Comparative analysis of the biological effects of the endodontic bioactive cements MTA-Angelus, MTA Repair HP and NeoMTA Plus on human dental pulp stem cells

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Abstract

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Aim To evaluate the biological effects *in vitro* of MTA-Angelus (MTA-Ang; Angelus, Londrina, PR, Brazil), MTA Repair HP (MTA-HP; Angelus) and NeoMTA Plus (NeoMTA-P; Avalon Biomed Inc, Bradenton, FL, USA) on human dental pulp stem cells (*h*DPSCs).

Methodology Cell viability and cell migration assays were performed using eluates of each material. To evaluate cell morphology and cell attachment to the different materials, hDPSCs were directly seeded onto the material surfaces and analysed by immunocytofluorescence and scanning electron microscopy, respectively. The chemical composition of the materials was determined by energy-dispersive X-ray (EDX), and eluates were analysed by inductively coupled plasma–mass spectrometry (ICP-MS). Statistical analysis was performed with the analysis of variance and Bonferroni or Tukey post-test ($\alpha < 0.05$).

Results Undiluted MTA-Ang, MTA-HP and NeoMTA-P displayed a significant increase in cell viability greater than that obtained using complete medium alone (control) ($^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$). Moreover, a cell migration assay revealed cell migration rates after incubation with extracts of MTA-Ang, MTA-HP and NeoMTA-P that were similar to levels obtained in the control group. In addition, stretched cytoskeletal F-actin fibres were detected in the cells treated with the three material extracts. SEM studies revealed a high degree of cell proliferation and attachment on all three materials. EDX analysis demonstrated similar weight percentages of C, O and Ca in all three materials, whilst other elements such as Al, Si and S were also found.

Conclusions MTA-Ang, MTA-HP and NeoMTA-P were associated with biological effects on *h*DPSCs in terms of cell proliferation, morphology, migration and attachment.

Keywords: bioactivity, cytotoxicity, mineral trioxide aggregate, stem cells.

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Introduction

Previous reports have described the physico-mechanical (Camilleri 2008a, Cutajar *et al.* 2011), chemical

(Camilleri 2008b) and biological (Camilleri *et al.* 2013, Collado-Gonzalez *et al.* 2017) properties of MTA. However, researchers have continued to focus on developing new MTA formulations to improve its physicochemical properties, without affecting its biocompatibility or bioactivity (Yamamoto *et al.* 2016).

In this context, new endodontic materials, such as NeoMTA Plus (Avalon Biomed Inc, Bradenton, FL, USA) and MTA Repair HP (Angelus Indústria de Produtos Odotontológicos S/A, Londrína, PR, Brazil), which are based on tricalcium silicates, have been introduced. NeoMTA Plus contains tantalum oxide as radiopacifier instead of bismuth oxide, avoiding the discolouration and producing calcium hydroxide, which is necessary to induce mineralized tissue formation (Camilleri 2015). MTA Repair HP replaces the bismuth oxide radiopacifier, being incorporating calcium tungstate. In addition, MTA Repair HP has greater plasticity than its predecessor white MTA-Ang, improving its handling and insertion into the tooth. These materials are proposed for use in treating dental pulps (pulp capping, cavity lining and pulpotomies) and root canals (perforation repair, root resorption and apexification). However, it should be pointed out that during vital pulp therapy, perforation repair and retrograde filling, the cytotoxicity of the material used might influence the viability of dental or periradicular cells, resulting in cell death by apoptosis or necrosis (Samiei *et al.* 2017). Therefore, it is important to avoid dental materials that are toxic to pulpal and periodontal cells (Rodriguez-Lozano *et al.* 2017).

Dental pulp stem cells (DPSCs), which are involved in dentine regeneration, are adversely affected by the chemicals present in dental cements such as bismuth oxide, which may induce cell death or cause alterations in cell viability, involving numerous pro-inflammatory cytokines and chemokines, which increase the likelihood of irreversible pulp inflammation (Camilleri *et al.* 2004, Niu *et al.* 2015).

In vitro cytotoxicity tests are used to detect toxic effects caused by a material or its extract in cell culture, facilitating the reproducibility of test results and providing highly reliable data from standardized protocols in a rapid and inexpensive way (ISO 10993-5 2005, Rodriguez-Lozano *et al.* 2017). However, there is no information available on the biological effects of MTA Repair HP and NeoMTA Plus on the phenotype and biological properties of human dental pulp stem cells.

The aim of this study was to assess and compare the cytotoxicity of three MTA-based materials: NeoMTA Plus (NeoMTA-P), MTA Repair HP (MTA-HP) and white MTA-Angelus (MTA-Ang) in contact with human dental pulp stem cells. The null hypothesis was that there would be no significant differences in cytotoxic effects between the materials.

Materials and methods

Material extracts

The materials tested in this study were MTA-HP (Angelus Indústria de Produtos Odotontológicos S/A), NeoMTA-P (Avalon Biomed) and MTA-Ang (Angelus Indústria de Produtos Odotontológicos S/A). Their compositions are described in Table 1.

The materials were mixed according to the manufacturer's instructions. Discs of each material were formed under aseptic conditions in sterile cylindrical rubber moulds 5 mm in diameter and a 2 mm high, and sterilized using ultraviolet irradiation for 15 min before storing in an incubator at 37 °C for 48 h to achieve complete setting. The eluates of the different materials were extracted in sterile conditions, using DMEM culture medium as the extraction vehicle. For the extraction of the eluates, the materials were stored in the culture medium for 24 h at 37 °C in a humid atmosphere containing 5% CO_2. The ratio of material surface area to medium volume was set at approximately 1.5 cm^2 mL^{-1} in accordance with the guidelines of the International Organization for Standardization 10993-5. The extraction medium was filtered with a 0.22-μm filter, and several concentrations (undiluted 1/1, 1/2, 1/4) were prepared.

Assessment of pH, osmotic pressure and inductively coupled plasma–mass spectrometry (ICP-MS) of extracts

Specimens of each sealer type measuring 5 mm in diameter and 2 mm high were prepared and then immersed in Hank's balanced salt solution (HBSS) without calcium or magnesium (Gibco, Gaithersburg, MD, USA) for 24 h. The pH of the HBSS and HBSS after immersion of the materials was assessed in triplicate (GLP21+ from Crison, Barcelona, Spain). The results correspond to the mean and standard deviation.

The osmolality of HBSS and HBSS after immersion of the test sealers was determined directly in 10 μL of HBSS (Osmometer Vapro 5520, Wescor, UT, USA). The presence of silicon, phosphorus, calcium and strontium was assessed using inductively coupled

Table 1 Tested materials

Materials	Manufacturer	Composition
White MTA	Angelus, Londrina, Parana, Brazil	Powder: SiO_2, K_2O, Al_2O, Na_2O, Fe_2O, SO_3, CaO, Bi_2O_3, MgO, insoluble residues of CaO, KSO_4, $NaSO_4$ and crystalline silica Liquid: Distilled water
MTA Repair HP	Angelus, Londrina, Parana, Brazil	Powder: Tricalcium silicate (Ca_3SiO_5), dicalcium silicate (Ca_2SiO_4), tricalcium aluminate ($3CaO{\cdot}Al_2O_3$), calcium oxide (CaO) and calcium tungstate ($CaWO_4$) Liquid: Water and polymer plasticizer
NeoMTA Plus	Avalon Biomed, Bradenton, Florida, USA	Powder: Tricalcium silicate (Ca_3SiO_5), dicalcium silicate (Ca_2SiO_4) and tantalum oxide (Ta_2O_5) Liquid: Water (H_2O) and proprietary polymers

plasma–mass spectrometry (ICP-MS-Agilent 7900, Stockport, UK).

Isolation and culture of *h*DPSCs

Human DPSCs (*h*DPSCs) were obtained from impacted third molars from healthy subjects ($n = 10$). Previously, donors gave their written informed consent according to the guidelines of the Ethics Committee of our Institution (internal code: 2017-3-8-HCUVA). Dental pulp (*h*DP) was obtained from pulp chamber and root canals using a barbed broach. After extraction, *h*DP was washed with Ca^{2+}/Mg^{2+}-free Hank's balanced salt solution, and subjected to collagenase-A digestion (3 mg mL^{-1}) (Sigma-Aldrich, St. Louis, MO, USA) for 1 h at 37 °C. Then, were cells seeded in 75-cm^2 culture flasks (Corning, New York, USA) containing alpha-minimum essential (α-MEM) medium supplemented with 100 U mL^{-1} penicillin and streptomycin, and 10% foetal bovine serum (FBS) in an incubator at 37 °C and 5% CO_2. This study was carried out with *h*DPSCs from the passage 4 of culture.

Flow cytometry assay

Human DPSCs were identified following the criteria recommended by the International Society of Cellular Therapy (ISCT) to confirm the mesenchymal phenotype of the cells (Horwitz *et al.* 2005, Dominici *et al.* 2006). The immunophenotype of cultured *h*DPSCs was analysed by flow cytometry using anti-human antibodies directed to markers CD73 (clone AD2), CD90 (clone DG3), CD105 (clone 43A4E1), CD14 (clone TEUK4), CD20 (clone LT20.B4), CD34 (clone AC136) and CD45 (clone 5B1) (Human MSC Phenotyping Cocktail, Miltenyi Biotec, Bergisch Gladbach, Germany). Flow cytometry analyses were performed using a flow cytometer FACSCanto II (BD Biosciences, San José, CA, USA).

Cell viability assay

The proliferation rate of *h*DPSCs growing in the presence of the different pulp capping eluates was evaluated using the MTT assay (MTT Cell Growth Kit, Chemicon, Rosemont, IL, USA). Briefly, *h*DPSCs were plated at a density of 1×10^4 cells per well and a volume of 100 µL in 96-well plates. After, cells were starved for 24 h with serum-free medium at 37 °C in a humidified 5% CO_2 atmosphere before the tests. Then, the serum-free medium was replaced with the different material elutes. Cells cultured in α-MEM medium containing 10% FBS served as a blank control. At each time-point (24, 48 and 72 h after cell seeding), MTT was added at a final concentration equal to 1 mg mL^{-1} and the cells were incubated for 4 h. Then, the culture medium containing MTT was removed and 100 µL DMSO was added to release formazan. The absorbance at 570 nm (Abs_{570}) was measured with an automatic microplate reader (ELx800; Bio-Tek Instruments, Winooski, VT, USA) using Abs_{690} as the reference wavelength.

Cell migration

Cell migration in the presence of the material extracts was assessed using an *in vitro* scratch wound healing assay. Human DPSCs were cultured on six-well plastic dishes until they formed a monolayer. A cross area was scratched with a pipette tip, the cell debris was removed with PBS and the specimens incubated with various dilutions of the eluates up to 48 h to allow cell migration back into the wound area. To quantify and compare the cell migration rates, images of the scratched area were captured at 0, 24 and 48 h using a phase-contrast microscopy (Nikon, Tokyo, Japan). Two images were taken per well at each indicated time. The software Image J (NIH, Bethesda, MD,

USA) was used to analyse the covered and uncovered area by the cells.

Cell morphology in the presence of extracts

To investigate morphological changes, *h*DPSCs were seeded directly on glass coverslips at a low density and cultured in culture medium containing the material extracts. Then, cells were fixed with 4% paraformaldehyde in PBS and permeabilized with 0.25% Triton-X-100 (Sigma-Aldrich). For the staining of the F-actin cytoskeleton and nuclei, cells were incubated with CruzFluor594-conjugated phalloidin (Santa Cruz Biotechnology, Dallas, TX, USA) and 4,6-diamidino-2-phenylindole dihydrochloride (DAPI) (Sigma-Aldrich), respectively. Confocal microscopy (Zeiss, Oberkochen, Germany) was used for visualization of the staining.

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

Samples of NeoMTA-P, MTA-HP and MTA-Ang were shaped into 1.6-mm thick and 5-mm diameter discs using rubber moulds. Fifteen discs of each material were prepared and subdivided into three groups, each containing five parallel samples. The *h*DPSCs were directly seeded onto each disc at a density of 5×10^4 cells mL^{-1}. After 72 h of culture, samples seeded with *h*DPSCs were removed from the culture wells, and fixed with 3% glutaraldehyde in PBS for 4 h. Then, samples were dehydrated in a graded series of ethanol concentrations up to 100%, immersed in 100% hexamethyldisilazane, air-dried, mounted on aluminium stubs and sputter coated with gold/palladium. Finally, gold/palladium-coated specimens were examined by SEM using a magnification of 100×. For SEM-EDX assay, samples of the four cell-free sealers were immersed in sterilized water obtained from Milli-Q integral purification system (MQ H_2O) without calcium or magnesium in a ratio 6 cm^2 mL^{-1} and stored at 37 °C for 24 h. Discs were coated with carbon in a CC7650 SEM carbon coater unit (Quorum Technologies Ltd, Laughton, UK), and each sample was examined using an scanning electron microscope (SEM, Jeol 6100 EDAX, Peabody, MA, USA) connected to a secondary electron detector for energy-dispersive X-ray analysis (EDX; Oxford INCA 350 EDX, Abingdon, UK) using computer controlled software ((Inca Energy Version 18, Inca Oxford Instruments, Abingdon, UK) with an accelerating voltage of 20 kV. The full scale for quantification was 4908 cts.

Statistical analysis

Data from the pH, MTT and cell migration assays were analysed using SPSS version 22.0 statistical software (SPSS, Inc., Chicago, IL, USA). Each experiment was performed with five replicates and carried out at least twice. Absorbance value readings are presented as the mean ± standard deviation (SD). Statistical differences between control and proliferation in the presence of different sealer extracts were analysed. Also, statistical differences between proliferation and migration in the presence of different extracts were analysed. In both cases, analyses were assessed by one-way ANOVA and Tukey's test ($*P < 0.05$, $**P < 0.01$, $***P < 0.001$). A P value <0.05 was considered significant.

Results

pH and inductively coupled plasma–mass spectrometry (ICP-MS) – mass of pulp capping extracts

Extracts of MTA-HP exhibited a slightly higher pH than NeoMTA-P and MTA-Ang ($P > 0.05$). In all cases, the pH was lower than that for the control. The results of the ICP-MS of the same samples are shown in Table 2.

Isolation and characterization of *h*DPSCs

After isolation and culture of *h*DPSCs, cell surface marker expression was analysed by flow cytometry. As reported previously, more than 95% of viable *h*DPSCs had a positive expression of the mesenchymal markers CD73, CD90 and CD105 and lack expression of the hematopoietic markers CD14, CD20, CD34 and CD45 (Fig. 1).

MTT assay

The MTT assays results of cell viability are presented in Fig. 2. No significant differences in cell viability were observed after incubation with 1/2 and 1/4 dilutions of NeoMTA-P, MTA-HP and MTA-Ang compared to the control group at the different time-points. In contrast, after 48–72 h of incubation with

Table 2 Assessment of pH and inductively coupled plasma–mass spectrometry (ICP-MS) of cement extracts

Material	pH	Concentration of element (mg L^{-1} solution)			
		Silicon	Phosphorus	Calcium	Strontium
HBSS	7.98 ± 0.15	61.94	27 314	32.3	4.99
NeoMTA Plus	7.13 ± 0.07	24.66	0.00	38.08	2.70
MTA Repair HP	7.57 ± 0.06	3.90	0.00	3.70	0.21
White MTA	7.29 ± 0.3	22.22	0.00	4.44	0.42

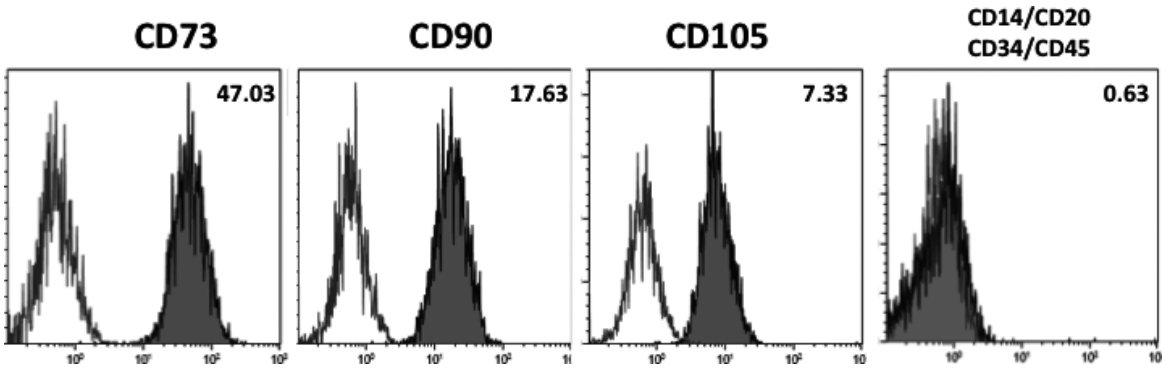


Figure 1 *h*DPSCs were stained with fluorescence-conjugated-specific antibodies for the mesenchymal markers CD73, CD90 and CD105 and the haematopoietic markers CD14, CD20, CD34 and CD45. Insert numbers represent mean fluorescence intensity values from viable cells. Figure shows representative flow cytometry histograms obtained after three independent experiments.

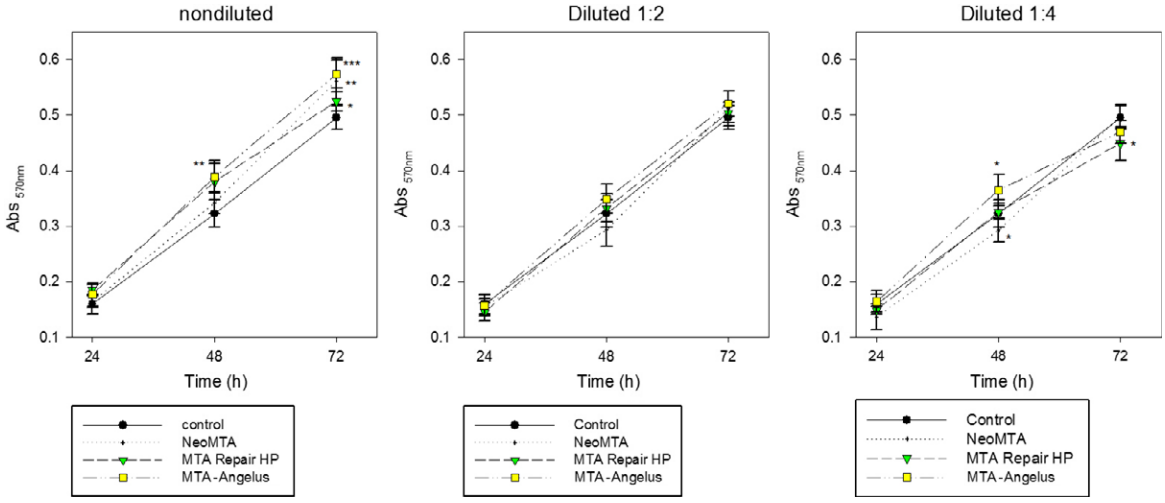


Figure 2 Cell viability was determined using the 3-(4,5-dimethyl-thiazol)-2,5-diphenyl-tetrazolium bromide (MTT) assays. After 72 h of culture, no significant differences in cell viability were observed with 1/2 and 1/4 dilutions of NeoMTA-P, MTA-HP and MTA-Ang compared with the control group. In contrast, undiluted MTA-Ang, NeoMTA-P and MTA-HP were associated with a significant increase in cell viability (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$, respectively).

undiluted MTA-Ang, NeoMTA-P and MTA-HP, *h*DPSCs underwent significant increases in cell viability compared to the control group (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$, respectively).

Cell migration

Treatment with the various dilutions of NeoMTA-P eluates and 1/4 dilution of MTA-HP for 24 h induced

a significantly lower cell migration rate than observed using complete medium (control) (*$P < 0.05$; **$P < 0.01$; Fig. 3). On the other hand, the extracts of the three materials were seen to have significantly promoted wound closure after 48 h of treatment, obtaining comparable levels of migration to those observed with the control extracts (**$P < 0.01$; **$P < 0.001$). Taken together, the results showed that NeoMTA-P, MTA-HP and MTA-Ang allowed the migration of *h*DPSCs.

Cell morphology

The cellular morphology and cytoskeletal organization were analysed by staining *h*DPSCs with phalloidin (red fluorescence) and DAPI (blue fluorescence) to visualize actin cytoskeleton and cell nuclei, respectively. In the control group, *h*DPSCs showed a gradual increase in growth over time, an extended morphology and a high content of F-actin, reaching confluence after 72 h of culture (Fig. 4a). Importantly, cells treated with undiluted extracts of NeoMTA-P (Fig. 4b), MTA-HP (Fig. 4c) and MTA-Ang (Fig. 4d) presented lower stress fibres with different degrees of cell shrinkage compared to the control group.

Cell attachment on materials and characterization of set materials

The scanning electron micrographs of the cells, in direct contact with the materials, are shown in Fig. 5. After 72 h of culture, *h*DPSCs were associated with cell attachment and merged with each other to reach subconfluence in all three materials. More cell monolayer structures were evident on the surface of MTA-Ang.

On the other hand, the EDX analysis revealed similar results for MTA-Ang, MTA-HP and NeoMTA-P in terms of percentage of weight of calcium, carbon and oxygen. Other elements found in minor amounts were silicon in MTA-Ang, aluminium and silicon in MTA-HP, and silicon and sulphide in NeoMTA-P (Fig. 6).

Discussion

The present study evaluated the biological effects of NeoMTA-P, MTA-HP and MTA-Ang on human dental pulp stem cells (*h*DPSCs). In vital pulp therapies, *h*DPSCs are involved in repairing dental pulp tissue. In fact, *h*DPSCs are multipotent stem cells with mesenchymal phenotype with the ability to migrate and differentiate into odontoblast-like cells, stimulating new dentine formation (Rodríguez-Lozano *et al.* 2011, Chen *et al.* 2016, Chen & Suh 2017).

Several tricalcium silicate-based cements have recently been used in direct pulp capping, to perform partial pulpotomies and to promote root formation in young teeth with open apices (Prati & Gandolfi 2015). In the previous studies, it was demonstrated that MTA cements are not genotoxic or cytotoxic to dental pulp cells, apical papilla cells or human mesenchymal cells (De-Deus *et al.* 2009, Bortoluzzi *et al.* 2015, Agrafioti *et al.* 2016, Peters *et al.* 2016). In the present study, *h*DPSCs were exposed to various cement extracts (undiluted, 1/2, 1/4,) in order to

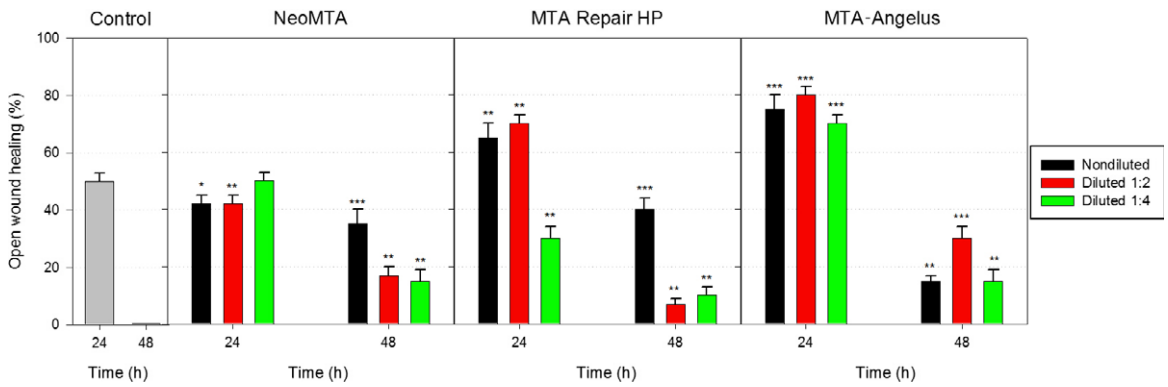


Figure 3 Migration of *h*DPSCs exposed to extracts of NeoMTA-P, MTA-HP and MTA-Ang evaluated by *in vitro* scratch wound healing assay. Confluent *h*DPSCs were wounded and incubated with different dilutions of the material extracts for up to 48 h. Cell migration is represented as the percentage of the open wound area for each condition compared with the control. (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$) analysed by one-way ANOVA.

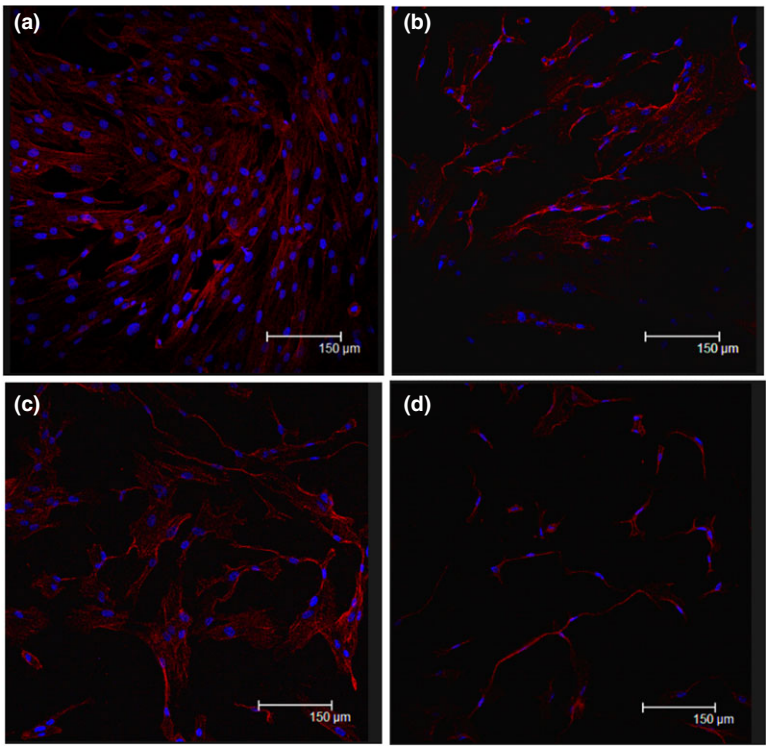


Figure 4 Representative immunofluorescence micrographs revealing cytoskeleton organization of *h*DPSCs exposed to control (a), NeoMTA-P (b), MTA-HP (c) and MTA-Ang (d) extracts. Cultured *h*DPSCs were treated with undiluted extracts of NeoMTA-P, MTA-HP and MTA-Ang for 24 h. After, cytoskeletal F-actin filaments were stained with phalloidin (red) and nuclei staining with 4,6-diamidino-2-phenylindole dihydrochloride (DAPI) (blue). Scale bar = 150 µm.

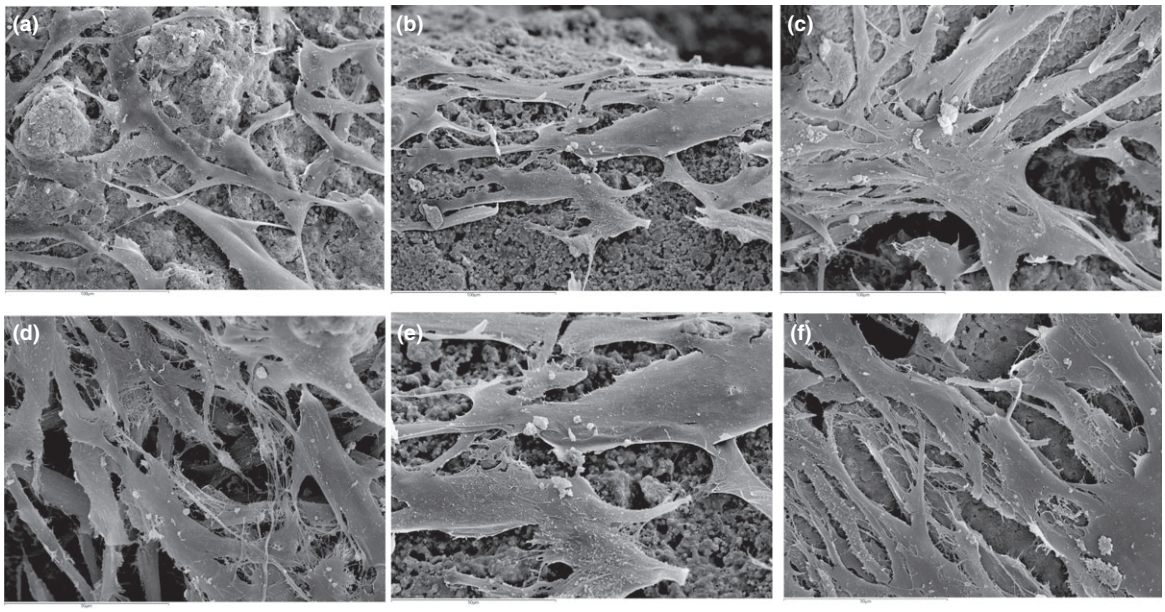
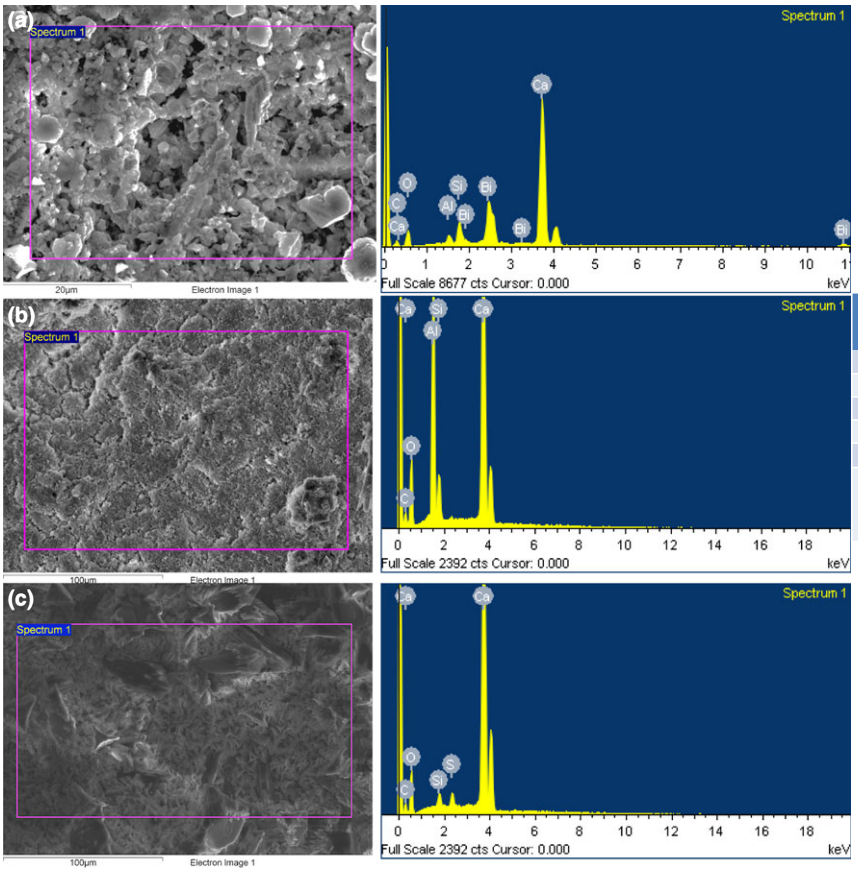


Figure 5 Cellular attachment of *h*DPSCs onto NeoMTA-P, MTA-HP and MTA-Ang specimens as observed by scanning electron microscopy. Scanning electron microscopic morphology images of *h*DPSCs attached to MTA-Ang (a, d), MTA-HP (b, e) and NeoMTA-P (c, f) surfaces were obtained at 72 h. Scale bar: 50 and 100 µm.



Element	Weight (%)	Atomic (%)
C	14.66	31.43
O	25.00	40.23
Al	0.99	0.95
Si	2.85	2.61
Ca	34.34	22.06
Bi	22.15	2.73
Totals	**100.00**	**100.00**

Element	Weight (%)	Atomic (%)
C	14.76	25.24
O	35.19	45.19
Al	13.75	10.47
Si	2.24	1.64
Ca	34.06	17.46
Totals	**100.00**	**100.00**

Element	Weight (%)	Atomic (%)
C	8.99	17.18
O	35.23	50.57
Si	0.64	0.52
S	0.95	0.68
Ca	54.19	31.05
Totals	**100.00**	**100.00**

Figure 6 Scanning electron microscope with energy-dispersive X-ray analysis (SEM-EDX) evaluating the chemical composition (spectra) and the element distribution (elemental mapping) of NeoMTA-P, MTA-HP and MTA-Ang.

simulate the clinical conditions where vital pulp therapy is performed. Cell viability and cell migration was confirmed using the MTT assay normalized to the number of cells in the sample and the wound healing assay, respectively. The MTT assay revealed that the extracts of MTA-Ang, MTA-HP and NeoMTA-P in the set form promoted the viability of *h*DPSCs. In addition, MTA-HP and NeoMTA-P promoted *h*DPSC migration, comparable with that of MTA-Ang.

Cell adhesion and migration require cytoskeletal reorganization and focal adhesion molecule expression, so the optimum state of dental pulp cells is a critical aspect that contributes to cell migration and finally to dental pulp repair (Zhang *et al.* 2015, Lv *et al.* 2017). In the present study, immunofluorescence staining revealed that undiluted extracts exhibited various morphological characteristics and cytoskeletal organization patterns when *h*DPSCs cells were treated with different materials. These findings suggest the satisfactory biocompatibility of the three different cements. This result was consistent with other previous studies, which revealed that MTA can promote dental pulp cell viability or osteoblast cells (Zhu *et al.* 2014a,b, Lv *et al.* 2017).

*h*DPSC attachment and spreading on material surfaces is another important aspect that plays a crucial role in dental pulp reparation (Ahmed *et al.* 2014). In this study, SEM analysis was used to examine the morphology of *h*DPSCs seeded on cement discs. At 72 h, a large number of flattened and spreading cells covered the surface of discs, particularly in the case of MTA-Ang. Other authors have reported similar cell degrees of attachment with MTA-Ang (Collado-Gonzalez *et al.* 2017).

Due to the composition of these materials and their possible influence on the cytotoxicity of *h*DPSCs, the chemical composition of the surface of set materials was analysed. The pH and ion release from extracts were also assessed because of the possible difference in cell response during cell interaction with extracts

compared with the surface material (Schembri-Wismayer & Camilleri 2017). When tricalcium silicate-based cements were in contact with the tissue fluids, they produced calcium silicate hydrate and calcium hydroxide (Camilleri 2015). The calcium hydroxide releases calcium ions, which are essential for cell attachment, migration, differentiation and proliferation of hard tissue-producing cells. Thus, the pH and the ion concentration of NeoMTA-P, MTA-HP and MTA-Ang extracts were tested. Depletion of phosphate in all pulp capping materials was observed. This effect is probably due to the reaction of the materials with the surrounding media (Arias-Moliz *et al.* 2017). The leaching of calcium ions was higher in NeoMTA-P than MTA-Ang and MTA-HP. This result is consistent with another study that demonstrated low early calcium ion release in MTA-Ang (Camilleri *et al.* 2015). Also, MTA-HP released minimum amounts of silicon and strontium in solution, whilst NeoMTA-P and MTA-Ang revealed a moderate level of silicon. Previous studies indicated that abundant silicon ions could be released from calcium silicate-based bioceramics upon exposure to an aqueous environment, and so significantly improve the biological performance of osteoblasts and periodontal ligament cells (Zhou *et al.* 2012, Zhu *et al.* 2014a).

The main components of MTA are tricalcium silicate, tricalcium aluminate, tricalcium oxide, silicate oxide and bismuth oxide (Khalil *et al.* 2016). The EDX results revealed that the major difference was that MTA-HP and NeoMTA-P are bismuth-free and that NeoMTA-P contains a significant amount of tantalum pentoxide rather than bismuth oxide. Tantalum has been used in sutures, plates and membranes in orthopaedics because of its inertness (Levine *et al.* 2006). The present study revealed that tantalum did not affect the biocompatibility of these cements. This result was consistent with a previous study showing that tantalum oxide promotes suitable physicochemical properties such as adequate radiopacity and calcium hydroxide production (Camilleri 2015). In addition, the presence of tantalum oxide in tricalcium cements promoted the production of mineralized nodules necessary in the pulp repair process (Tanomaru-Filho *et al.* 2017). Also, EDX analyses revealed aluminium in MTA-HP and MTA-Ang, indicating the use of a Portland type of cement, but not in NeoMTA-P. The use of tricalcium silicate rather than Portland cement as the main cementitious phase avoids the presence of trace elements that are usually found in Portland cement and MTA (Arias-Moliz *et al.* 2017). Finally, substantial amounts of calcium (Ca) were found in all three biomaterials. The release of calcium hydroxide is important not only because it plays a role in cell differentiation and dentine bridge formation but also because it is responsible for the antimicrobial activity of these tricalcium silicate-based pulp capping materials (Arias-Moliz *et al.* 2017).

Conclusions

MTA-HP, MTA-Ang and NeoMTA-P were cytocompatible and promoted adequate biological responses in *h*DPSCs in terms of cell proliferation, morphology, migration and attachment.

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Conflict of interest

The authors have stated explicitly that there is no conflict of interests in connection with this article.

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