Received: 11 June 2020 | Accepted: 22 June 2021

DOI: 10.1111/iej.13592

ORIGINAL ARTICLE

INTERNATIONAL ENDODONTIC JOURNAL | WILEY

Concentrated MTA Repair HP reduced biofilm and can cause reparative action at a distance

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Funding information
This study was supported by Conselho Nacional de Desenvolvimento Tecnológico (CNPq); Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES – grant 409196/2018-5); Fundação de Amparo do Distrito Federal (FAPDF – grant 0193.001702/2017) and Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul (FUNDECT).

Abstract

Aim: To evaluate *in vitro* whether MTA Repair HP can induce repair processes at a distance, including its effects on biofilm, cell viability, migration, production of TGF-β, phosphate and ALP, evaluated through MTA diluted extracts.

Methodology: Initially, antibacterial tests were performed with the bacterium *Streptococcus mutans* (ATCC 25175) in the presence of MTA extracts (dilutions of 1:1, 1:2 and 1:4). Growth inhibition assay by microdilution in broth, antibiofilm plate assay of young biofilm and antibiofilm assay in confocal microscopy of mature biofilm were carried out. Then, pulp cells were stimulated in the presence of several MTA dilutions, and cell viability (MTT assay), proliferation and migration capacity (scratch assay) were evaluated. To evaluate the capacity of 1:1, 1:2 and 1:4 dilutions of MTA Repair HP to promote the production of important agents of odontogenic differentiation and mineralization, ALP activity, TGF-β secretion and phosphate quantification were measured. Statistical differences were verified using one-way and two-way ANOVA and Tukey's post-tests.

Results: The test dilutions of MTA Repair HP did not inhibit planktonic *S. mutans* growth but were able to reduce young and mature *S. mutans* biofilm ($p < 0.001$). In addition, none of the MTA Repair HP dilutions was cytotoxic for pulp cells. The 1:2 and 1:4 dilutions of MTA Repair HP induced migration and proliferation of pulp cells ($p < 0.05$). ALP activity and TGF-β secretion were independent of the tested dilution

($p < 0.001$). Diluted 1:4 MTA Repair HP produced less phosphate than the more concentrated 1:1 and 1:2 MTA dilutions ($p < 0.001$).

Conclusions: Undiluted MTA Repair HP reduced *S. mutans* biofilm, when compared to 1:2 and 1:4 MTA dilutions. Furthermore, none of the tested dilutions was cytotoxic to pulp cells. MTA Repair HP promoted cell migration and proliferation at a distance, assessed through the dilution of the MTA. Even from a distance, MTA Repair HP has the ability to participate in some events related to repair, such as migration, proliferation and TGF production.

KEYWORDS

antibiofilm, bioactivity, bioceramic materials, cytotoxicity, mineral trioxide aggregate

INTRODUCTION

Mineral trioxide aggregate (MTA) is a common bioceramic material used in Dentistry, most commonly for endodontic practice (Schwendicke et al., 2016; Takita et al., 2006). MTA is a calcium silicon-based cement with tricalcium silicate, tricalcium aluminate, silicate oxide, bismuth oxide and small amounts of other oxides (Rodrigues et al., 2016). Its bioactivity is related to its properties, such as cytocompatibility, sealing and biomineralization (Camilleri et al., 2011; Gandolfi et al., 2010; Rodrigues et al., 2016; Takita et al., 2006), and is indicated as a pulp capping material for pulpotomy, crown and root perforations, root resorption, immature permanent teeth (apexification, apexogenesis and pulp revascularization therapies) and as a root-end filling material (Caliskan & Guneri, 2017; Parirokh et al., 2018; Stringhini Junior et al., 2019).

MTA contributes to tissue repair in several clinical situations. Initially, due to its high pH, when in direct contact with tissue, MTA causes a necrotic surface layer and a transient inflammatory process (Benetti et al., 2019; Cintra et al., 2017). Then, the presence of silicate and calcium induces the formation of hydroxyapatite and promotes the regeneration and remineralization of hard tissues (Asgary et al., 2006, Parirokh & Torabinejad, 2010). Calcium ions can mediate this process by modulating levels of osteopontin (OPN) and bone morphogenetic protein-2, which are responsible for inducing dentinogenesis (Niu et al., 2014). In addition, the induction of dental pulp cell proliferation and odontoblast-like cell differentiation through the secretion of morphogenetic proteins and growth factors, such as BMP-2 and transforming growth factor-beta, may lead to repair processes and dentine formation (Rodrigues et al., 2016).

However, MTA has disadvantages, including the potential for dentine discoloration, handling difficulties, long setting time, high cost and difficulty in removing it (Parirokh & Torabinejad, 2010; Park et al., 2014). Technological advances can reduce dentinal discoloration by reducing iron oxide and preventing the formation of aluminoferrite, a factor responsible for the greyish coloration of grey MTA (Darvell & Wu, 2011). White MTA still demonstrates tooth discoloration due to the bismuth oxide, used as a radiopacifier agent (Marciano et al., 2014, 2015; Valles et al., 2013). In order to reduce these disadvantages, in 2016, MTA Repair HP was developed by the Brazilian company Angelus. MTA Repair HP (Angelus) contains calcium tungstate, as a radiopacifier agent, and calcium oxide, which reduces material setting time and provides a plasticizer effect in water to improve the handling of this material (Tomás-Catalá et al., 2017). MTA Repair HP has higher levels of free calcium oxide. This contributes to increasing the alkalinity of the medium, as well as causing suppression of RANKL and reducing the process of osteoclastogenesis (Rezende et al., 2020; Tanomaru-Filho et al., 2015). These properties of MTA Repair HP can contribute to antibiofilm and repair effects.

MTA Repair HP is a new bioceramic material needing both laboratory and clinical investigations on its properties and biologic activities. Bioactive materials used for repair should enhance dentinogenesis and contribute to the repair process, such as migration, osteogenesis, differentiation and mineralization. Furthermore, so that all repair activities can take place, bacterial control can provide higher success rates in endodontics, which is a desirable property (Parirokh & Torabinejad, 2010). However, classical MTA formulations have controversial antibacterial results. A study reported antimicrobial effectivity against *Streptococcus salivarius* and *Streptococcus sanguis* determined by a diffusion method (Luczaj-Cepowicz et al., 2008). Other investigations did not demonstrate any antibacterial effect on planktonic bacteria, such as *Enterococcus faecalis* and *Actinomyces viscosus* (Pimenta et al., 2015; Shin et al., 2017). It is known that the presence of bacteria is the main reason for failure in endodontic procedures (Momoi et al.,

2012). Bacteria located in deep caries can induce severe inflammatory reactions in the pulp and even cause pulp necrosis (Abbott & Yu, 2007). Therefore, the prevention of initial bacterial infections can modify treatment plans, cause conservative treatments and improve pulp prognosis (Yang et al., 2014). Thus, *Streptococcus mutans* was chosen, since it is the major pathogen that causes human tooth caries, in order to observe the effect of MTA Repair HP antibacterial powder at the beginning of endodontic diseases. This study aims to evaluate *in vitro* whether MTA Repair HP can induce repair processes at a distance, such as antibiofilm activity, cell viability, migration and production of TGF-β, phosphate and ALP, evaluated through dilution of extracts. These results may shed light on how this recent MTA formulation may contribute to a better prognosis in endodontics.

MATERIAL AND METHODS

Primary culture of human dental pulp cells

Cells from nonerupted open-apex third molar teeth donated by patients aged 18 to 20 years were used. This project was approved by the Human Research Ethics Committee of the Universidade Católica de Brasília (CEP-UCB 2.209.368/2017). Cells were cultured using the explant technique (Jiang et al., 2008). Dental pulp tissue was removed and washed in phosphate-buffered saline (PBS). Pulp samples were sectioned with a scalpel blade and transferred to a six-well plate in DMEM medium containing 25 mmol. L^{-1} high glucose (Sigma Aldrich), 50 U. mL^{-1} penicillin (Gibco), 50 μg. mL^{-1} streptomycin (Gibco) and 20% foetal bovine serum (FBS; Gibco). Confluent cells were subcultured in DMEM medium supplemented with antibiotics and 10% FBS. Cell cultures between the third and sixth passages were used for all experiments. However, experiments that required cell differentiation, such as TGF-β, ALP and phosphate measurement, were performed between the third and fourth passages.

Preparation of MTA extracts

MTA Repair HP (Angelus) weighing 0.085 g was divided into four equal parts of 0.02125 g, using a precision balance. Next, each portion was prepared according to the manufacturer's instructions. Each portion of mixed MTA was inserted in a 24-well plate (16.2 mm in diameter and 2 mm in height) and incubated at 37°C for 30 min (Braga et al., 2014). After setting, 2.5 mL of medium was added (supplemented DMEM medium [Sigma], Brain Heart Infusion medium (BHI) [Sigma] or Todd Hewitt broth media limited in nutrients (BM2); 62 mmol. L^{-1} potassium phosphate [VETEC], 7 mmol. L^{-1} $[NH_4]$ $2SO_4$ [VETEC], 2 mmol. L^{-1} $MgSO_4$ [Sigma], 10 μmol L^{-1} $FeSO_4$ [VETEC] and 0.5% glucose [Sigma]). Plates were incubated at 37°C for 24 h according to the International Organization for Standardization (ISO) 10993-5. After 24 h of MTA contact with the medium, the supernatant from each well was united and filtered with sterile 0.22 mm filters. After this filtration, the new medium was named MTA 1:1 or undiluted MTA. From the MTA 1:1, higher dilutions of 1:2 and 1:4 were created (Braga et al., 2014; Tomás-Catalá et al., 2018). MTA extracts were analysed by matrix-assisted laser desorption ionization (Figure S1). The same method was also used to assess pH, using the following mediums: ultrapure water, DMEM medium and BM2 medium. The pH of the MTA extract was evaluated at 30 min, 1, 24, 48 and 72 h with a pH meter (Digimed). Samples containing DMEM medium and BM2 medium were used as controls.

Antimicrobial and antibiofilm assays

Antibacterial assay against planktonic bacteria

Streptococcus mutans (ATCC 25175) was first grown in BHI broth (Sigma) placed in a shaker at 220 rpm overnight at 37°C. Next, 100 μL of the pre-inoculum was added to 4900 mL of BHI medium (Sigma) and incubated at 37°C, at 220 rpm, until reaching growth log phase (0.25–0.30 nm at 595 nm). Bacteria were plated at 2×10^5 CFU per well with MTA extract (Ji et al., 2011). The following controls were used: negative control – *S. mutans* in BHI medium (Sigma); and positive control – *S. mutans* in BHI medium (Sigma) added to 30 μg. mL^{-1} chloramphenicol (Kim et al., 2015). Experiments were performed in 96-well culture plates (TPP, Trasadingen, Switzerland) incubated for 18 h at 37°C under medium shaking in a microplate reader (Bio-Tek PowerWave HT) at 595 nm. The minimal inhibitory concentration was determined according to no growth compared to controls.

Young antibiofilm activity

Antibiofilm assays were performed with a pre-inoculum of *S. mutans* on BHI medium (Sigma) placed in a shaker at 220 rpm 37°C overnight. Bacterial suspension was diluted in BM2 minimal medium (Reffuveille et al., 2014) to 1/100 v/v per well and cultivated in a 96-well plate with U-bottom at 37°C, for 24 h. Preformed biofilm

was exposed to MTA Repair HP (Angelus) extracts (1:1, 1:2 and 1:4 dilutions). Chloramphenicol (30 μg. mL^{-1}) was also used as a negative control (NCCLS, 2003) and *S. mutans* bacterium (1/100 v/v) in BM2 medium as a positive control. Subsequently, to evaluate the biofilm viability, 100 μL of BM2 medium and 10 μL 3-(4,5-di methylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma) (500 μg per well) were added to each well, and the microplates incubated for 4 h, at 37°C in the absence of light (Mosmann, 1983). After the complete solubilization of the cell product, the absorbance was read at 570 nm.

Mature antibiofilm activity

Ten third molars with caries-free crowns were used. The crowns were cut in the transverse direction with a diamond disc at the limit of the pulp horn. Discs of 1 mm thickness and 4 mm in diameter were used. A disc of each tooth was obtained, and all adjacent enamel was removed. Discs were treated with 0.5 mol L^{-1} EDTA for 60 s for both sides and then washed with distilled water (Dos Santos et al., 2019). Subsequently, the discs were individually wrapped and sterilized in an autoclave (saturated steam under pressure).

After that, the discs were inserted into the bottom of a 24-well plate. Sequentially, bacterial suspensions of *S. mutans* (ATCC 25175) were diluted in BHI medium to 10/990 v/v per well and cultivated under the disc at 37°C for 7 days. After the time elapsed, the dentine disc and biofilm were removed from the well and gently washed with PBS and inserted in a new 24-well plate. The biofilm was then exposed to the MTA extract 1:1, 1:2 and 1:4 for 24 h. *S. mutans* in BHI medium was the positive control. After 24 h, the disc was washed in PBS twice to remove culture medium and non-adherent cells. Then, the surface of the disc was stained with 50 μL live/dead BacLight Bacterial Viability Kit (Thermo Fisher Scientific). SYTO 9 is a green fluorescent stain, labelling both live and dead microorganisms; propidium iodide is a red fluorescent nucleic acid stain and penetrates only the cells with damaged membranes (dead microorganisms). Thus, the adherent cells were incubated at room temperature for 10 min, and then they were rinsed with PBS and observed using an inverted confocal laser scanning microscope (Leica TCS-SPE; Leica Biosystems CMS). Five images were made of each sample with a 40× oil lens. Each image was representative of a 275 × 275 μm^2 field. Images were then transferred to the Imaris 7.2 software (Bitplane Inc.). The biofilm analysis tool was used to evaluate the five fields of each sample. The test was performed in two individual replicates, conducted on different days. The results for each group generated a unique average, representative of 10 fields in each sample. The variable presented was green biovolume, representing the volume occupied by living cells (Arias-Moliz et al., 2016).

Cell viability assay

Cell viability was determined according to MTT assay (Sigma). Pulp cells were grown in 96-well plates (1×10^4 cells per well) and, after 24 h, the MTA Repair HP extracts were inserted. Plates were incubated at 37°C and 5% CO_2 for 24 and 72 h. The culture medium was removed, and then, 100 μL DMEM supplemented with 100 U. mL^{-1} penicillin (Invitrogen), 100 μg. mL^{-1} streptomycin (Invitrogen), 10% FBS and 10 μL MTT (5 mg. mL^{-1}; Sigma) was added. Cells and MTT solution were incubated at 37°C and 5% CO_2 for 4 h. The absorbance of dissolved MTT formazan crystals was read at 570 nm. Data obtained for each group were normalized based on cultures containing only cells and culture medium (de Souza Costa et al., 2014).

Cell migration assay

Pulp cells (2.5×10^5 cells per well) were seeded in six-well culture plates (Prolab, Brazil) in DMEM medium (Gibco), supplemented with 100 U. mL^{-1} penicillin (Invitrogen) and 100 μg. mL^{-1} streptomycin (Invitrogen) and 10% FBS. Cultures were maintained until the formation of a confluent monolayer. Next, an artificial wound was reproduced on well surfaces with a plastic micropipette tip. Remaining cells were washed three times, and MTA Repair HP extracts were added to cultures with supplemented DMEM with 50 U. mL^{-1} penicillin and 50 μg. mL^{-1} FBS-free streptomycin. Cultures were incubated and monitored for up to 48 h. Microscopy photographs were taken at 0, 24 and 48 h for analysis using Image J software (National Institutes of Health; Kajiya et al., 2010).

Pulp cell proliferation

Pulp cells (1×10^4 cell/well) were cultured in 96-well plates for 24 h. Then, the culture was exposed to the MTA Repair HP extract (dilutions 1:1, 1:2 and 1:4). Cell cultures were incubated at 37°C and 5% CO_2 for 24 and 48 h. Cell proliferation was evaluated using Trypan Blue stain (Sigma) under a microscope at 40× magnification, after cells were recovered by trypsin (0.025%) and EDTA (0.01%; Crowley et al., 2016).

Odontogenic differentiation

Pulp cells, between the third and fourth passage, were plated in triplicate in 24-well plates, at a density of 5×10^4 cell/well. After reaching cellular confluence, cells were treated with supplemented DMEM medium (Sigma), containing osteogenic inducers: 100 Nm dexamethasone (Sigma-Aldrich), 10 mM 2-β-glycerol-phosphate (Sigma-Aldrich) and 50 μmol L^{-1} ascorbic acid (Sigma-Aldrich), for 14 days (Moraes et al., 2016). Odontogenic differentiation was assessed by alkaline phosphatase activity and TGF-β cytokine secretion – enzyme-linked immunosorbent assay (ELISA) and phosphate quantification.

Alkaline phosphatase activity

Alkaline phosphatase enzyme (ALP) activity was measured by the colorimetric method of p-nitrophenyl phosphate (pNPP) using the Alkaline Phosphatase Diethanolamine Activity Kit (Sigma). After 14 days of cell contact with the osteogenic medium, diluted MTA Repair HP extract (1:1, 1:2 and 1:4), cells were washed twice with PBS and incubated in 0.05% Triton X-100 for 20 min at room temperature, with shaking. Cells were transferred to a 1.5 mL tube, vortexed for 20 s, centrifuged for 15 min at 4°C and 2500 rpm and kept on ice for 20 min. Aliquots of cell lysate were incubated with pNPP as the substrate at 37°C for 60 min. The reaction was stopped by adding 5 μL 1 N NaOH and absorbance was measured at 405 nm using the spectrophotometer (SpectraMax M2, Molecular Devices). Normalization of alkaline phosphatase activity was held by total protein assessed by Qubit method (Moraes et al., 2016).

TGF-β cytokine secretion – ELISA

After 14 days, supernatants were used for cytokine TGF-β quantification by the enzyme-linked immunosorbent assay using TGF-β Human Kit (R&D Systems) according to the manufacturer's instructions. Results were expressed in pg. mL^{-1}.

Phosphate quantification

The determination of the phosphate concentration was verified from the supernatant of the pulp cell cultures after 14 experimental days under osteogenic conditions. The test was performed using the Phosphate Colorimetric Assay Kit (Sigma-Aldrich), according to the manufacturer's instructions. Phosphate levels were expressed in millimolar (mmol. L^{-1}) after comparison with the standard curve proposed by the kit.

Statistical analysis

All experiments were carried out in three technical replicates and repeated at three independent moments. According to statistical analysis, normal distribution was verified by Shapiro–Wilk test. Data were described as mean and standard deviation (SD). Statistical analysis of the data was performed by using one-way analysis and two-way analysis, when necessary, variance and Tukey's multiple-comparison post-test were also used, with significance of $p < 0.05$.

RESULTS

MTA repair HP antimicrobial activity against *S. mutans*

MTA Repair HP extract was not capable of inhibiting *S. mutans* (ATCC 25175) growth under planktonic conditions in 1:1, 1:2 and 1:4 dilutions in microdilution assays (data not shown). Only 7.6% of the *S. mutans* biofilm performed in U-bottom 96-well plates for 24 h was viable. However, dilutions of MTA extract (1:2 and 1:4) left 60% and 61% of viable preformed biofilms respectively. There was a significant difference between diluted MTA Repair HP (1:1, 1:2 and 1:4) with the control, represented by a culture of *S. mutans* ($p < 0.001$). It was also possible to observe a significant difference between diluted MTA (1:2 and 1:4) and MTA (1:1; $p < 0.001$; Figure 1a).

In order to reproduce biofilm growth on dentine surfaces, the antibiofilm capacity of MTA was also evaluated on mature biofilm grown on dentine discs for 7 days. More significant biofilm reduction was noted when mature biofilm was exposed to undiluted MTA ($p < 0.001$). Note that when using MTA 1:1, 39% of the *S. mutans* biofilm was still viable. An increase in viable cells to 58% was observed when in the presence of diluted MTA 1:2. This increased to 87% of viable biofilm when using MTA 1:4 in mature *S. mutans* biofilm. Thus, the lower the MTA dilution in the extract, the more effective it was against *S. mutans* biofilm (Figure 1b,c).

MTA repair HP toxicity and proliferative and migratory activity on pulp cells

MTA Repair HP extract did not interfere in the viability of pulp cells even in high concentrations after 24 and 72 h (Figure 2), demonstrating no toxicity. Therefore, its effects on cell migration were evaluated. It was observed that pulp cell migration was inversely proportional to the MTA

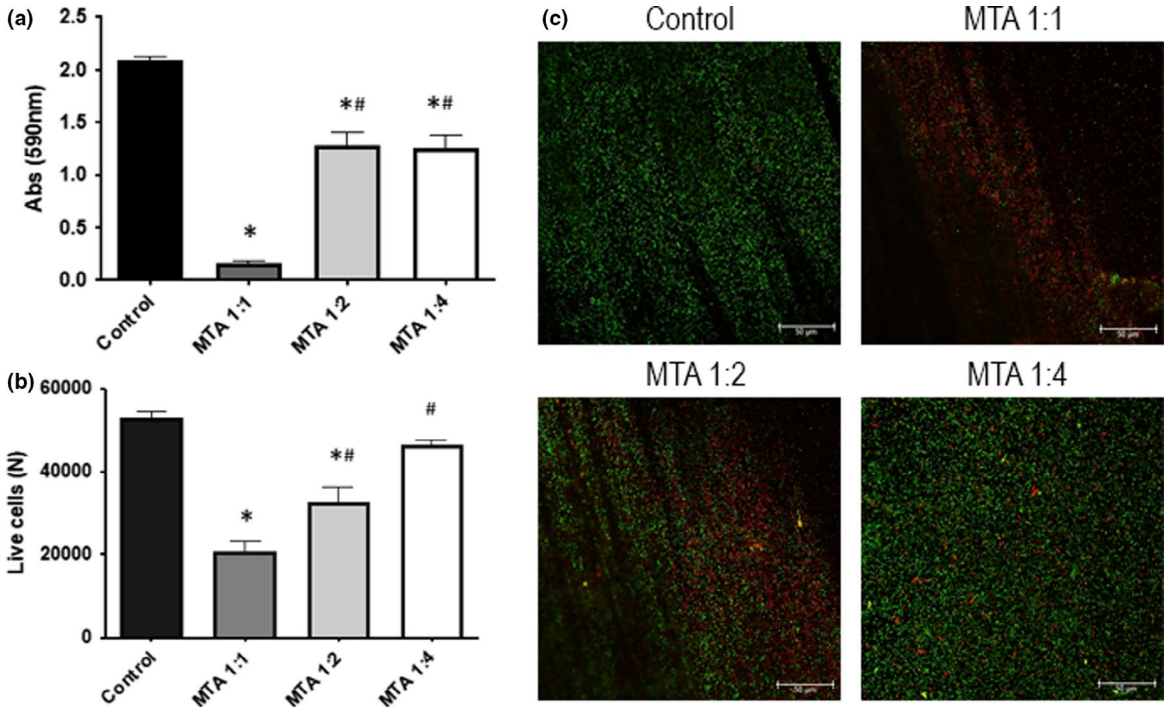

FIGURE 1 Evaluation of the antibiofilm activity of MTA Repair HP 1:1, 1:2 and 1:4. (a) Bacterial cell viability of *S. mutans* after 24 h exposure of MTA Repair HP in preformed biofilm in U-bottom 96-well plates. (b) Total biovolume (μm^3) of living cells from *S. mutans* biofilm on dentin slices after 7 days followed by MTA Repair HP exposure for 24 h, evaluated by confocal microscopy. Biofilm eradication was calculated according to five random views of *S. mutans* biofilms for each independent replicate. Representation of two independent replicas. Data were presented as mean ± SD. $^{*}p < 0.001$ in relation to control and $^{\#}p < 0.001$ in relation to MTA Repair HP 1:1, by one-way ANOVA and post-Tukey tests. Positive control for (a) and (b) was represented by *S. mutans* bacterium in BHI medium. (c) Confocal microscopy images of *S. mutans* biofilm. Green images represent living cells and red images represent dead cells. The scale bar represents 50 μm

extract dilution. The undiluted MTA extract did not stimulate cell migration. However, diluted MTA extract 1:2 and 1:4 demonstrated 51% and 42% higher migration rates, respectively, than the control group after 24 h incubation ($p < 0.04$; Figure 3a,c). Similar results were observed after 48 h incubation, showing an increase in migration rates of 34% and 51% in the presence of MTA extract, 1:2 and 1:4 dilutions ($p < 0.04$), respectively (Figure 3a,b). When evaluating the capacity of cell proliferation after exposure of pulp cells to MTA Repair HP, it was noted that dilutions 1:2 and 1:4 were able to promote greater cell proliferation in 24 and 48 h ($p < 0.04$) when compared to control and undiluted MTA extract ($p < 0.05$; Figure 3c).

MTA repair HP effect on culture medium pH

MTA Repair HP extract was able to increase pH in a dose-dependent manner. The undiluted MTA Repair HP extract showed a higher pH (9.22 ± 0.09) in the first 30 min when compared to the 1:2 (8.72 ± 0.10) and 1:4 (8.35 ± 0.00) dilutions. It was also possible to evaluate the pH reduction in all dilutions after 24 h reaching neutral pH (7.0) in 72 h (Table 1). Controls presented stable pH until 24 h.

Odontogenic differentiation – ALP, TGF-β secretion and phosphate quantification

In order to evaluate the capacity of the MTA Repair HP 1:1, 1:2 and 1:4 dilutions to promote the production of important agents of odontogenic differentiation and mineralization, ALP activity, TGF-β secretion and phosphate quantification were measured. There was no difference in the alkaline phosphatase activity between different dilutions of MTA Repair HP (1:1, 1:2 and 1:4; Figure 4a). A higher secretion of TGF-β was observed in the presence of diluted MTA Repair HP 1:1 and 1:2 ($p < 0.001$) compared to pulp cell culture in osteogenic medium (Figure 4b). However, there were no differences between TGF-β secretion in undiluted and diluted MTA. Thus, it was noted that ALP activity and TGF-β secretion were independent of the MTA dilution used. However, diluted MTA Repair HP 1:4 produced less phosphate than the more concentrated MTA extracts 1:1 and 1:2 ($p < 0.001$; Figure 4c).

DISCUSSION

The use of MTA has been extended in endodontic practice (Li et al., 2015; Mente et al., 2014), including its use in conservative pulp therapies (Caliskan & Guneri, 2017). In order to establish a comparison with clinical practice, MTA Repair HP dilutions of 1:2 and 1:4 were used to compare different concentrations of MTA which can be observed in direct and indirect pulp tissue contact. The higher MTA concentration (1:1) can be compared to clinical direct capping or pulpotomy conditions. MTA dilutions of 1:2 and 1:4 can be compared to cases of pulp capping performed in deep and medium cavities. For clinical use of MTA, several formulation options are available, but most of them come from the initial formulation, represented by a powder mixture consisting of hydrophilic particles, including a Portland cement clinker, bismuth oxide and gypsum. Its mechanism of action is related to by-products of insoluble calcium silicate hydrate and alkaline calcium hydroxide (Ji et al., 2011). Therefore, MTA presents good stability and sealing ability, antibacterial properties, biocompatibility and a high capacity to enhance hard-tissue healing (Tomás-Catalá et al., 2017; Torabinejad et al., 1995).

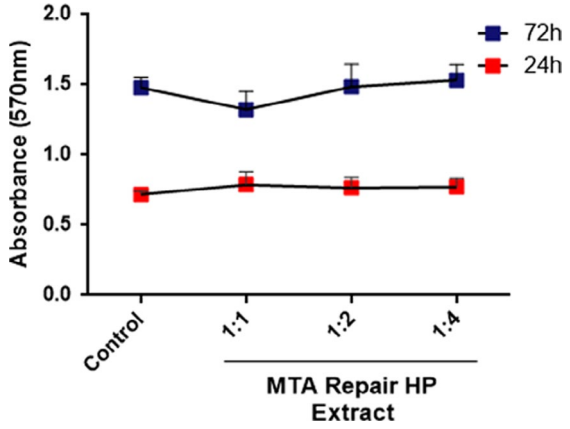

FIGURE 2 Cell viability after exposure to MTA Repair HP extracts at 1:1, 1:2 and 1:4 dilutions. Control represented by pulp cell culture without additional stimulus. Red dot represents the cell viability evaluated after 24 h. Blue square represents cell viability after 72 h of MTA extract exposure. Data shown represent the mean ± SD of three independent experiments performed in triplicate with dental pulp from three tissue donors. No significant differences were observed between the MTA extract dilutions and control after two-way ANOVA and Tukey's post-tests.

Despite the satisfactory results obtained with the use of MTA, it has continued to be developed over the years. Improvements have been made in terms of handling and the removal of components, such as bismuth oxide, which causes dentinal chromatic changes (Marciano et al., 2014, 2015). Changes in MTA composition caused changes in consistency and improved the handling of the material, leading to MTA Repair HP (Marciano et al., 2014). Another study demonstrated alkaline pH, antibacterial activity against *E. faecalis*, low solubility and no cytotoxic

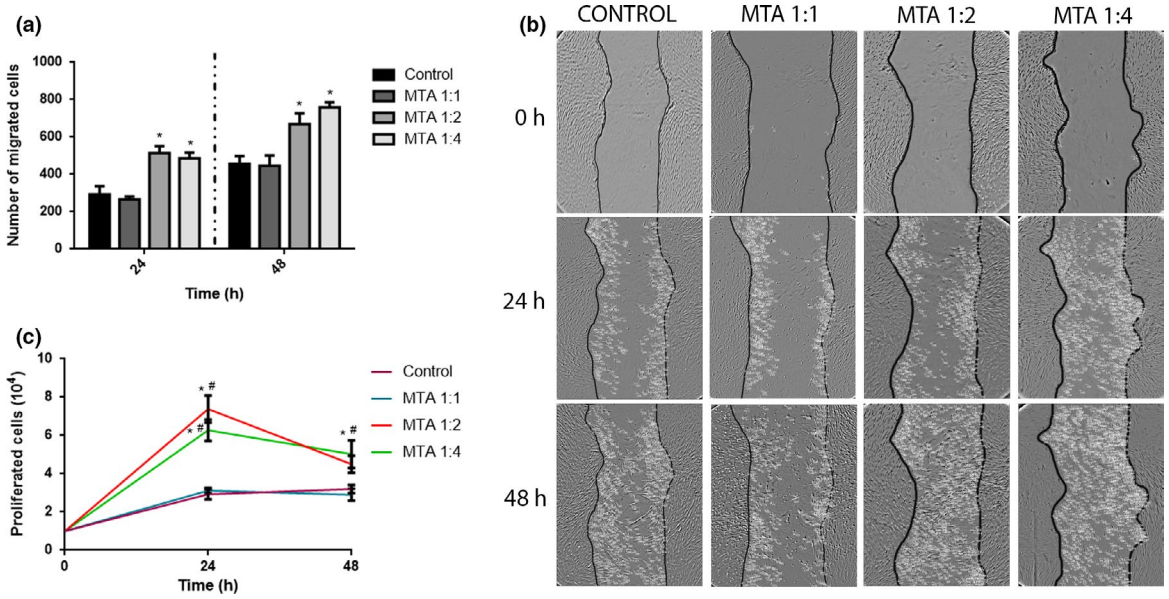

FIGURE 3 MTA Repair HP's migratory and proliferative capacity. (a) Number of cells migrated after contact with different dilutions of MTA extract in pulp cell culture, after 24 and 48 h. Control group represented by pulp cell culture without any stimulation. $^{*}p < 0.04$, by two-way ANOVA and post-Tukey tests. (b) Representation of cell proliferation after exposure of MTA Repair HP 1:1, 1:2 and 1:4 in pulp cell culture. Evaluated by Trypan Blue at 24 and 48 h $^{*}p < 0.04$ and $^{\#}p < 0.05$. (c) Representative photos analysed by Imagen J demonstrating pulp cell culture migration using the scratch method after 0, 24 and 48 h. Data shown represent the mean ± SD of three independent experiments performed in triplicate with dental pulp from three tissue donors

TABLE 1 pH assessment of MTA Repair HP extract at 1:1, 1:2 and 1:4 dilutions and used culture mediums (DMEM and BM2 medium) at 30 min, 1, 24, 48 and 72 h incubation

Solution	30 min	1 h	24 h	48 h	72 h
MTA 1:1	$9.22 \pm 0.09^{\#\Delta}$	$8.45 \pm 0.12^{\Delta}$	$7.16 \pm 0.03^{\#\Delta}$	$7.1 \pm 0.09^{\Delta}$	$6.99 \pm 0.09^{\#\Delta}$
MTA 1:2	$8.72 \pm 0.10^{*\Delta}$	$8.22 \pm 0.04^{\Delta}$	$6.87 \pm 0.06^{*\Delta}$	6.71 ± 0.01	$6.76 \pm 0.03^{*\Delta}$
MTA 1:4	$8.35 \pm 0.00^{*\#}$	$7.85 \pm 0.01^{*\#}$	$6.34 \pm 0.26^{*\#}$	$6.54 \pm 0.06^{*}$	$6.35 \pm 0.30^{*\#}$
BM2 medium	7.14 ± 0.01	7.15 ± 0.03	7.05 ± 0.14	6.87 ± 0.24	6.77 ± 0.06
DMEM medium	8.27 ± 0.09	8.25 ± 0.19	8.20 ± 0.04	8.16 ± 0.02	7.98 ± 0.03
Ultrapure water	7.11 ± 0.09	7.15 ± 0.08	6.52 ± 0.05	6.45 ± 0.06	6.04 ± 0.30

Significant differences verified by two-way ANOVA and Tukey's post-tests. Statistical analyses were performed comparing the dilutions MTA 1:1 (*), MTA 1:2 (#) and MTA 1:4 (Δ), in each experimental period.

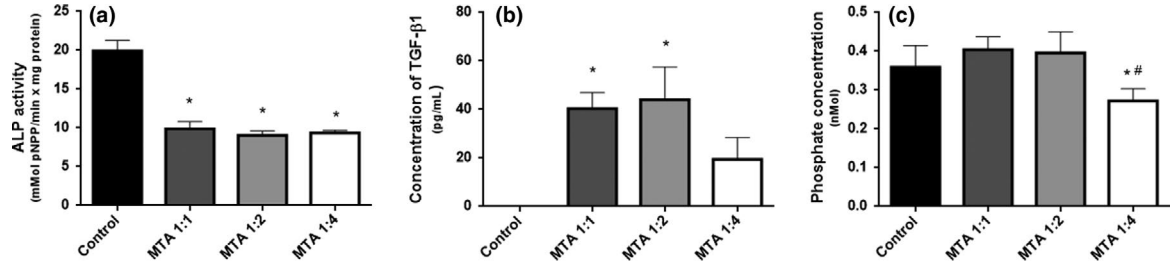

FIGURE 4 Quantitative evaluation of molecules related to osteogenesis. (a) Alkaline phosphatase (ALP) activity in cells cultured in osteogenic medium for 14 days. ALP activity (mMol p-nitrophenol released per min) was normalized for protein. (b) ELISA analysis of the production of TGF-β (pg. mL^{-1}) by pulp cells differentiated by osteogenic medium and exposure to MTA Repair HP 1:1, 1:2 and 1:4 for 14 days. (c) Phosphate quantification after exposure to MTA Repair HP for 14 days. Data shown represent the mean ± standard error of three independent experiments performed in triplicate with dental pulp from three tissue donors. $^{*}p < 0.001$ in relation to control and $^{\#}p < 0.001$ in relation to MTA Repair HP 1:1 by one-way ANOVA and post-Tukey tests

effect of MTA Repair HP and radiopacifiers (zirconium oxide, calcium tungstate and niobium oxide) added to tricalcium silicate (Queiroz et al., 2021). In this context, to test this recent MTA formulation, pulp cells and the bacterium *S. mutans* were exposed to MTA extract following ISO 10993-5 standardization.

However, little is known about MTA Repair HP and the way in which it acts on the pulp repair process. This process tends to start as soon as the bacterial population is reduced to subcritical levels and the pulp inflammatory response reaches homeostasis (Simon et al., 2009). In addition, it is known that most endodontic failures are attributable to bacterial invasion or irritant leakage into the root canal (Ji et al., 2011). For this reason, antibacterial effects on *S. mutans* were initially evaluated. However, this study did not demonstrate bactericidal or bacteriostatic activity against planktonic cultures of *S. mutans*. The literature on the antibacterial effect of MTA is controversial (Kim et al., 2015; Parirokh & Torabinejad, 2010). Some investigations have reported that MTA has an antimicrobial effect against some microorganisms, such as *Lactobacillus rhamnosus*, *Lactobacillus paracasei*, *S. mutans* and *Porphyromonas gingivalis* (Kim et al., 2015). A previous study demonstrated that MTA Repair HP has antibacterial activity against planktonic *Enterococcus faecalis* (Queiroz et al., 2021). In addition, studies on facultative and strict anaerobic bacteria have shown the antibacterial effect of MTA on some facultative bacteria, but no effect was observed on strict anaerobic bacteria (Torabinejad et al., 1995). However, other studies did not observe any antibacterial effect of MTA on planktonic *S. mutans*, using an agar diffusion test methodology, corroborating results evaluated in the present study (Pimenta et al., 2015; Shin et al., 2017). Yang et al. (2014) also reported the absence of antibacterial activity of MTA in planktonic *S. mutans* using the broth-based method (Yang et al., 2014).

The ability of MTA Repair HP extract in preformed *S. mutans* biofilm was evaluated using two different approaches. The first corresponded to an evaluation of MTA Repair HP 1:1, 1:2 and 1:4 in young biofilm of *S. mutans*, grown in appropriate plates and medium poor in nutrients, which favoured the formation of biofilm after 24 h (Reffuveille et al., 2014). In this assay, undiluted MTA extract reduced *S. mutans* biofilm after 24 h. However, this reduction was diminished as soon as the MTA extract was diluted. The second assay corresponded to the evaluation by confocal microscopy of the exposure of MTA Repair HP 1:1, 1:2 and 1:4 in biofilm culture for 7 days on dentine discs. This trial demonstrated the ability of undiluted MTA to reduce biofilm by approximately 61%. However,

the more MTA that was in the diluted extract, the lower its ability to reduce biofilm. The possible reason for this reduction was the higher pH value (9.22) in the presence of undiluted MTA extract after 30 min. The presence of free calcium oxide present in MTA Repair HP may also have promoted an increase in the alkalinity of the medium, as evaluated in another study (Tanomaru-Filho et al., 2015). As assessed in the present study, Tanomaru-Filho et al. (2015) also demonstrated that MTA reduces its pH after 3 h, and Tomás-Catalá et al. (2017) reported pH of the MTA Repair HP extract at 7.57 after 24 h. However, another study reported a high pH of 11 after 7 days (Guimaraes et al., 2018). That study used a maceration of MTA Repair HP in the medium, which may have caused a difference in relation to the present study, which used only the pH verification of the material extract. The initial alkalinity may be related to its composition and mechanism of action (Niu et al., 2014). The hydration of tricalcium silicate particles leads to a rapid exchange of Ca^{2+} with H_3O^+ ions. The reaction of Ca^{2+} ions with OH^- ions derived from water results in the formation of $Ca(OH)_2$, and creates a highly alkaline environment (Niu et al., 2014). Probably, the alkalization medium imposed by the MTA Repair HP extract promoted biofilm disturbance, leading to the destruction or disorganization of proteins, polysaccharides and nucleic acids present in the extracellular matrix of the biofilm (Jardine et al., 2019). Thus, biofilm dissipation and cell fragilization lead to bacterial death process. However, there is a need for further studies to confirm the MTA mechanism of action in *S. mutans* or multibacteria biofilms.

Once MTA Repair HP had been seen to promote the reduction in *S. mutans* biofilm, a new question was raised. Does this biomaterial also lead to pulp cell disruption? As MTA is commonly used to induce pulp regeneration and repair, it must be biocompatible and bioactive causing cell migration, proliferation and differentiation (da Rosa et al., 2018). Results showed that MTA was not toxic to pulp cells after 24 and 72 h. Tomás-Catalá et al. (2017, 2018) observed similar results regarding the viability of pulp cells after exposure to 1:1, 1:2, and 1:4 dilutions of MTA Repair HP extracts. Another study compared MTA Repair HP with White MTA, which also did not reveal a reduction in cell viability after contact with both biomaterials (Ferreira et al., 2019).

The release of calcium ions by MTA has been not only related to the differentiation and proliferation of cells but also to the capacity for cell migration (Collado-Gonzalez et al., 2019). The migratory and proliferative potential of pulp cells exposed to the MTA extract reveals promising results for all clinical MTA indications. It was observed that the *in vitro* migratory and proliferative capacity of pulp cells increased after they had been exposed to MTA extracts, mainly to diluted MTA extracts (1:2 and 1:4). This result also demonstrates that the use of MTA in indirect conditions can assist in the repair process. The processes of cell migration and proliferation are essential for the repair process (da Rosa et al., 2018). The migratory process observed in the presence of MTA extracts was probably favoured by the neutral pH observed after 24 h. Tomás-Catalá et al., (2018) also reported similar rates of migration of pulp cells in the presence of extracts of this material (1:2 and 1:4 dilutions), with greater cell migration in 24 h. Besides, after 48 h, similar pulp cell migration was reported after exposure to undiluted MTA extract (Tomás-Catalá et al., 2018). Studies show that the direct contact of MTA with pulp tissue initially causes a process of superficial tissue necrosis (Benetti et al., 2019; Cintra et al., 2017). A previous study demonstrated the presence of superficial tissue necrosis *in vivo* up to 7 days, and a moderate inflammatory infiltrate decreases after the 30th day in rat models (Cintra et al., 2017). This information may clarify why cell migration occurred modestly in pulp cells, when exposed to undiluted MTA Repair HP.

To evaluate if MTA Repair HP has the ability to stimulate responses related to repairing near to and distant from the pulp tissue, ALP activity, TGF-β secretion and phosphate production were evaluated. ALP is an enzyme presented in the early stages of osteoblast/odontoblast cell differentiation. It is responsible for supplying inorganic phosphate to form hydroxyapatite, initiating mineralization (Salles et al., 2012). High levels of ALP were observed in the control related to pulp cells. A study demonstrated high levels of ALP in pulp culture, in addition to showing that the presence of TGF-β can negatively regulate ALP activity (Shirakawa et al., 1994). This fact corroborates the present results, including high levels of TGF-β secretion after MTA exposure. The same ALP production was evaluated, regardless of the dilution used. Furthermore, undiluted and 1:2 diluted MTA led to higher TGF-β production, when compared to 1:4 diluted MTA. Studies have reported that phosphate has the ability to induce the expression of important genes for cell proliferation, energy metabolism and mineralization in cells similar to osteoblasts. Thus, the phosphate together with calcium form hydroxyapatite crystals, and for this reason, it was quantified (Chande & Bergwitz, 2018). The present study demonstrated that 1:1 and 1:2 MTA dilutions were able to induce higher phosphate production than 1:4 diluted MTA. Thus, even 1:2 diluted MTA Repair HP demonstrated alkaline phosphatase activity, TGF-β secretion and phosphate production similar to undiluted MTA.

MTA has important biological properties, such as proliferation, differentiation of dental pulp cells in odontoblast-like cells and induction of mineralized tissue formation (Rodrigues et al., 2016; Tomás-Catalá et al.,

2018). It was possible to evaluate the ability of even diluted MTA Repair HP to cause migration and proliferation of pulp cells, in addition to causing TGF-β secretion and phosphate production similar to undiluted MTA. Thus, the use of MTA Repair HP for conservative pulp treatments, such as indirect capping, may assist in the pulp repair process.

CONCLUSION

Undiluted MTA Repair HP promoted a greater reduction in *S. mutans* biofilm when compared to 1:2 and 1:4 MTA dilutions. Furthermore, none of the tested dilutions was cytotoxic to pulp cells. MTA Repair HP promoted cell migration and proliferation at a distance, assessed through the dilution of the MTA. It was also noted that even at a distance, MTA Repair HP may have the ability to participate in the pulp repair process because ALP activity, TGF-β secretion and phosphate production were similar between MTA 1:1 and MTA 1:2.

ETHICAL STATEMENT

Authors affirm that this is an original work, which has not been previously published elsewhere. Furthermore, the paper reflects the authors' research and analysis wholly and truthfully. All sources used are appropriately disclosed and cited. We also affirm that authors have been personally and actively involved in substantial word leading to the paper and will take public responsibility for its content.

AUTHOR CONTRIBUTION

All authors have contributed to the development of this original research. Furthermore, all authors have read and approved the manuscript. Poliana Amanda Oliveira Silva is the first author and was responsible for teeth collection, methodology development and writing of the manuscript. Stella Maris de Freitas Lima prepared all figures and part of the manuscript. Danilo César Mota Martins was responsible for teeth collection and human dental pulp cells isolation and culture. Ingrid Aquino Amorim and Cristiano Castro Lacorte were responsible for assisting in the development of the biofilm eradication methodology. Jeeser Alves de Almeida contributed to data analysis and statistics. Octávio Luiz Franco and Taia Maria Berto Rezende were involved in data analysis, financial support and in manuscript preparation.

CONFLICT OF INTEREST

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

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REFERENCES

Abbott, P.V. & Yu, C. (2007) A clinical classification of the status of the pulp and the root canal system. *Australian Dental Journal*, 52, S17–S31.

Arias-Moliz, M.T., Morago, A., Ordinola-Zapata, R., Ferrer-Luque, C.M., Ruiz-Linares, M. & Baca, P. (2016) Effects of dentin debris on the antimicrobial properties of sodium hypochlorite and etidronic acid. *Journal of Endodontics*, 42, 771–775.

Asgary, S., Parirokh, M., Eghbal, M.J., Ghoddusi, J. & Eskandarizadeh, A. (2006) SEM evaluation of neodentinal bridging after direct pulp protection with mineral trioxide aggregate. *Australian Endodontic Journal*, 32, 26–30.

Benetti, F., Queiroz, I.O.A., Cosme-Silva, L., Conti, L.C., Oliveira, S.H.P. & Cintra, L.T.A. (2019) Cytotoxicity, biocompatibility and biomineralization of a new ready-for-use bioceramic repair material. *Brazilian Dental Journal*, 30, 325–332.

Braga, J.M., Oliveira, R.R., Martins, R.C. & Ribeiro Sobrinho, A.P. (2014) The effects of a mineral trioxide aggregate-based sealer on the production of reactive oxygen species, nitrogen species and cytokines by two macrophage subtypes. *International Endodontic Journal*, 47, 909–919.

Caliskan, M.K. & Guneri, P. (2017) Prognostic factors in direct pulp capping with mineral trioxide aggregate or calcium hydroxide: 2- to 6-year follow-up. *Clinical Oral Investigations*, 21, 357–367.

Camilleri, J., Gandolfi, M.G., Siboni, F. & Prati, C. (2011) Dynamic sealing ability of MTA root canal sealer. *International Endodontic Journal*, 44, 9–20.

Chande, S. & Bergwitz, C. (2018) Role of phosphate sensing in bone and mineral metabolism. *Nature Reviews Endocrinology*, 14, 637–655.

Cintra, L.T.A., Benetti, F., de Azevedo Queiroz, Í.O., de Araújo Lopes, J.M., Penha de Oliveira, S.H., Sivieri Araújo, G. et al. (2017) Cytotoxicity, biocompatibility, and biomineralization of the new high-plasticity MTA material. *Journal of Endodontics*, 43, 774–778.

Collado-González, M., López-García, S., García-Bernal, D., Oñate-Sánchez, R.E., Tomás-Catalá, C.J., Moraleda, J.M. et al. (2019) Biological effects of acid-eroded MTA Repair HP and ProRoot MTA on human periodontal ligament stem cells. *Clinical Oral Investigations*, 23, 3915–3924.

Crowley, L.C., Marfell, B.J., Christensen, M.E. & Waterhouse, N.J. (2016) Measuring cell death by trypan blue uptake and light microscopy. *Cold Spring Harbor Protocols*, 2016, 643–646.

Darvell, B.W. & Wu, R.C. (2011) "MTA"—an Hydraulic Silicate Cement: review update and setting reaction. *Dental Materials*, 27, 407–422.

dos Santos, A.F., Pacheco, J.M., Silva, P.A.O., Bedran-Russo, A.K., Rezende, T.M.B., Pereira, P.N.R. et al. (2019) Direct and transdentinal biostimulatory effects of grape seed extract rich in proanthocyanidin on pulp cells. *International Endodontic Journal*, 52, 424–438.

Ferreira, C.M.A., Sassone, L.M., Gonçalves, A.S., de Carvalho, J.J., Tomás-Catalá, C.J., García-Bernal, D. et al. (2019) Physicochemical, cytotoxicity and in vivo biocompatibility of a high-plasticity calcium-silicate based material. *Scientific Reports*, 9, 3933.

Gandolfi, M.G., Taddei, P., Tinti, A. & Prati, C. (2010) Apatite-forming ability (bioactivity) of ProRoot MTA. *International Endodontic Journal*, 43, 917–929.

Guimaraes, B.M., Prati, C., Duarte, M.A.H., Bramante, C.M. & Gandolfi, M.G. (2018) Physicochemical properties of calcium silicate-based formulations MTA Repair HP and MTA Vitalcem. *Journal of Applied Oral Science*, 26, e2017115.

Jardine, A.P., Montagner, F., Quintana, R.M., Zaccara, I.M. & Kopper, P.M.P. (2019) Antimicrobial effect of bioceramic cements on multispecies microcosm biofilm: a confocal laser microscopy study. *Clinical Oral Investigations*, 23, 1367–1372.

Ji, D.-Y., Wu, H.-D., Hsieh, S.-C., Teng, N.-C., Chen, C.-C., Ke, E.-S. et al. (2011) Effects of a novel hydration accelerant on the biological and mechanical properties of white mineral trioxide aggregate. *Journal of Endodontics*, 37, 851–855.

Jiang, L., Zhu, Y.Q., Du, R., Gu, Y.-X., Xia, L., Qin, F. & et al. (2008) The expression and role of stromal cell-derived factor-1alpha-CXCR4 axis in human dental pulp. *Journal of Endodontics*, 34, 939–944.

Kajiya, M., Shiba, H., Komatsuzawa, H., Ouhara, K., Fujita, T., Takeda, K. et al. (2010) The antimicrobial peptide LL37 induces the migration of human pulp cells: a possible adjunct for regenerative endodontics. *Journal of Endodontics*, 36, 1009–1013.

Kim, R.J., Kim, M.O., Lee, K.S., Lee, D.Y. & Shin, J.H. (2015) An in vitro evaluation of the antibacterial properties of three mineral trioxide aggregate (MTA) against five oral bacteria. *Archives of Oral Biology*, 60, 1497–1502.

Li, Z., Cao, L., Fan, M. & Xu, Q. (2015) Direct pulp capping with calcium hydroxide or mineral trioxide aggregate: a meta-analysis. *Journal of Endodontics*, 41, 1412–1417.

Luczaj-Cepowicz, E., Pawinska, M., Marczuk-Kolada, G., Leszczynska, K. & Waszkiel, D. (2008) Antibacterial activity of two Mineral Trioxide Aggregate materials in vitro evaluation. *Annales Academiae Medicae Stetinensis*, 54, 147–150, discussion 50–51.

Marciano, M.A., Costa, R.M., Camilleri, J., Mondelli, R.F., Guimaraes, B.M. & Duarte, M.A. (2014) Assessment of color stability of white mineral trioxide aggregate angelus and bismuth oxide in contact with tooth structure. *Journal of Endodontics*, 40, 1235–1240.

Marciano, M.A., Duarte, M.A. & Camilleri, J. (2015) Dental discoloration caused by bismuth oxide in MTA in the presence of sodium hypochlorite. *Clinical Oral Investigations*, 19, 2201–2209.

Mente, J., Hufnagel, S., Leo, M., Michel, A., Gehrig, H., Panagidis, D. et al. (2014) Treatment outcome of mineral trioxide aggregate or calcium hydroxide direct pulp capping: long-term results. *Journal of Endodontics*, 40, 1746–1751.

Momoi, Y., Hayashi, M., Fujitani, M., Fukushima, M., Imazato, S., Kubo, S. et al. (2012) Clinical guidelines for treating caries in adults following a minimal intervention policy–evidence and consensus based report. *Journal of Dentistry*, 40, 95–105.

Moraes, D.A., Sibov, T.T., Pavon, L.F., Alvim, P.Q., Bonadio, R.S., Da Silva, J.R. et al. (2016) A reduction in CD90 (THY-1) expression results in increased differentiation of mesenchymal stromal cells. *Stem Cell Research & Therapy*, 7, 97.

Mosmann, T. (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, 65, 55–63.

NCCLS. (2003) *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard*. Pennsylvania, USA. NCCLS.

Niu, L.-N., Jiao, K., Wang, T.-d., Zhang, W., Camilleri, J., Bergeron, B.E. et al. (2014) A review of the bioactivity of hydraulic calcium silicate cements. *Journal of Dentistry*, 42, 517–533.

Parirokh, M. & Torabinejad, M. (2010) Mineral trioxide aggregate: a comprehensive literature review–part III: clinical applications, drawbacks, and mechanism of action. *Journal of Endodontics*, 36, 400–413.

Parirokh, M., Torabinejad, M. & Dummer, P.M.H. (2018) Mineral trioxide aggregate and other bioactive endodontic cements: an updated overview—part I: vital pulp therapy. *International Endodontic Journal*, 51, 177–205.

Park, S.J., Heo, S.M., Hong, S.O., Hwang, Y.C., Lee, K.W. & Min, K.S. (2014) Odontogenic effect of a fast-setting pozzolan-based pulp capping material. *Journal of Endodontics*, 40, 1124–1131.

Pimenta, H.C., Borges, A.H., Bandeca, M.C., Neves, A.T., Fontes, R.G., da Silva, P.V. & et al. (2015) Antimicrobial activity of filling materials used in primary teeth pulpotomy. *Journal International Oral Health*, 7, 54–57.

Queiroz, M.B., Torres, F.F.E., Rodrigues, E.M., Viola, K.S., Bosso-Martelo, R., Chavez-Andrade, G.M. et al. (2021) Physicochemical, biological, and antibacterial evaluation of tricalcium silicate-based reparative cements with different radiopacifiers. *Dental Materials*, 37, 311–320.

Reffuveille, F., de la Fuente-Nunez, C., Mansour, S. & Hancock, R.E. (2014) A broad-spectrum antibiofilm peptide enhances antibiotic action against bacterial biofilms. *Antimicrobial Agents and Chemotherapy*, 58, 5363–5371.

Rezende, T.M.B., Ribeiro Sobrinho, A.P., Vieira, L.Q., Sousa, M. & Kawai, T. (2020) Mineral trioxide aggregate (MTA) inhibits osteoclastogenesis and osteoclast activation through calcium and aluminum activities. *Clinical Oral Investigations*, 25, 1805–1814.

Rodrigues, E.M., Cornélio, A.L.G., Mestieri, L.B., Fuentes, A.S.C., Salles, L.P., Rossa-Junior, C. et al. (2016) Human dental pulp cells response to mineral trioxide aggregate (MTA) and MTA Plus: cytotoxicity and gene expression analysis. *International Endodontic Journal*, 50, 780–789.

da Rosa, W.L.O., Piva, E. & da Silva, A.F. (2018) Disclosing the physiology of pulp tissue for vital pulp therapy. *International Endodontic Journal*, 51, 829–846.

Salles, L.P., Gomes-Cornélio, A.L., Guimarães, F.C., Herrera, B.S., Bao, S.N., Rossa-Junior, C. et al. (2012) Mineral trioxide aggregate-based endodontic sealer stimulates hydroxyapatite nucleation in human osteoblast-like cell culture. *Journal of Endodontics*, 38, 971–976.

Schwendicke, F., Brouwer, F., Schwendicke, A. & Paris, S. (2016) Different materials for direct pulp capping: systematic review and meta-analysis and trial sequential analysis. *Clinical Oral Investigations*, 20, 1121–1132.

Shin, M., Chen, J.-W., Tsai, C.-Y., Aprecio, R., Zhang, W.U., Yochim, J.M. et al. (2017) Cytotoxicity and antimicrobial effects of a new fast-set MTA. *BioMed Research International*, 2017, 2071247.

Shirakawa, M., Shiba, H., Nakanishi, K., Ogawa, T., Okamoto, H., Nakashima, K. et al. (1994) Transforming growth factor-beta-1 reduces alkaline phosphatase mRNA and activity and

stimulates cell proliferation in cultures of human pulp cells. *Journal of Dental Research*, 73, 1509–1514.

Simon, S., Smith, A.J., Lumley, P.J., Berdal, A., Smith, G., Finney, S. et al. (2009) Molecular characterization of young and mature odontoblasts. *Bone*, 45, 693–703.

de Souza Costa, C.A., Hebling, J., Scheffel, D.L., Soares, D.G., Basso, F.G. & Ribeiro, A.P. (2014) Methods to evaluate and strategies to improve the biocompatibility of dental materials and operative techniques. *Dental Materials*, 30, 769–784.

Stringhini Junior, E., Dos Santos, M.G.C., Oliveira, L.B. & Mercade, M. (2019) MTA and biodentine for primary teeth pulpotomy: a systematic review and meta-analysis of clinical trials. *Clinical Oral Investigations*, 23, 1967–1976.

Takita, T., Hayashi, M., Takeichi, O., Ogiso, B., Suzuki, N., Otsuka, K. et al. (2006) Effect of mineral trioxide aggregate on proliferation of cultured human dental pulp cells. *International Endodontic Journal*, 39, 415–422.

Tanomaru-Filho, M., Garcia, A.C., Bosso-Martelo, R., Berbert, F.L., Nunes Reis, J.M. & Guerreiro-Tanomaru, J.M. (2015) Influence of addition of calcium oxide on physicochemical properties of Portland cement with zirconium or niobium oxide. *Journal of Conservative Dentistry*, 18, 105–108.

Tomás-Catalá, C.J., Collado-González, M., García-Bernal, D., Oñate-Sánchez, R.E., Forner, L., Llena, C. et al. (2017) 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. *International Endodontic Journal*, 50(Suppl. 2), e63–e72.

Tomás-Catalá, C.J., Collado-González, M., García-Bernal, D., Oñate-Sánchez, R.E., Forner, L., Llena, C. et al. (2018) Biocompatibility of new pulp-capping materials NeoMTA plus, MTA repair HP, and biodentine on human dental pulp stem cells. *Journal of Endodontics*, 44, 126–132.

Torabinejad, M., Hong, C.U., Pitt Ford, T.R. & Kettering, J.D. (1995) Antibacterial effects of some root end filling materials. *Journal of Endodontics*, 21, 403–406.

Valles, M., Mercade, M., Duran-Sindreu, F., Bourdelande, J.L. & Roig, M. (2013) Color stability of white mineral trioxide aggregate. *Clinical Oral Investigation*, 17, 1155–1159.

Yang, Y., Huang, L.I., Dong, Y., Zhang, H., Zhou, W., Ban, J. et al. (2014) In vitro antibacterial activity of a novel resin-based pulp capping material containing the quaternary ammonium salt MAE-DB and Portland cement. *PLoS One*, 9, e112549.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Silva, P.A.O., Lima, S.M.D.F., Martins, D.C.M., Amorim, I.A., Lacorte, C., de Almeida, J.A., et al. (2021) Concentrated MTA repair HP reduced biofilm and can cause reparative action at a distance. *International Endodontic Journal*, 00, 1–12. https://doi.org/10.1111/iej.13592