells per material and dilution were added to 96 well-plates in quintuplicate. Cells cultured in DMEM medium without any eluate and incubated for the same time and temperature served as negative controls. The MTT assay is a well-reported non-radioactive colorimetric assay for measuring cell viability and cytotoxicity (Lopez-Garcia et al., 2019b). Briefly, 10 μL of MTT reagent (Thermo Fisher Scientific) at a final concentration of 1 mg/ml was added to the 200-μL growth medium/well after 24, 48 or 72 h of culture and incubated for additional 4 h at 37 °C. At the end of the incubation period, medium was removed and dimethyl sulfoxide solution (DMSO) (Sigma-Aldrich) (100 μL/well) was added and incubated for 30 min at 37 °C to dissolve formazan crystals. Finally, the optical density at 570 nm was measured in a spectrophotometric microplate reader (Synergy H1, BioTek Instruments, Winooski, VT, United States). Each experimental condition was carried out in triplicate for each material and analyzed in three independent experiments.

2.5. Cell migration assay

Scratch migration assay was used to determine HGF migratory ability in presence of the different cement eluates, as described before (Rodriguez-Lozano et al., 2017). HGFs were initially seeded at a concentration of 1×10^5 cells per well in 6-well culture plates (n = 3), and cultured to achieve confluent cell monolayers. Thereafter, a wound was made in the center of each well by scratching with a 200 μL pipette tip. Then, cell monolayers were washed twice with PBS to remove detached cells or debris. To measure the extent of cell migration, images of the wound areas were captured in an inverted microscope (Nikon, Tokyo, Japan) at 0 h, 24 h, 48 h, and 72 h in presence of the different cement eluates (1:1; 1:2 and 1:4) or DMEM culture medium without any extract (negative control). Cell migration was analyzed by measuring the wound closure areas on 6-well plates using ImageJ (National Institutes of Health, Bethesda, MD, United States). Wound closure areas were analyzed separately during three periods of time: 0–24 h (first period), 24–48 h (second period), and 48–72 h (third period).

2.6. Confocal microscopy analysis

To analyze changes in cell attachment, cell morphology and in the F-actin cytoskeleton content of HGFs confocal microscopy experiments was performed as previously described (Tomas-Catala et al., 2017). For this purpose, three discs were immersed in culture medium for 24 h. In brief, HGFs were seeded at a density of 1.0×10^4 cells/well on 24-well plates in culture medium containing undiluted eluates of the different cements. Then, cells were fixed with a solution of 4% paraformaldehyde (Sigma-Aldrich) in PBS, washed twice with PBS, and permeabilized with 0.25% Triton X-100 (Sigma-Aldrich) in PBS for 10 min. Subsequently, cells were incubated with CruzFluor594-conjugated phalloidin (Santa Cruz Biotechnology, Dallas, TX, United States) and 4,6-diamidino-2-phenylindole dihydrochloride (DAPI) (Sigma-Aldrich). Finally, cells were observed using an Axio Imager M2 Zeiss microscope (Carl Zeiss, Oberkochen, Germany). Each experimental condition was carried out in triplicate for each material and analyzed in three independent experiments.

2.7. Scanning electronic microscopy and scanning electronic microscopy/energy-dispersive X-ray analysis (SEM/EDX)

To estimate the effect of surface chemistry of the different cements on cell adhesion and growth, scanning electronic microscopic (SEM) was used. A total of 5×10^4 HGFs were directly seeded to each disk surface and cultured for 72 h. Then, specimens were post-fixed with 2.5% glutaraldehyde in PBS for 30 min at 4 °C, dehydrated, air-dried, and sputter-coated with gold/palladium. Finally, cell morphology was evaluated using 100X and 300X magnifications by SEM. For Morphological and chemical analysis (EDX), cement surfaces were analyzed using SEM (JEOL-6100 EDAX, Peabody, MA, United States) equipped with an energy-dispersive X-ray analysis (EDX; Oxford INCA 350 EDX, Abingdon, United Kingdom) and computer-controlled software (Inca Energy Version 18, Oxford INCA 350 EDX) with an acceleration voltage of 20 kV. The full scale for quantification was 8760 cts. For this assay, six discs of each material were used.

2.8. Evaluation of reactive oxygen species (ROS) production

To determine the levels of intracellular reactive oxygen species (ROS) produced by the different cements, 2×10^4 HGF/well in 6-well plates were allowed to adhere for 24 h and after incubated in 2 ml of each eluate for 72 h at 37 °C. After, cells were detached, resuspended in 1 ml of pre-warmed PBS at 37 °C and incubated with a final concentration of 5 μM of the general fluorescent oxidative stress indicator CM-H2DCFDA (Invitrogen, Molecular Probes, Eugene, CA, United States) for 30 min at 37 °C. After CM-H2DCFDA labeling, HGFs were washed twice, returned to pre-warmed complete DMEM growth medium, and analyzed in a flow cytometer (FACSCanto II™, BD Biosciences, San Jose, CA, United States). Fluorescence levels displayed by untreated CM-H2DCFDA-unloaded cells and CM-H2DCFDA-loaded cells cultured in control conditions were used as negative controls. Each experimental condition was carried out in triplicate for each material and analyzed in three independent experiments.

2.9. Statistical analysis

The data obtained were acquired using Graph-Pad Prism (version 8.1.0, GraphPad Software, San Diego, CA, United States) and statistically analyzed by one-way analysis of variance (ANOVA) followed by Tukey´s range tests. Statistical significance was considered when $p < 0.05$. All assays were assessed at least three times. Sample size analysis was calculated using the web site www.openepi.com with a confidence interval of 95% and power of 80%.

3. Results

3.1. Cytotoxicity assays

The metabolic activity of HGFs exposed to the studied cement eluates was analyzed after 24, 48 and 72 h of culture (undiluted (1:1), 1:2 and 1:4 dilutions). From 24 h onwards, all diluted eluates of Panavia significantly decreased the viability of HGFs compared to the control condition (reductions of 60% viability at 24 h, 69% at 48 h and 83% at 72 h in the 1:1 elutes compared to the control, $^{**}\ p < 0.01$, $^{***}\ p < 0.001$), with the only exception of the 1:4 dilution after 72 h of culture, which showed a comparable viability than control cells. On the other hand, Multilink eluates did not affect cell viability in the first 24 h, but caused a transient and significant reduction of cell viability at 48 h with the undiluted (10%) and 1:2 dilution (6%) ($^{**}\ p < 0.01$, $^{***}\ p < 0.001$) and that after 72 h was comparable to the control. Eluates of SoloCem significantly reduces

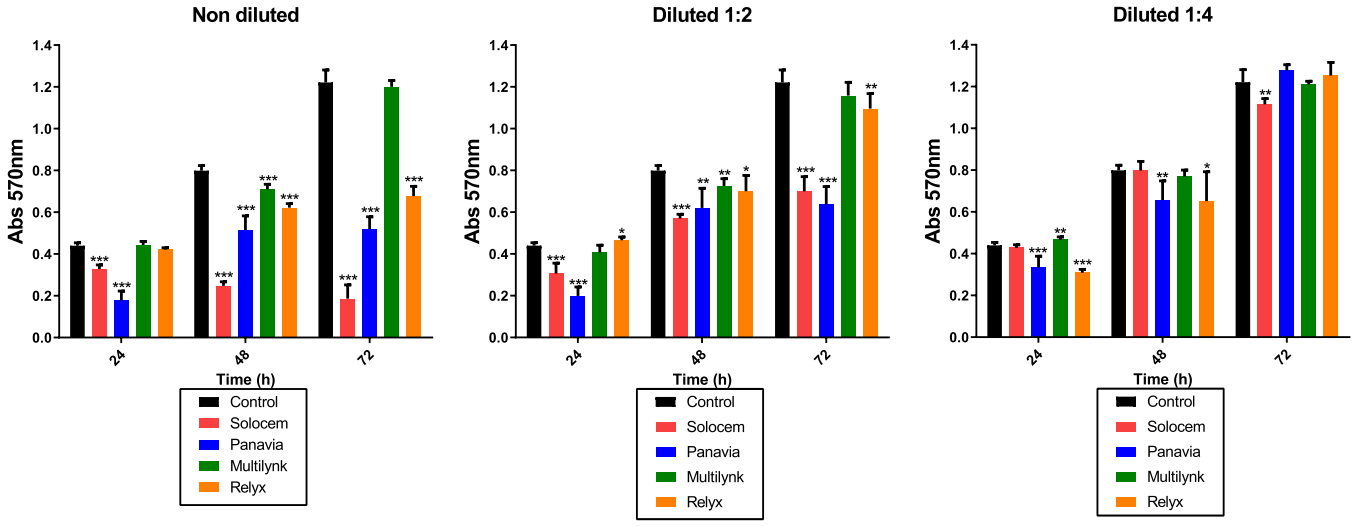

Fig. 1. Metabolic activity of HGFs exposed to cement eluates after 24, 48, and 72 h of culture (undiluted (1:1), 1:2, and 1:4 dilutions). Absorbance values were significantly different from those obtained in the control groups, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ by one-way analysis of variance followed by Tukey´s range tests. Results are represented as mean ± standard deviation and are representative from n = 3 separate experiments using n = 3 HGF samples isolated from different healthy donors.

the viability of HGFs at any time using the undiluted (22% at 24 h, 69% at 48 h) and 1:2 dilution (20% at 24 h, 33% at 48 h) (*** $p < 0.001$). However, 1:4 dilution did not affect cell viability after 24 or 48 h of culture. Finally, eluates of Relyx displayed significant differences from 48 h of culture compared to the control, mainly when using 1:1 eluates (24% at 48 h, 46% at 72 h) (*** $p < 0.001$) (Fig. 1).

3.2. Cell migration assays

To assess the effects of the different abutment cements to HGF migration, scratch migration assays were performed. As shown in Fig. 2, migration speed of HGFs in presence of undiluted SoloCem eluates was significantly lower than in the control at all studied time periods (*** $p < 0.001$), showing a 88% open wound area after 24 h of

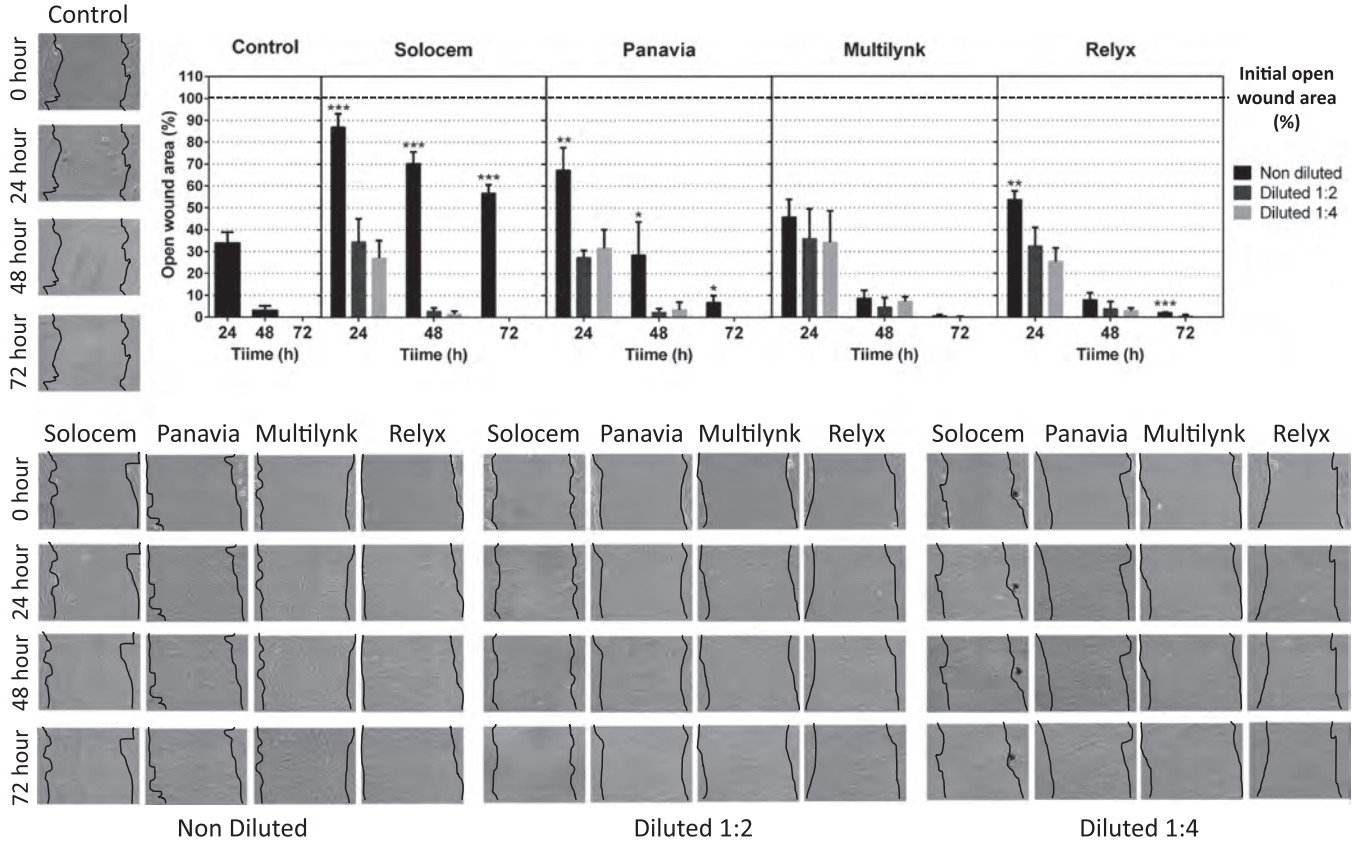

Fig. 2. Effect of different cement eluates on HGF migratory ability. HGFs were seeded and allowed to grow in culture to achieve confluent monolayers. After, a scratch was made with a pipette tip. The wound closure area was measured in the absence (control groups) or presence of the different cement eluates (1:1; 1:2 and 1:4). Cell migratory ability was determined and expressed as the open wound area percentage for each condition compared with the control,* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ by one-way analysis of variance followed by Tukey´s range tests. Results are represented as mean ± standard deviation and are representative from n = 3 separate experiments using n = 3 HGF samples isolated from different healthy donors.

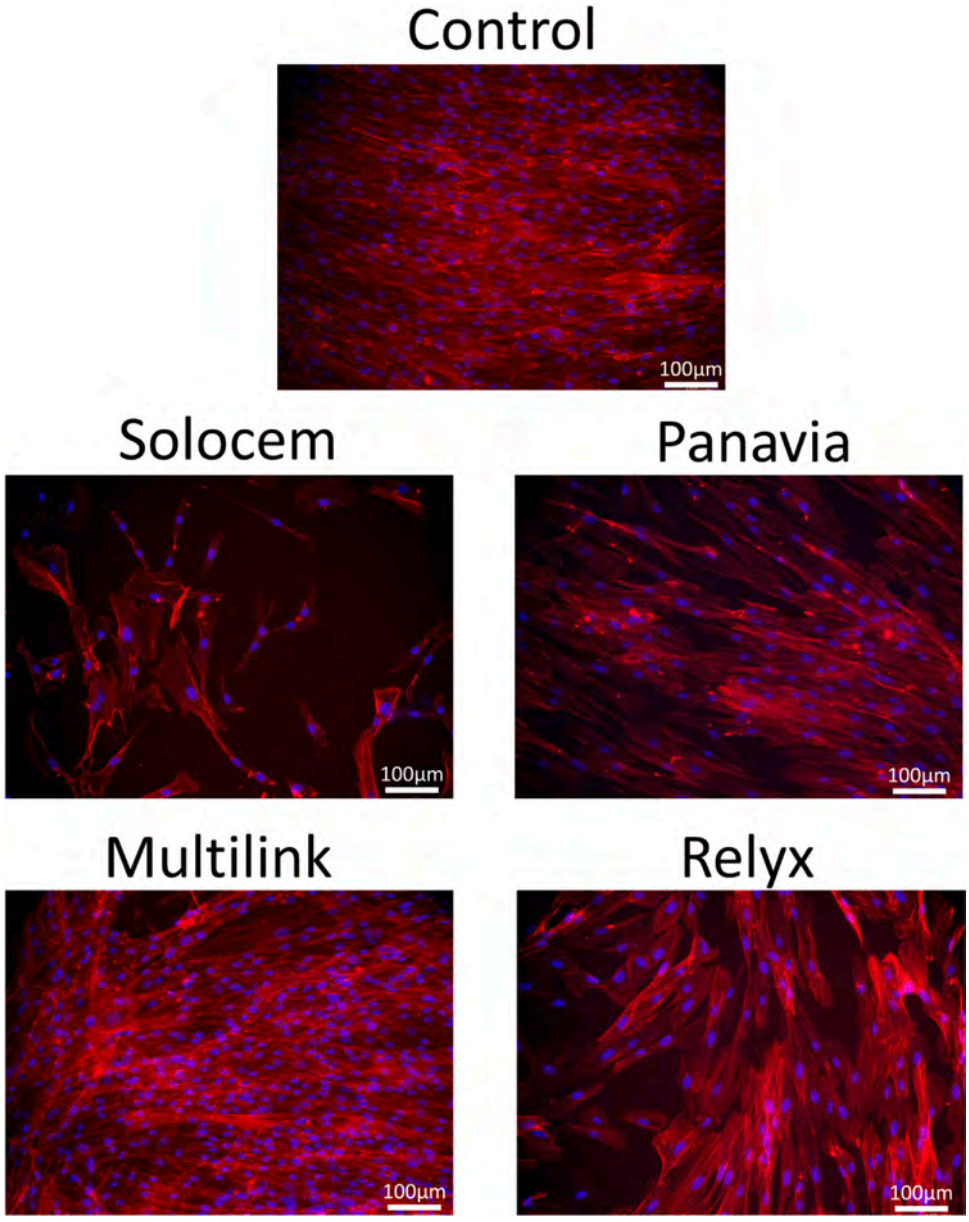

Fig. 3. Confocal fluorescence microscopy analysis was used to analyze changes in the actin cytoskeleton of HGFs cultured with different abutment cement eluates. After fixation and permeabilization, HGFs were incubated with CruzFluor594-Phalloidin (redfluorescence) to stain F-actin filaments and 4,6-diamidino-2-phenylindole dihydrochloride (DAPI) (bluefluorescence) to stain cell nuclei. Representative confocal fluorescence microscopy images of HFGs isolated from different healthy donors and cultured in presence of each cement eluate are shown. Scale bar: 100 µm.

culture. Contrarily, migration speed with Multilynk eluates was similar to that of the control group at all time periods and all dilutions, being these differences not statistically significant (Fig. 2). In the Panavia group, and only with the non diluted eluates, significant differences were found, showing a 68% open wound area at 24 h (**p < 0,01), 29% at 48% (*p < 0.05) and 9% at 72 h (*p < 0.05); meanwhile, no statistical differences were observed in the 1:2 and 1:4 dilutions when compared with the control group open wound areas. Finally, significant differences were detected in undiluted Relyx eluates in comparison to the control at 24 h, with a 54% open wound area at 24 h (** $p < 0.01$), and 4% at 72 h (*** $p < 0.001$).

3.3. Confocal analysis

HGFs cultured in presence of SoloCem eluates displayed a fibroblastic spindle-shaped morphology although with a lower cell density compared to control cells. However, HGFs in contact with the other experimental cements showed higher cell numbers and a similar well-organized F-actin filaments compared to the control group (Fig. 3).

3.4. SEM and EDX assays

The analysis of cell adherence and morphology of HGFs on the surfaces of the different cement specimens showed the presence of lower numbers of attached cells and cell debris in the SoloCem group, evidencing, although indirectly, a greater cytotoxic effect of this material. Conversely, abundant and more functionally oriented cells were evidenced in the Relyx group. Also, a moderate cell growth and elongated cells were detected in Multilink and Panavia groups (Fig. 4). Concerning the morphological appearance of surfaces, SEM showed morphological variations among the different analyzed specimens. Micrographs of Relyx and SoloCem surface cements evidenced spherical and irregular aggregates of various

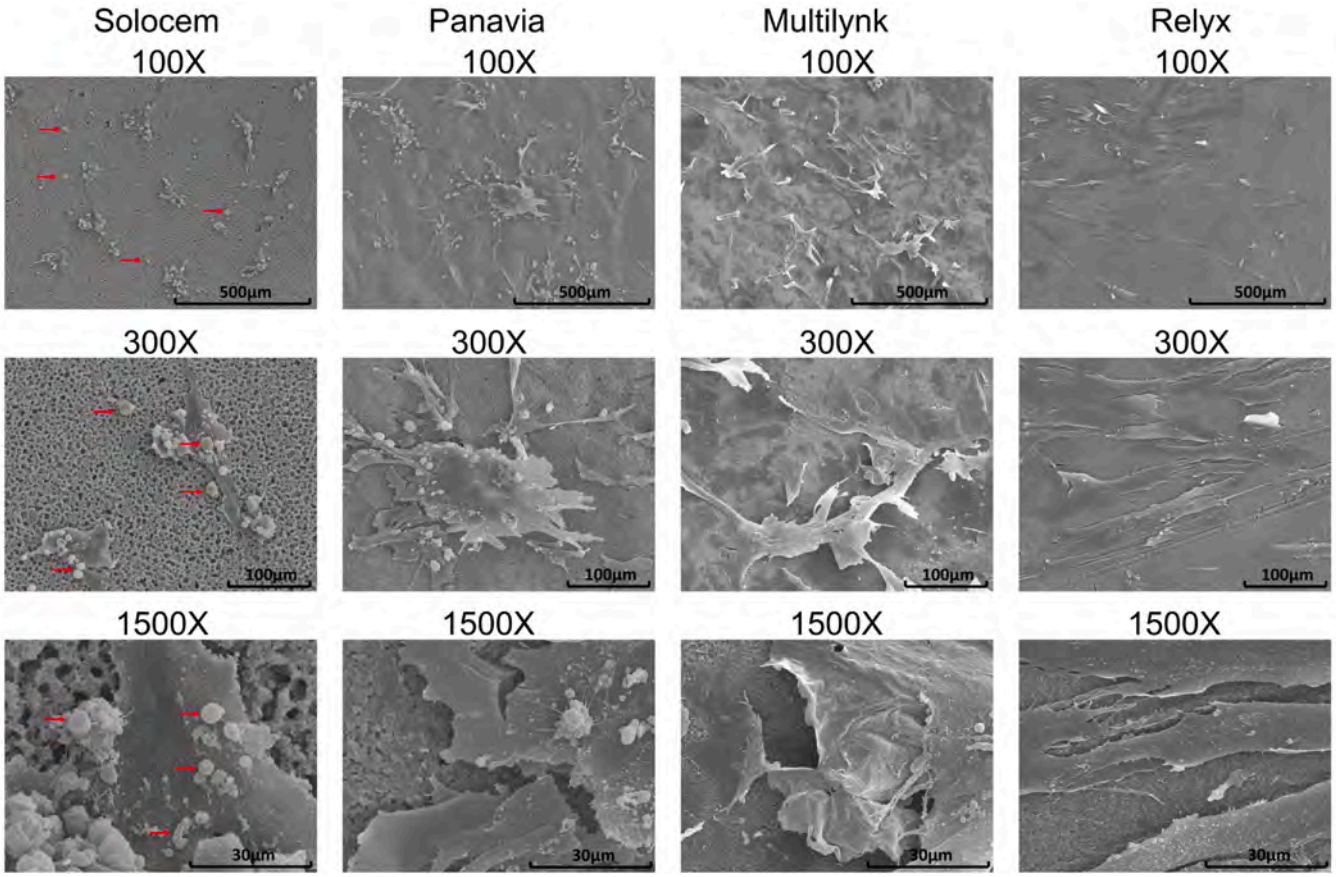

Fig. 4. Scanning electronic microscopic (SEM) analysis. HGFs were directly added to the disk surface of the different cements and cultured for 72 h. After, cell morphology was evaluated using different magnifications by SEM (100X, 300X and 1500X). Results shown are representative from n = 3 separate experiments using n = 3 HGF samples isolated from different healthy donors.

sizes. Irregular-shaped particles of various sizes could be observed with Multilink and Panavia cements (Fig. 4).

Regarding its chemical composition, the elemental analysis of Multilink showed silicon and high amounts of titanium. However, SoloCem displayed high amounts of terbium and Silicon. Relyx presented carbon, oxygen, aluminium, silicon, phosphorus, fluoride, sodium, sulfur, calcium, strontium, lanthanum, and tungsten in its composition. Finally, in the case of Panavia, the main difference in terms of composition was the presence of high amounts of barium (Fig. 5).

3.5. Analysis of reactive oxygen species (ROS) production

As shown in Fig. 6, the percentages of CM-H2DCFDA positive HGFs and mean fluorescence intensity (MFI) obtained in presence of SoloCem eluates (dilutions 1:1 and 1:2) or Relyx (dilution 1:1) were significantly enhanced compared to the control conditions ($^{***}p < 0.001$). Conversely, when HGFs were cultured with Multilynk or Panavia undiluted or diluted eluates, percentages and MFI of CM-H2DCFDA positive HGFs were not different from that observed on control cells.

4. Discussion

Most studies confirm a very high rate of peri-implantitis after dental implant therapy (Kordbacheh Changi et al., 2019), an inflammatory response which subsequently affects the osseo-integration of the implants, and that can finally cause supporting bone destruction (Schwarz et al., 2018). One of the most frequent reasons that cause this inflammation is the excess of cement used and the later inability to remove it completely from the gingival tissue. It has been previously reported that distinct oral stromal cell populations (e.g., mesenchymal stem cells and gingival fibroblast) may participate and/or potentiate the peri-implantitis incipient inflammation by a heightened secretion of cytokines, chemokines, matrix metalloproteinases and other growth factors that lead to the infiltration of inflammatory leukocytes to the tissue around the implant, resulting in a harmful self-feeding cycle (Bordin et al., 2009). Thus, it is crucial to perform an in-depth analysis of how the different chemical composition of the employed cement could influence the biological properties of different oral soft/gingival tissue-resident cells, i.e. HGFs (Ramer et al., 2014; Wadhwani et al., 2012; Wasiluk et al., 2017; Wilson, 2009).

The great variety of cements used in clinical practice, and their continuous evolution in their chemical formulations, can lead to great differences between cements of the same type as observed in our study between SoloCem and Multilink. Toxic effects of cements due to their chemical composition or ions release could provoke inflammation or induce apoptosis in gingival tissues (Oguz et al., 2020). In this regard, ROS production in response to different products/biomaterials used nowadays by dentists in the clinical practice has been tightly related with their induced cytotoxicity and cell apoptosis in some types of dental tissue-derived mesenchymal stem cells (Llena et al., 2019). ROS are chemically unstable reactive chemical species including superoxide anions, hydroxyl radicals, superoxide ions or singlets oxygen produced as natural molecules of the cell metabolism itself, but also in response to different exogenous stimuli. Thus, different cell stressors or environmental conditions can critically induce ROS accumulation, resulting in cell damage, senescence, loss of stemness, cytotoxicity and mutagenic

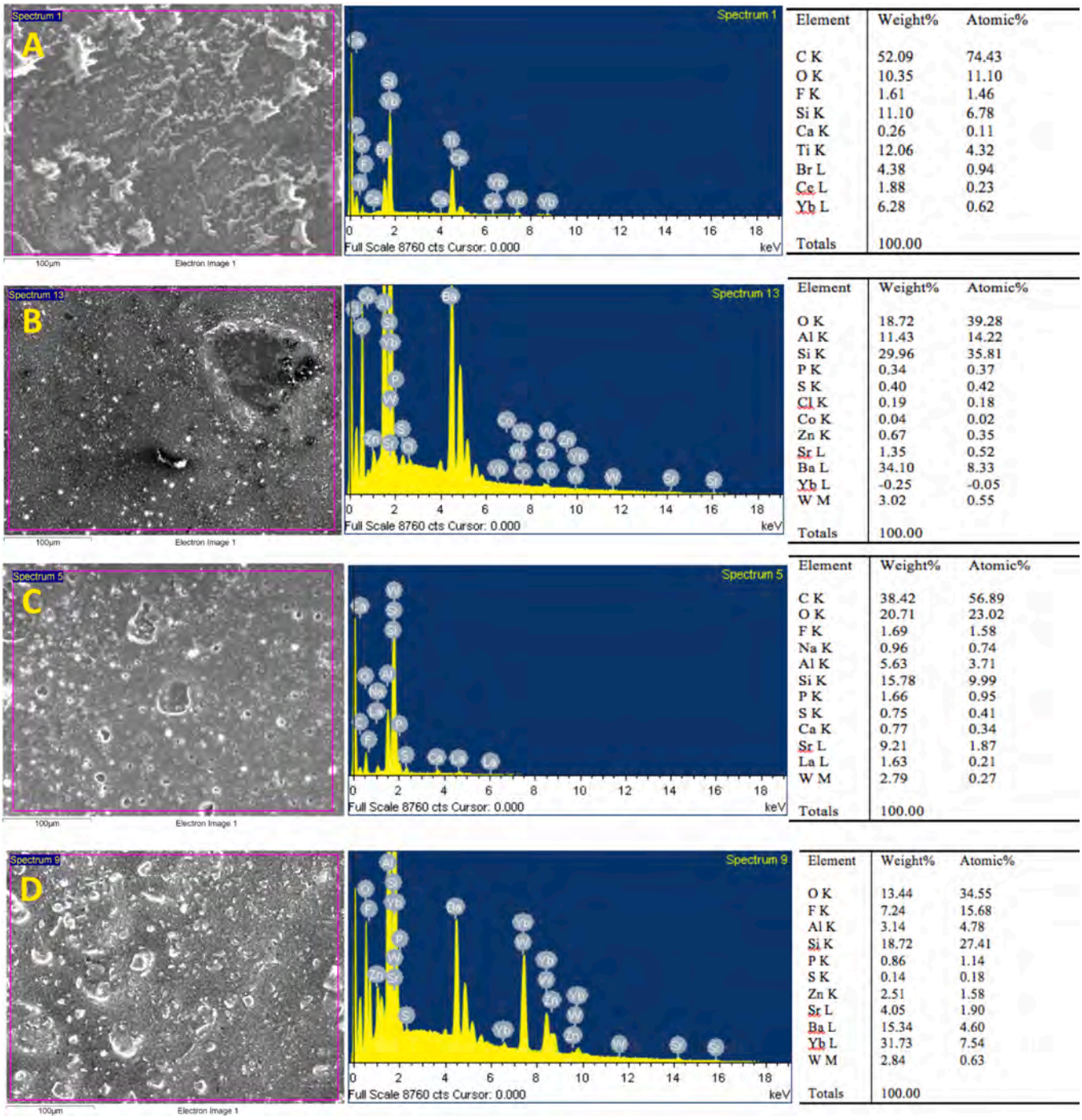

Fig. 5. Morphological and chemical analysis by EDX. Surfaces of the different abutment cements were analyzed using SEM equipped with an energy-dispersive X-ray analysis. Results shown are representative from n = 3 separate experiments using n = 3 HGF samples isolated from different healthy donors.

damage (Hou et al., 2013). In the present study, we shown that ROS levels in Relyx-treated HGFs, and mainly in SoloCem-treated HGFs, were significantly augmented compared to those levels detected in the control cells or cells treated with the other cements, a cell event that was directly related to the cytotoxicity exhibited by these biomaterials.

On the other hand, the type of curing does not appear to be a determining factor in cytotoxicity since SoloCem and Multilink are both self-curing cement and display opposite results. The colorimetric MTT assay is the most used technique for the quantitative determination of cell metabolic activity or cell viability (Kumar et al., 2018), while other authors also used this technique to evaluate cytotoxic activity of other dental cements (Marvin et al., 2019).

Cell migration assay is a test with a great clinical impact that involves a number of controlled events such as reorganization of the F-actin cytoskeleton, formation of a leading edge and assembly/disassembly of focal contacts. These cellular events are particularly important for a variety of cell processes such as angiogenesis and tissue remodeling (Leprince et al., 2012). When an implant-supported crown is placed, the gingival tissue undergoes pressure, and remodeling occurs. The objective is to create a barrier to the implant and a papilla forms with adjacent teeth (Welander et al., 2008).

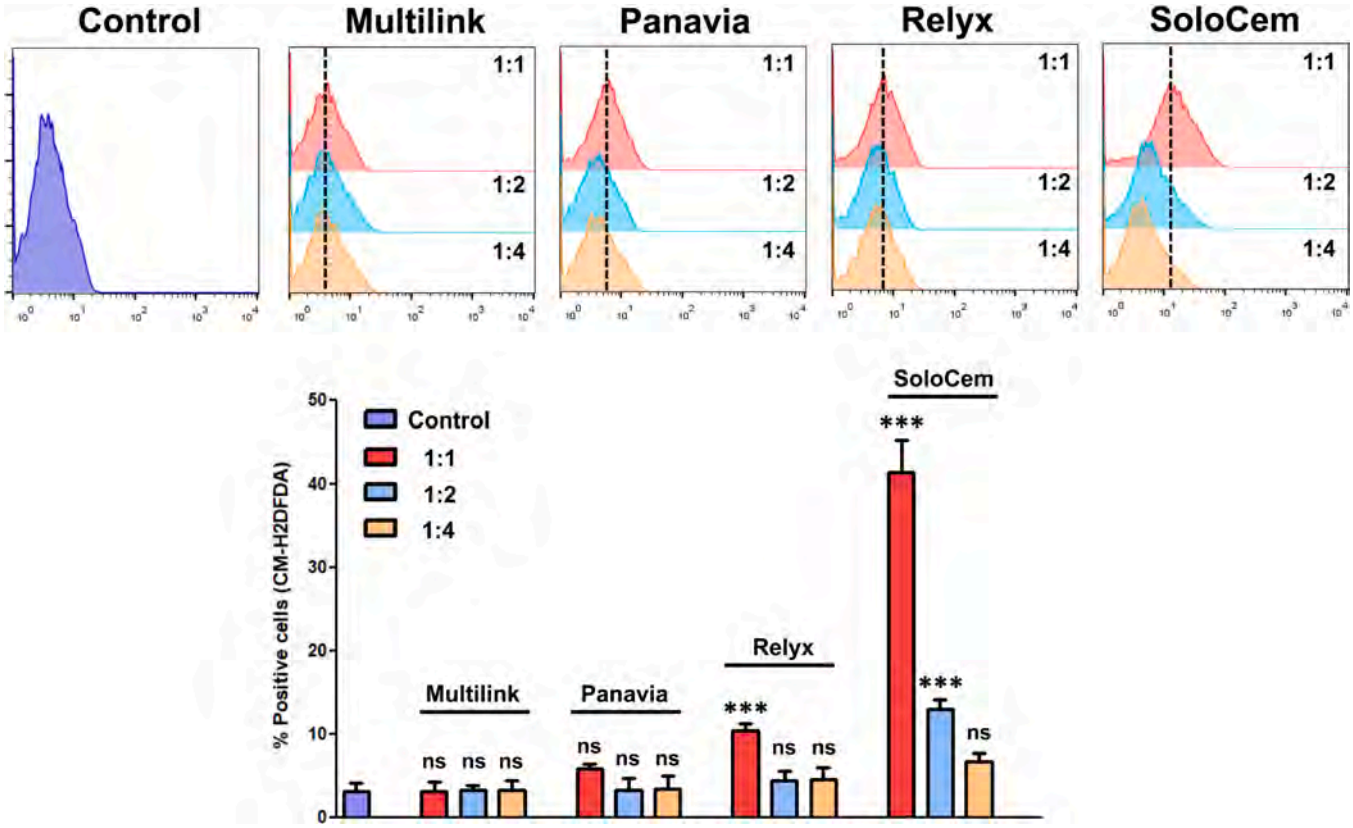

Fig. 6. Levels of intracellular reactive oxygen species (ROS) were evaluated in HGFs cultured in presence of different cement eluate dilutions by flow cytometry. Representative flow cytometry histograms and percentages of CM-H2DFDA positive cells obtained in each experimental condition from three separate analyses and n = 3 HGF samples isolated from different healthy donors are shown. Data are represented as the mean + /- standard deviation. Percentages of CM-H2DFDA positive cells increased significantly compared to the control conditions, $^{***}p < 0.001$ by one-way analysis of variance followed by Tukey´s range tests.

Negative results, such as those obtained when using the SoloCem cement, can prevent the achievement of these objectives (Cabanes-Gumbau et al., 2019; Hartlev et al., 2014; Rompen et al., 2007).

The chemical composition seems to be more important in their subsequent biological effects. Taking into account the analysis of the composition of the studied materials (Table 1), it is obvious that methacrylates are major ingredients of these materials. In line with our results, numerous studies in this field concluded that the time of contact with the cells affects their toxicity (Pagano et al., 2019). Scheinder et al. (Schneider et al., 2019) reported a study using different monomers, and showed that TEGDMA (present in SoloCem and Panavia) was less cytotoxic than Bis-GMA (present in Panavia). In agreement with these findings, others authors studied these monomers and concluded that they were cytotoxic (Chang et al., 2012; Harorli et al., 2009; Sun et al., 2018). Also, the cytotoxic effects observed, especially in SoloCem, could be related to the presence of elements such as Zn^{2+}. Previous reports have been evidenced that ZnO caused DNA damage and increased oxidative stress (Agnihotri et al., 2019). Furthermore, Zn^{2+} was detected in the early setting stage of dental materials, suggesting that the release of zinc or zinc oxides plays a role in reducing cell viability (Gong and Franca, 2017; Lee et al., 2016). However, the main limitation of this study was the scarce information about these materials.

Some studies investigated other dental resin-cements and concluded that the composition of the materials may cause different cytotoxic effects, so the formulation of the material should be adjusted to avoid these adverse effects (Marvin et al., 2019; Rohr et al., 2020; Sun et al., 2018). Finally, only one study investigated the cytotoxicity of the same dental resin-cements using other cell types. When SoloCem and Relyx were compared, SoloCem showed worse results in all the different cells tested (Diemer et al., 2021), as we shown in our study.

5. Conclusions

Among them all, Multilink has better biocompatibility than the rest of the cements studied. Conversely, SoloCem showed worse results in all parameters compared to the control group and the other cements. It would be preferable to use Multilink in areas that will be subgingival.

Ethics approval and consent to participate

Not applicable.

CRediT authorship contribution statement

SLG, JG, MPL, conceived and designed the data; DGB, MRPL, JG, acquiredthe data; DGB, FJRL, analyzed and interpreted the data; DGB, MRPL, JG, drafted the article; FJRL, SLG, JG, MRPL, MPL, critically revised and edited the study. All authors: approved the manuscript.

Declaration of Competing Interest

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

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