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ARTICLE

BIODENTINE STIMULATES THE MIGRATORY AND BIOLOGICAL RESPONSES OF HUMAN GINGIVAL FIBROBLAST.

Biodentine estimula la respuesta migratoria y biológica de fibroblastos gingivales humanos.

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ABSTRACT:

Introduction: Biodentine (BD), a dentin substitute, is currently used to treat external cervical root resorption, but its effects on gingival fibroblasts (GFs) are not fully known.

Objective: To investigate and compare BD and MTA (mineral trioxide aggregate) in terms of proliferative, migratory, and adhesion effects on human pulpal and gingival cells.

Material and Methods: Cells were incubated directly on the surface of BD and MTA disks. Adhesion (4 and 24 h) and proliferation (3, 5, 7, 14, 21) were evaluated with crystal violet and MTT assays (n=9 X each group). A wound-healing assay was performed for cell migration, with 0.2 and 2 µg/ml MTA or BD (n=6 X each group). The cut-off point for statistical significance was set at $p<0.05$, $p<0.01$ and $p<0.001$.

Results: The best adhesion and proliferation results for gingival fibroblast (GFs) were obtained with BD ($p<0.01$). MTA and BD enhanced the migration of GFs in a dose-dependent manner, with superior results with BD, and 2 µg/ml was the optimal concentration for enhancing the migration of GFs.

Conclusion: Results indicate that BD and MTA exhibit excellent compatibility in terms of cell adhesion, proliferation, and cellular migration. Also, the results suggested that BD is associated with better results than MTA in GFs. The results support the clinical application of BD in areas colonized with GFs.

KEYWORDS:

Biodentine; Mineral trioxide aggregate; Cell migration; Fibroblasts; Cell culture techniques; Pulp Capping and Pulpectomy Agents.

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RESUMEN:

Introducción: Biodentine® (BD), un sustituto de la dentina se usa para tratar la reabsorción de la raíz cervical externa, pero sus efectos sobre los fibroblastos gingivales (FG) no se conocen por completo.

Objetivo: investigar y comparar BD y MTA (agregado de trióxido mineral) en términos de efectos proliferativos, migratorios y de adhesión en células pulpares y gingivales humanas.

Material y Métodos: Las células se incubaron directamente sobre la superficie de discos BD y MTA. La adherencia (4 y 24 h) y la proliferación (3, 5, 7, 14, 21) se evaluaron con ensayos de cristal violeta y MTT (n=9 X cada grupo). Se realizó un ensayo de cicatrización de heridas para la migración celular, con 0,2 y 2 µg/ml de MTA o BD (n=6 X cada grupo).

Resultados: Los mejores resultados de adhesión y proliferación para fibroblastos gingivales (GFs) se obtuvieron con BD (p<0,01). MTA y BD mejoraron la migración de FG de manera dependiente de la dosis, con resultados superiores con BD, y 2 µg/ml fue la concentración óptima para mejorar la migración de GF.

Conclusión: Los resultados indican que BD y MTA exhiben una excelente compatibilidad en términos de adhesión celular, proliferación y migración celular. Además, los resultados sugirieron que BD se asocia con mejores resultados que MTA en FG. Los resultados apoyan la aplicación clínica de BD en áreas colonizadas con FG.

PALABRAS CLAVE:

Biodentina; Agregado de trióxido mineral; Migración celular; Fibroblastos; Técnicas de cultivo celular; Agentes de recubrimiento pulpar y pulpectomía.

INTRODUCTION.

The field of endodontics has been an area of active research and development of dental materials, generating a significant impact on dentistry. The most popular materials are the calcium silicate-based formulations, with various compositions, as they resemble mineral trioxide aggregate (MTA), the gold standard for endodontic treatments.[1] Today, endodontic cements allow clinicians to obtain successful treatments that previously had reserved prognoses, such as root resorption, perforations, and direct pulp-capping.[2]

In this regard, MTA is used extensively for vital pulp therapies, protecting scaffolds during regenerative endodontic procedures, apexification, restorative procedures, periodontal defects, treatment of vertical and horizontal root fracture, apical barriers in teeth with necrotic pulps and open apices, perforation repair root canal filling, root-end filling during surgical endodontics, and for the treatment of external cervical root resorption or palatogingival grooves.[3-5]

Calcium silicate-based materials have gained popularity due to their resemblance to MTA, their better handling properties, and their lower cost. Different products have been launched, but the majority of the research focuses on Biodentine (BD).[1]

For example, in a study using extracted molars, the histological evaluation showed a minimal inflammatory reaction with MTA and BD, in which the materials provoked a response in the tissue without damage.[6] The overall conclusion was that BD might be considered as an alternative to MTA for pulp-capping.[3,4]

Moreover, tridimensional (3D) culture models suggest that biocompatibility and gene expression after BD or MTA stimulation are similar in dental pulp fibroblasts (PF), also indicating their use for direct pulp-capping.[4,7] BD was designed as a "dentine replacement material"; however, it has been used in a wide range of clinical applications, such as root perforations, apexification, resorptive lesions, and retrograde fillings, the majority of these presented

as case reports.[4,8] In particular, the use of BD for the treatment of external cervical root resorption or palatogingival grooves has been successful in individual cases.

However, to our knowledge, no clinical trials have been effectively conducted.[9] During treatments that require contact of the material with the soft tissue, the material has the potential to affect the proliferation and migration of connective tissue cells.

The material should also promote the regeneration of bone and gingival tissues, preventing the formation of granulation tissue. Therefore, to achieve tissue regeneration and the development of functional periodontal and dental tissues, cells such as fibroblast and pulp cells must migrate and adhere to target sites.[10-12]

Fibroblasts have different developmental origins, including the neural crest, the lateral plate mesoderm, and the dermatomyotome. Their differences in origin correlate with varying HOX gene expression patterns, and different types of fibroblasts exhibit different biological responses. In this regard, local biological reactions are mainly exhibited in pulp, gingival, and oral mucosa cells.[11]

Gingival fibroblasts have a higher sensitivity than established cell lines, such as L929, and they are more accessible to culture and have a higher survival rate than pulp fibroblasts.[13-15] Hence, the fibroblast model produces the majority of the extracellular matrix in connective tissue and is essential for wound-healing and tissue regeneration.

In addition, and despite the increasing literature regarding the effects of BD and MTA in proliferation and adhesion, studies on the effects on the migratory capability of cells stimulated with BD or MTA are scarce.[10-12]

Many studies have been conducted *in vitro*, confirming the biocompatibility of BD in dental PF and osteoblasts, but the results are contradictory and difficult to compare.[12-16] Also, the majority of *in vitro* studies are conducted for 7 days or less. Hence, there is a gap of information on the cytotoxicity of bioactive endodontic cements in primary cell lines in longer culture times (21 days).

In this study, we test the hypothesis of whether BD promotes proliferation at long culture times and the possible effect on migration in GF. The null hypothesis is that adhesion and proliferation at 21 days are unrelated to BD in GF stimulation. The second null hypothesis is that migration is unrelated to BD stimulation in GF.

In this regard, the present study aims to contribute to the analysis of the biological response of primary GF cells and conduct a long culture time experiment. For purposes of comparison, MTA and PF were also used in the present study.

MATERIALS AND METHODS.

Ethical requirements and sample calculation

The recollection of human dental PF and GF was previously reported, following the appropriate ethical requirements.[15] Briefly, the collection of cells was performed based on the ethical standards of the World Medical Association Declaration of Helsinki (as revised in Brazil 2013) and under the Mexican legislation for research in human subjects (NOM-012-SSA3-2012).

Written informed consent was obtained from the patients after receiving approval from the Research and Ethics Committee of the Faculty of Dentistry, UNAM (Number, CIE/1110/2017). For the experimental procedures, GF and PF were grown in Minimum Essential Medium, Alpha (Alpha-MEM; Corning, NY, USA) supplemented with 10% fetal bovine serum (FBS) (Sigma-Aldrich, St Louis, MO, USA) and 5% antibiotic–antimycotic solution (10,000 units/ml of Penicillin, 10,000 µg/ml of Streptomycin and 25 µg/ml of Fungizone (Gibco/Invitrogen, Gibco Life Technologies, Grand Island, NY, USA).

Experimental groups

The biological response of fibroblasts to the dental materials MTA-Angelus (MTA) (Angelus, Londrina, PR, Brazil) and BD (Septodont, St. Maur des Fosses, France) was analyzed according to the following experimental groups:

Group 1: MTA disks

Group 2: BD disks

Group 3: Control, plastic culture plate.

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The cements were prepared according to the manufacturer's instructions. The mixing cements were dispensed in prefabricated silicon molds in the shape of a disk, until setting (Figure 1).

The disks of MTA or BD were prepared with a diameter of 5 mm and a height of 2 mm. The disks were exposed to ultraviolet light (UV) for 30 min to prevent bacterial contamination (Figure 1). Each group consists of nine samples to perform three repetitions in triplicate.

Biocompatibility tests

To determine the effects of BD and MTA on the adhesion of GF and PF, the cells were seeded onto BD or MTA disks, and the results were compared with those for plastic culture plates. For all groups, 1 × 103 cells/ml were seeded and incubated for 4 hours and 24 hours. Briefly, GF or PF were seeded onto MTA or BD disks inserted into 96-well culture plates.

Then, the cells were allowed to adhere under standard culture conditions for 4 hours and 24 hours. Following the established experimental times, the cells were rinsed with phosphate-buffered saline (PBS) to remove non-adherent cells, and fixed with 4% paraformaldehyde for 4 hours at 4 °C.

Subsequently, the samples were rinsed with deionized water and incubated with a 100% methanol/0.1% crystal violet solution for 15 min. Finally, the dye was extracted with a 2% sodium dodecyl sulfate solution. Optical absorption was measured in a spectrophotometer at 595 nm (CHROMATE; Awareness Technology, Inc.).

Cells seeded in 96-well plates were used as a control. The background of the crystal violet was subtracted from disks treated with crystal violet and free of cells. After background correction, the results are presented as the percentage of adherent cells, according to the following formula:

% Adherent Cells = Optical Density (OD) in disks/ OD in control plate × 100.

Cell proliferation, MTT (methylthiazolyldiphenyl-tetrazolium bromide) salt powder (Sigma-Aldrich, St. Louis, MO, USA), which was dissolved in PBS (pH 7.4) to obtain a 5-mg/ml stock solution.

The GF or PF were seeded at a density of 1 × 104 cells/ml onto MTA or BD disks inserted into 96-well plates, and incubated for 3, 5, 7, 14, or 21 days. Then, 200 µl of fresh culture medium was added to each well, followed by 20 µl of MTT stock solution. The samples were incubated at 37°C in the dark for 4 h; subsequently, the supernatant was removed, and 150 µl of dimethyl sulfoxide (DMSO; Merck, USA, 102952) was added to each well. The OD value was measured at a wavelength of 570 nm using a spectrophotometer (CHROMATE; Awareness Technology, Inc.). Cells seeded in 96-well plates were considered as controls. All experiments were performed in triplicate and with three repetitions each time.

Wound-healing assay

A wound-healing assay was performed according to Kramer *et al.*,[14] Briefly, GFs or PFs were plated in 24-well plates at a density of 5 x 10^4 cells/well for 24 h. Then, the cells were starved with an FBS-free medium. After 24 h, the cells were exposed to different concentrations of MTA or BD (2 and 0.2 µg/ml), and at different times (0, 3, 6, 12, and 24 h) a vertical scratch was performed with the tip of a 200-µl pipette. Cells cultured with 10% FBS were used as controls.

Finally, cells were fixed with 2% glutaraldehyde and then stained with 0.5% crystal violet for 5 min. The experiment was performed three times for every condition and with two repetitions each time. Scratch area analysis was done with an inverted microscope equipped with digital analysis software (AmScopeX). Briefly, representative microphotographs at 4-magnification were taken of each well. The micro-photograph was taken in the center of the plate, from an area where the scratch was straight, or where the scratch showed parallel borders.

A total of six measures along the scratch were made for each image, and a total of 20 images were analyzed for each experimental group. Then, the mean of the results for each condition was calculated. The results are presented as a percentage of the wound

width. Significant results were found only after 24 hours of culture. Therefore, only the measures from 0 hours and 24 hours were considered for inclusion in the manuscript.

Statistical analysis

The studies for adhesion and proliferation were set up to include three wells per condition, and each experiment was independently repeated a minimum of three times. The studies for migration were set up to include two wells per condition, and each experiment was independently repeated a minimum of three times.

The data were analyzed with GraphPad Prism ver. 6.0c statistical software (GraphPad Software, San Diego, CA, USA) using repeated-measures ANOVA at different time intervals, followed by the Tukey test, to compare differences between groups, with the level of significance set at $p<0.05$, $p<0.01$ and $p<0.001$.

RESULTS.

Biocompatibility of MTA and BD

As shown in Figure 2, the results for the cellular-adhesion response of GF demonstrated that BD induced an increased response in GF during the first 4 h of culture. There was a positive effect on the adhesion of GF (146.94%) to BD disks (Group 2), compared to the control (100%) (Group 3), and

Figure 1. Graphic representation of the fabrication of Biodentine (BD) and mineral trioxide aggregate (MTA) disks. MTA was mixed with a 1:1 solution of cement powder and distilled water provided by the manufacturer. BD was mixed with 5 drops of distilled water in a capsule and mixed with an amalgamator. The cements were condensed in in prefabricated silicon molds for 24 h. Subsequently, the disks made of MTA or BD were inserted into the wells of culture plates and sterilized under ultraviolet (UV) light.

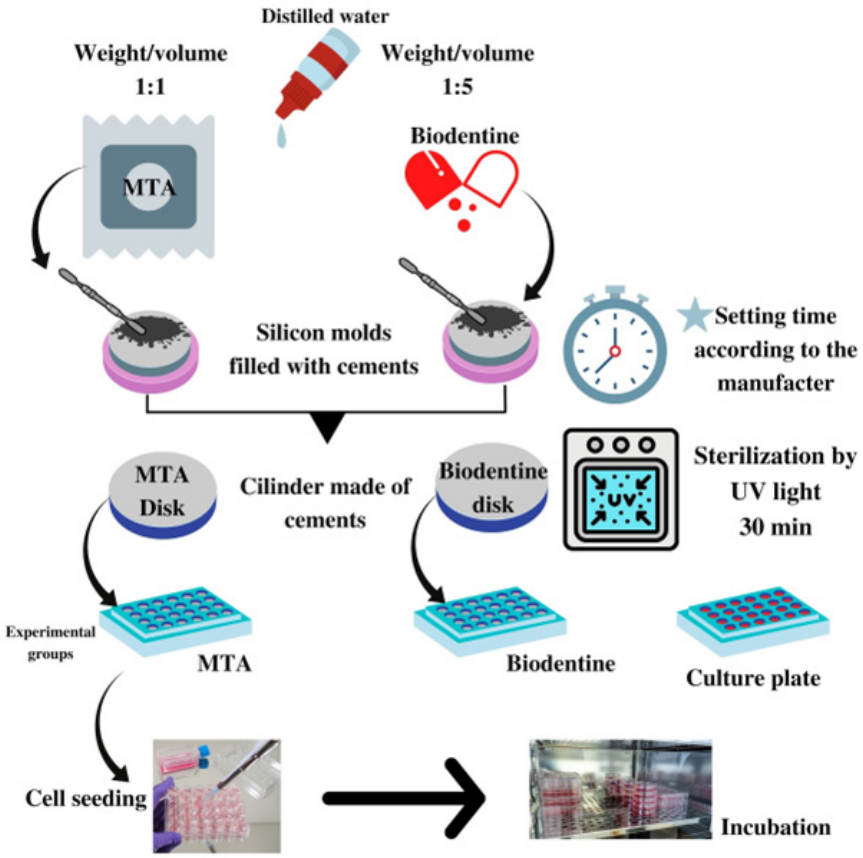

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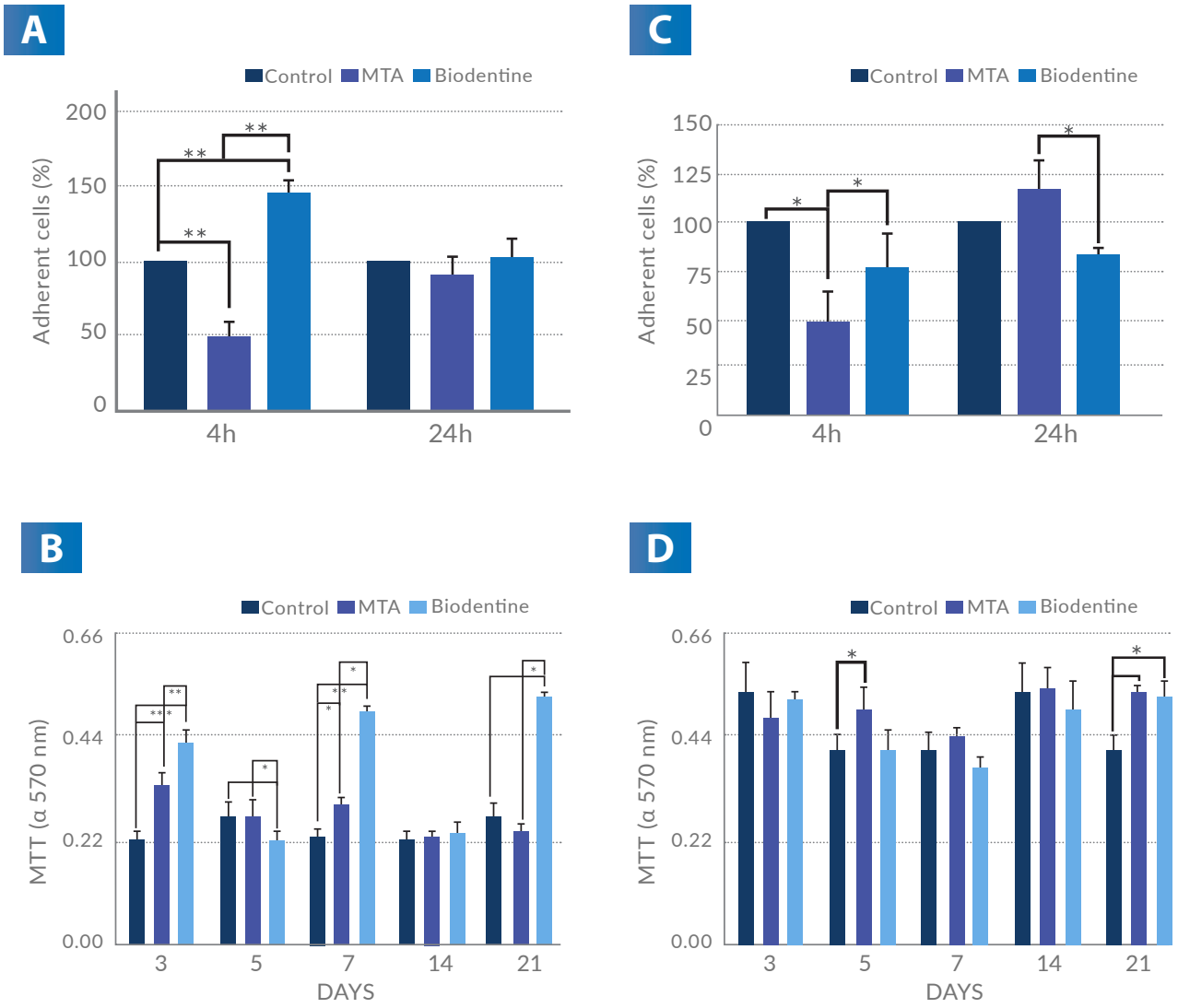
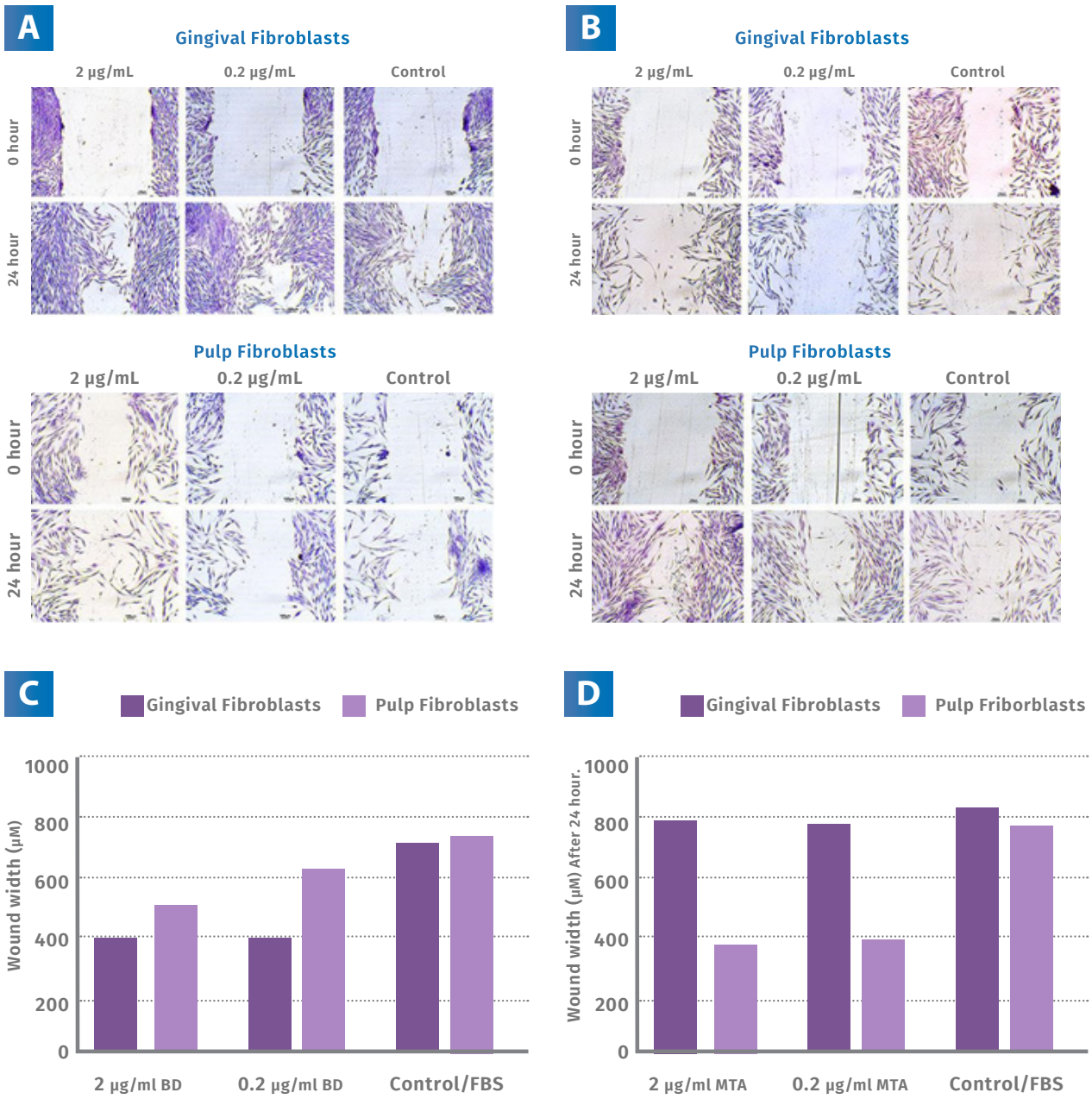
Figure 2. Analysis of adhesion and proliferation assays of gingival (GF) (A and B) and dental pulp fibroblast (PF) (C and D). GF or PF were seeded directly onto Biodentine (BD) or mineral trioxide aggregate (MTA) for 4 or 24 h. The response of PFs and GFs to the materials is different. As expected, PFs were more sensitive to exposure to dental materials than GFs; due to the localization of the cells, GFs are more exposed to environmental aggression of the oral cavity; hence GFs are more resistant.



A: There was a decrease in adhesion of GFs at 4 h; however, the differences disappeared 24 h after culture.
B: The proliferation of GFs seeded onto BD disks increased at 3, 5, 7, and 21 days of culture, compared to the control. The highest proliferation rate of GF seeded onto BD was observed after 21 days of culture.
C: In the adhesion assay of PF seeded onto BD or MTA disks. There was low adhesion at 4 h of culture; subsequently, the adhesion increased, with best results observed in MTA disks.
D: MTA disks stimulated the proliferation of PFs at 5 and 21 days of culture, compared with the control group. Also, at 21 days of culture, BD stimulated PF proliferation, which was higher than in the control group; there were no differences between the proliferation of MTA- and BD-stimulated PFs after 21 days of culture. (*) $p< 0.05$, (**) $p<0.01$, and (***) $p<0.001$; upper brackets indicate differences between experimental groups and the control, or between experimental groups.

Figure 3. Representative image of the density of 5 x 10^4 cells/well performed on gingival (GF) and pulp fibroblasts (PF) stimulated with 2 and 0.2 µg/ml Biodentine (BD) (A and C) or mineral trioxide aggregate (MTA) (B and D), and compared with 10% fetal bovine serum (FBS) stimulation (control) at 0 and 24 h after the scratch.



A: The largest filling area was observed in GF stimulated with 2 µg/ml BD. The GF migrated in a diagonal pattern, individually in collectively with a sheet pattern, and the majority of PF migrated individually, in a horizontal pattern.
B: GF and PF at 0 and 24 h after the scratch, and stimulation with 2 or 0.2 µg/ml MTA. GF stimulated with 2 µg/ml MTA showed individual cells migrating into the center of the wound, although there were no significant differences between MTA concentrations and the control. The smallest wound-width area as observed in PF stimulated with 0.2 µg/ml MTA, and the cells migrated in a diagonal and horizontal pattern. The filling area of the PFs stimulated with 2 µg/ml MTA was similar to that of the control after 24 h of incubation.
C: Graphic representation of wound width in µm after 24 h of stimulation with BD.
D: Graphic representation of wound width in µm after 24 h of stimulation with MTA.

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Table 1. Wound fill areas comparing MTA and Biodentine in vitro treatments.

		MINERAL-TRIOXIDE AGGREGATE						BIODENTINE					
		10% Fetal Bovine Serum		0.2 µg/ml		2 µg/ml		10% Fetal Bovine Serum		0.2 µg/ml		2 µg/ml	
		Wound with (µm)	Percentage of wound width. (%)	Wound with (µm)	Percentage of wound width. (%)	Wound with (µm)	Percentage of wound width. (%)	Wound with (µm)	Percentage of wound width. (%)	Wound with (µm)	Percentage of wound width. (%)	Wound with (µm)	Percentage of wound width. (%)
Gingival Fibroblasts	0 hour	1042.98	100	1039.59	100	1005.60	100	983.54	100	971.35	100	939.29	100
	24 hour	835.91	80.14	790.40	78.59	781.25	75.14	701.54	60.27	390.69	40.30	372.04	38.30
Pulp Fibroblasts	0 hour	1013.22	100	969.29	100	961.11	100	1102.43	100	1085.43	100	1094.20	100
	24 hour	781.25	77.10	493.76	50.94	409.60	42.61	728.85	66.11	635.98	58.59	510.59	46.66

µm: micrometers.

to MTA (48.83%) (Group 1) after 4 h of culture (Figure 2A).

However, after 24 hours of culture, adhesion was similar in MTA (92.03%) and BD (102.72%) disks and control plates (100%). As for PFs, adhesion decreased after 4 h of culture in MTA (48.72%) and BD disks (77.63%), compared to the control (100%) (Figure 2B). Subsequently, after 24 h of culture, the percentage of adhesion of PF seeded onto MTA (98.82%) and BD (84.14%) disks was similar to that in the control experiment (100%), with no statistical differences between groups. These results suggest that after 4 h of culture, the surviving PF cells could overcome the initial cytotoxic effect of BD and MTA materials.

The results of the MTT assay suggest that MTA and BD disks were not toxic to GFs or PFs. As presented in Figure 2, the proliferation of GFs increased constantly in BD disks (group 2), with better results observed after 3, 7, and 21 days of culture, compared to the control ($p< 0.001$ and $p< 0.01$) (Group 3).

Also, the proliferation of GF seeded onto MTA disks (Group 1) increased after 3 ($p<0.0001$) and 7 ($p<0.0001$) days of culture, compared to the control ($p<0.0323$, and $p<0.0013$). However, after 21 days, proliferation decreased in MTA disks, compared to the control and BD disks ($p<0.0481$).

Furthermore, GF seeded onto BD disks were statistically different to those in the control ($p>0.01$) and MTA groups ($p>0.05$) after 21 days of culture (Figure 2C). As for PF, intragroup comparisons revealed that MTA disks (Group 1) allowed for significant cell proliferation at 5 ($p> 0.0430$) and 21 ($p>0.0057$) days of culture (Figure 2).

Although no statistically significant differences were observed at 7 or 14 days of culture, a slight increased proliferation was observed in MTA disks (Group 1), (Figure 2). Cell survival of PF remained constant in both materials at different evaluation times, with superior results for MTA disks (Figure 2D). Interestingly, after 21 days of culture, the proliferation of PF seeded onto MTA (Group 1) and BD (Group 2) increased compared to the control (Group 3) ($p< 0.05$).

These results demonstrate that GF and PF maintained a stable proliferation rate when placed in contact with MTA and BD. Significant results were observed for GF seeded onto BD disks; however, PF exhibited a better interaction with MTA over a short and long culture time.

Monolayer Wound-healing Assay

The migration of GF and PF treated with BD or MTA was determined based on the pattern of migration and wound width. The wound-healing assay for GF and PF showed that neither GF nor PF exhibited a significant migration rate at 3, 6, and 12 hour of culture (data not shown). Table 1 summarizes the results of the wound-healing assay.

GF treated with 2 and 0.2 µg/ml BD demonstrated

the best migration motility and wound closure after 24 hours compared with the FBS control. Mean wound width was 372.04 µm and 390.69 for 2 and 0.2 µg/ml BD, respectively, and the control showed a mean wound width of 701.71 µm. The migration of GF stimulated with MTA 2 µg/ml, 0.2 µg/ml MTA, and FBS showed a mean wound width of 781.25, 790.40, and 835.91 µm, respectively, after 24 h of culture (Figure 3).

As for PF, 2 µg/ml MTA in the medium resulted in a smaller unclosed area (409.60 µm), compared with 0.2 µg/ml (493.76 µm) and FBS (781.25 µm) after 24 h; 2 µg/ml BD in PF reduced the mean wound width (510.59 µm), compared with the concentration of 0.2 µg/ml (635.98 µm) and the FBS control (728.85 µm) after 24 h (Figure 3).

The wound-closure response of GFs reveals that the cells migrate into a central area both individually and in the form of cell sheets and replenish the wound area from the center to the edges of the wound. In contrast, PF exhibited a predominantly horizontal pattern along all wound edges, mostly individual cells moving from all the edges of the wound to the center (Figure 3).

DISCUSSION.

BD has emerged as a material with properties comparable to those of MTA. However, studies comparing MTA with BD in the context of their clinical applications are scarce and varied in methodology.[10,12,15] The majority of the available literature regarding the biocompatibility of BD and MTA favors the materials, considering them as non-toxic and supporters of tissue-healing and repair.[6] Also, *in vitro* evaluations consistently report that BD and MTA can increase the cell proliferation and biomineralization of dental pulp cells.[6-17]

In addition, the cells' features are important to consider; previously reported data showed that GF have distinct functional activities during the regeneration and repair of periodontal tissues, and that PF can differentiate to diverse cell types, including osteoblast, depending on the external stimuli.[15-18]

In the present study, we used an *in vitro* model to compare the effects of MTA and BD in GF, and compared the results with the effects on PF.

The present findings demonstrate the response of GF and PF to BD after short and long culture times (Figure 2B). The results exhibit light toxicity in the first hours after seeding GF and PF, which disappeared after 24 hours of culture, and this is consistent with previously reported research.[19-21] It has been suggested that MTA and BD release toxic substances before complete setting, and that the toxicity disappears with time.[4-22] In this regard, few studies have evaluated the cytotoxic effects of bioactive endodontic cements on primary cell lines at 168 hours.

To the best of our knowledge, our study is the first one to evaluate the effects after 21 days, and it is unique in this respect.[23] Mozayeni *et al*.,[24] exposed L929 fibroblasts to BD for 1, 24 and 168 h of culture; the viability increased with no statistical differences. Saberi e*et al*.,[23] found that MTA increases the viability in GF after 24h, and the results in the present study confirm their observations.

The setting time for BD cement is observed after 1 day, and the initial cytotoxic effects of BD are related to the immediate and abundant release of calcium hydroxide.[25]

The MTA and BD final setting might explain the difference in adhesion at a short culture time. It has been reported that MTA's final setting time is up to 175 min and 10.1 min for BD.[20,26-28] Also, soluble products derived from the MTA or BD could cause damage to the cells. Once the soluble products are no longer available, the cell adhesion is facilitated.

Nunez *et al*.,[22] reported similar findings and showed that the viability of 3T3 fibroblasts seeded onto coverslips coated with BD and MTA after 1, 3, and 7 days was similar to the control culture plate. In addition, Zhou *et al*.,[16] found that GFs exposed to BD and MTA have no differences from the control in terms of cytotoxicity at 1, 3, and 7 days of culture.

The prolonged incubation period of GF and PF in the present study favors a homogeneous reaction at 14 days of culture.

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This result could be related to the low metabolic activity of GF and PF, rather than to a proliferation activity. However, to confirm this hypothesis, more molecular studies are needed. For example, apoptosis and reactive oxygen species production assays and the function of GF, such as collagen production and inflammatory-markers expression, need to be confirmed.

Also, an *in vivo* model is required to assess the clinical effect of BD. Our results demonstrated that MTA and BD provide an excellent surface for stimulating cell attachment and proliferation of GF and PF. The present results agree with the results by Zhou *et al.*,[16] who suggested that BD and MTA surfaces support GF cell adhesion and growth, which is more favorable than the results obtained with glass ionomer surfaces.

The results presented herein also demonstrate that the biological response of GF is more favorable for BD, and that PF interacts better with MTA. The results of the study are in agreement with the findings reported by Zanini *et al.*,[29] who evaluated the cytotoxicity of BD against immortalized murine pulp cells.

Briefly, BD initially inhibits cell proliferation in the first 2 days; then, the proliferation increased, and in our study, this was constant until 21 days of culture. The response of PF and GF to the materials is different and might depend on the cell's characteristics.

For example, PF stimulation supports differentiation into osteogenic/odontogenic cells and promotes the formation of mineralized extracellular matrix (ECM). Hence, the composition of MTA favors mineral-producing cells, consequently aiding in the formation of the dentin/pulp-like complex. In addition, the PF can stimulate angiogenesis of endothelial cells.[30-32]

In the case of GF, previously reported research demonstrated that these cells could suppress the activation and function of inflammatory Th1 and Th17 cells and promote regulatory T-cell proliferation. The combined effects of GF on both macrophages and T cells suggest that GF could be employed for several inflammatory-disease models, such as colitis, contact-allergic-dermatitis, oral mucositis, and skin wound-healing.[30]

Moreover, the results of the present study showed continuous cell growth during 21 days of culture in MTA and BD disks, suggesting that neither material is cytotoxic and that both materials are biologically compatible and better than the control used. These results are in concordance with previously published research.[20-26]

Studies on the effects of MTA and BD on cell migration are scarce.[18,26,33,34] The concentrations used in this study were selected based on similar, previously reported studies. In this regard, it has been suggested that concentrations over 2 µg/ml of MTA extract had toxic effects on mammalian cells. Also, the intermediate concentration of 1 mg/ml of MTA was found to favor viability. Therefore, we selected a low concentration of 0.2 µg/ml and a previously reported optimal concentration for PF of 2 µg/ml. In the present study, the optimal concentration for stimulating migration was also 2 µg/ml for both materials.

The existing research suggests that there is a significant increase in the migration ability of dental-pulp stem cells after stimulation with 0.2 or 0.02 µg/ml BD, compared with an FBS control.[33,34] The results from the wound-healing assay of the present study revealed that BD and MTA at concentrations of 0.2 and 0.02 µg/ml enhance the migration of GF and PF after 24 h. However, the effects of BD were superior in GF. Luo *et al.*,[33] demonstrated similar results for BD in human dental-pulp stem cells, corroborating the material's ability to stimulate cell migration.

PF stimulated with 2 µg/ml MTA demonstrated a higher number of replenished wound areas than 0.2 µg/ml MTA and the control, or BD stimulation. The results suggest that MTA is better at stimulating the migration of PF.

Though the interactions between capping materials and injured pulp tissue are not well understood, the evidence suggests a role of growth factors, with TGF-β1 playing a major role.[1] The

BD cement can modulate TGF-β1 secretion in PF, inducing an early form of reparative dentin-like synthesis. BD stimulates the reparative response, *i.e.*, the migration of inflammatory cells.[31]

The results of the migration assays might also reflect the characteristics of the cells, *i.e.*, PF are committed to odontoblast differentiation and do not require mobile capabilities. On the other hand, GF are related to the immune response, in which migration is a key feature of the cells involved.

CONCLUSION.

The majority of studies regarding newly introduced bioactive endodontic cements have used MTA for comparison. Consequently, present evidence in English literature is insufficient for reaching a consensus.

However, the present study confirmed that MTA and BD are favorable materials for dental applications. Also, the results support their reduced cytotoxicity and enhance bioavailability at a long culture time.

In this regard, the results of the present in-vitro study validate the clinical effects of BD and suggest several mechanisms of action. For example, BD has a limited impact on the cellular proliferation of fibroblasts derived from dental pulp, but a concentration of 2µg/ml stimulates the migration of these fibroblasts. Also, in the present study BD increased the migration of GF along the edges, in an oblique and collective pattern, an effect that is not achieved with MTA, nor with FBS stimulation.

Moreover, PF revealed better adaptation to MTA, while GF showed better adaptation to BD.

Proper migration of connective tissue cells and adequate signals that stimulate healing, rather than chronic inflammation, can reduce the formation of granulation tissue.

Hence, the positive effects of BD on the migration, proliferation, and adhesion of GF suggest a possible advantage of the material depending on its clinical application. More studies on the effect of BD on the migration of human fibroblasts may expand the findings of this *in vitro* study.

As for the present study, the results suggest that BD, at a concentration of 2 mg/mL is biocompatible with PF cells and can be employed as root-filling material, due to its involvement in the proliferation, migration, adhesion, and differentiation of the PF cells. The limitations of this study lie in that the models for evaluating cell growth do not reproduce the same conditions as in the oral cavity.

Studies with 3D culture models are needed to confirm an absolute advantage of BD over MTA regarding GF migration, and to support the clinical application of the cement in the treatment of different endodontic treatments.

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All authors declare no conflict of interest.

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Authors' contributions:

Vazquez-Vazquez F: Was involved in the conceptualization, formal analysis, investigation, writing original draft, review and editing of the final draft, and funding acquisition.

Rodríguez-Hidalgo A: Contributed to review and to edit the final draft.

Suaste-Olmos F: Contributed substantially to methodology, investigation, and review and editing of the final draft.

Alvarez-Perez M: Was involved in the review and editing of the final draft and funding acquisition.

González-Alva P: Was involved in the conceptualization, formal analysis, data curation, investigation, writing original draft, and review and editing the final draft.

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