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Research Article

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Biological assessment of a new ready-to-use hydraulic sealer

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No potential conflict of interest relevant to this article was reported.

ABSTRACT

Objectives: This study compared the cytotoxicity, biocompatibility, and tenascin immunolabeling of a new ready-to-use hydraulic sealer (Bio-C Sealer) with MTA-Fillapex and white MTA-Angelus.
Materials and Methods: L929 fibroblasts were cultivated and exposed to undiluted and diluted material extracts. Polyethylene tubes with or without (the control) the materials were implanted into the dorsa of rats. At 7 days and 30 days, the rats were euthanized, and the specimens were prepared for analysis; inflammation and immunolabeling were measured, and statistical analysis was performed ($p < 0.05$).
Results: MTA-Fillapex exhibited greater cytotoxicity than the other materials at all time points ($p < 0.05$). The undiluted Bio-C Sealer exhibited greater cytocompatibility at 6 and 48 hours than white MTA-Angelus, with higher cell viability than in the control ($p < 0.05$). White MTA-Angelus displayed higher cell viability than the control at 24 hours, and the one-half dilution displayed similar results at both 6 and 48 hours ($p < 0.05$). At 7 days and 30 days, the groups exhibited moderate inflammation with thick fibrous capsules and mild inflammation with thin fibrous capsules, respectively ($p > 0.05$). At 7 days, moderate to strong immunolabeling was observed ($p > 0.05$). After 30 days, the control and MTA-Fillapex groups exhibited strong immunolabeling, the white MTA-Angelus group exhibited moderate immunolabeling ($p > 0.05$), and the Bio-C Sealer group exhibited low-to-moderate immunolabeling, differing significantly from the control ($p < 0.05$).
Conclusions: Bio-C Sealer and white MTA-Angelus exhibited greater cytocompatibility than MTA-Fillapex; all materials displayed adequate biocompatibility and induced tenascin immunolabeling.

Keywords: Biocompatibility; Cytotoxicity; Hydraulic sealer; Mineral trioxide aggregate; Tenascin

INTRODUCTION

Mineral trioxide aggregate (MTA) is widely used in the development of calcium silicate materials due to its relative biocompatibility and sealing properties [1]. These materials,

Author Contributions

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known as bioceramics or hydraulic sealers, are highly bioactive, capable of promoting cell differentiation and inducing osteoconductive effects [1,2].

Hydraulic materials are composed of tricalcium and dicalcium silicate, or tricalcium aluminate [3], and can produce hydroxyapatite [4]. This enables a functional integration with dentin [5], favoring the clinical use of hydraulic materials as either restorative materials or sealers for root canal fillings.

MTA-Fillapex (Angelus, Londrina, PR, Brazil) is a hydraulic sealer that has been extensively studied [6-8]. This sealer contains MTA particles with dicalcium and tricalcium silicate, resin (salicylate resin and diluted resin) [9], and bismuth oxide as a radiopacifier (**Table 1**). MTA-Fillapex was developed with the objective of combining the physicochemical properties of a root canal sealer and the bioactivity of MTA [6]. However, despite its satisfactory dimensional changes, radiopacity, and solubility, no consensus has been reached regarding its biocompatibility relative to other sealers [7,8]. For instance, high cytotoxicity of MTA-Fillapex was observed in *in vitro* studies, owing to its inability to release the ions necessary for apatite formation [7,8]. Studies have attributed these results to the presence of resin in the composition of MTA-Fillapex [10,11]. In contrast, an *in vivo* analysis has demonstrated that this material is biocompatible and capable of inducing biomineralization in rat connective tissue [9].

In the search for an ideal sealer material, several bioceramic materials have been introduced to the market [12]. To this end, a new hydraulic sealer was developed containing silicates, aluminate, calcium oxide, ferric oxide, zirconium, and silicon dioxide in addition to other dispersing agents (**Table 1**). This sealer, known as Bio-C Sealer (Angelus), is available in a syringe, with no need for spatulation.

Studies using rat subcutaneous tissue have highlighted important results regarding the effects of different endodontic materials on markers of inflammation and biomineralization [13-16]. For example, a comparative analysis of collagen maturation or fibroblast growth factor in rat subcutaneous tissue revealed that materials with different compositions could influence tissue response [17]. Furthermore, studies have shown that MTA could induce the expression of tenascin [18,19], a non-collagenous glycoprotein of the extracellular matrix that plays important roles in migration, adhesion, cell proliferation [18,20], and mineralization

Table 1. Composition of the materials and manufacturers' instructions for use

Material	Composition	Instructions for use
MTA Fillapex	· Salicylate resin, diluting resin, natural resin, bismuth trioxide, nanoparticulated silica, MTA	· Dual syringe: Press plunger to extrude material directly onto a glass slab, onto a mixing pad, or into the tooth canal. The cement should be used immediately after mixing. · Tubes: Mix equal-volume units (1:1). Mix for 30 seconds to attain a homogeneous consistency.
Bio-C Sealer	· Calcium silicates, calcium aluminate, calcium oxide, zirconium oxide, iron oxide, silicon dioxide and dispersing agent	· Ready for use.
White MTA-Angelus	· Powder: tricalcium silicate, dicalcium silicate, tricalcium aluminate, calcium oxide, bismuth oxide · Liquid: distilled water	· For 30 seconds, mix the content of 1 sachet of MTA-Angelus (or 1 spoon of MTA-Angelus) with 1 drop of distilled water. The final mixture should be homogeneous and have a consistency similar to wet sand.

MTA, mineral trioxide aggregate.

[18,21]. Therefore, in addition to indicating a tissue healing process, the expression of this protein may induce biomineralization. However, these phenomena have not been evaluated for endodontic sealers.

Despite its ease of clinical handling and the considerable attention that Bio-C Sealer has attracted in endodontics, its manufacturers do not provide information regarding the sealer's filler material, pigments, and diluents. For this reason, an initial analysis of its components is challenging. Thus, the aim of the present study was to evaluate the cytotoxicity, biocompatibility, and potential of Bio-C Sealer for the immunolabeling of tenascin compared to MTA-Fillapex and white MTA-Angelus.

MATERIALS AND METHODS

In vitro study: cell viability assay

The Bio-C Sealer used in this study was acquired in ready-to-use form, while the MTA-Fillapex and white MTA-Angelus materials were mixed according to the manufacturers' recommendations (**Table 1**). The material discs were prepared using sterile and cylindrical polyethylene tubes (diameter and height, 5 and 3 mm, respectively) [13,22] and incubated at 37°C and 100% relative humidity for 6 hours. These were then sterilized using ultraviolet irradiation for 1 hour, and material extracts were prepared according to International Organization for Standardization (ISO) specifications [13,22,23]. Three different dilutions (undiluted, one-half, and one-fourth) of the material extracts were used.

For the viability assay, fibroblasts from the L929 cell line were cultured under standard cell culture conditions using Dulbecco's modified Eagle medium (DMEM; Sigma-Aldrich, St. Louis, MO, USA) supplemented with fetal bovine serum (10%, Sigma-Aldrich), streptomycin, and penicillin (temperature, 37°C; humidity, 100%; air, 95%; and CO_2, 5%). Cells were subsequently seeded and incubated in 96-well plates (Sigma-Aldrich) at 10^4 cells/well for 24 hours under standard conditions. Next, serial extract dilutions were prepared using either the culture or DMEM without material extracts (the control). After 6, 24, and 48 hours, a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma-Aldrich) assay [13,22] was performed to determine cell viability. Each condition was analyzed in triplicate.

In vivo study

1. Biocompatibility analysis

Sixteen 2-month-old male Wistar rats (each weighing approximately 280 g) were used in the biocompatibility assay. Previous studies were used to determine the sample size that should be used [13,22]. The animals were housed in a controlled environment (22ºC–24ºC and a light cycle of 12 hours light/dark), with water and food provided *ad libitum*. All experimental procedures were approved by the ethics committee of the local institution (CEUA 937-2017) and were performed in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (Bethesda, MD, USA).

A total of 48 polyethylene tubes (Abbott Laboratories, São Paulo, SP, Brazil) filled with Bio-C Sealer, MTA-Fillapex, or white MTA-Angelus, as well as 16 empty tubes (used as the control), were randomly and immediately implanted into the rat subcutaneous tissue. Each rat received 4 tubes: 3 material-filled tubes and 1 empty tube. For this purpose, the rats were anesthetized, their dorsa were shaved, and a 2.0-cm incision was made in a head-to-tail

orientation with a no. 15 blade; the skin was reflected to create 2 pockets on the right and left sides, after which the randomly selected tubes were implanted [13,22].

After 7 and 30 days, the rats (n = 8) were euthanized with an overdose of an anesthetic solution. The tubes, along with the surrounding tissue, were removed and fixed in a neutral formalin solution. Then, the specimens were processed and embedded into paraffin. The blocks were oriented parallel to the long axis of the tubes, and longitudinal serial sections of 5-μm thickness were obtained from the central areas of the implants. The histological sections were stained using hematoxylin and eosin.

The inflammatory response in the tissues that were in contact with the material in the tube insertion region was evaluated as follows [13,22]: 1) no/few inflammatory cells, no inflammation; 2) < 25 cells, mild inflammation; 3) 25–125 cells, moderate inflammation; and 4) ≥ 126 cells, severe inflammation. Fibrous capsules < 150 μm were considered thin, while those ≥ 150 μm were considered thick. The specimens were examined by a single calibrated operator in a blinded manner using light microscopy (DM 4000 B; Leica Microsystems, Wetzlar, Germany).

2. Immunohistochemical analysis

Immunohistochemical analysis was performed using an indirect immunoperoxidase technique [24]. The histological sections were deparaffinized using xylene and hydrated in ethanol. Antigen retrieval was performed using a citrate buffer solution (Antigen Retrieval Buffer; Spring Bioscience, Pleasanton, CA, USA) in a pressurized chamber (Decloaking Chamber™; Biocare Medical, Concord, CA, USA). The sections were then immersed in 3% H_2O_2 solution (for 1 hour and 20 minutes) and in 1% bovine serum albumin (for 12 hours) to block endogenous peroxidase activity and non-specific sites, respectively. Next, the sections were incubated with the primary antibody anti-tenascin (rabbit; Sigma-Aldrich) for 24 hours, after which they were incubated with biotinylated secondary antibody (1 hour and 30 minutes) and streptavidin-horseradish peroxidase conjugate (1 hour and 30 minutes) (Universal Dako labeled streptavidin-biotin kit; Dako Laboratories, Santa Clara, CA, USA). The reaction was developed using the chromogen 3,3′-diaminobenzidine tetrahydrochloride (DAB Chromogen kit; Dako Laboratories) and counterstained with hematoxylin. The negative controls consisted of specimens that underwent the previously described procedures without treatment with the primary antibody.

Analyses were performed by a single calibrated and blinded operator using light microscopy (×400 magnification; DM4000 B). The immunolabeling of tenascin was defined as the presence of a brownish color in the cytoplasm of the cells and extracellular matrix. A semi-quantitative analysis revealed the numbers of immunolabeled cells and immunolabeling intensity of the extracellular matrix, as follows [24]: 1) no immunolabeling; 2) mild immunolabeling; 3) moderate immunolabeling; 4) strong immunolabeling; and 5) very strong immunolabeling.

Statistical analysis

The data regarding cytotoxicity were statistically analyzed using 2-way analysis of variance, followed by the Bonferroni correction. The Kruskal-Wallis and Dunn tests were used to analyze biocompatibility and immunohistochemical data, and p values of < 0.05 were considered to indicate statistical significance.

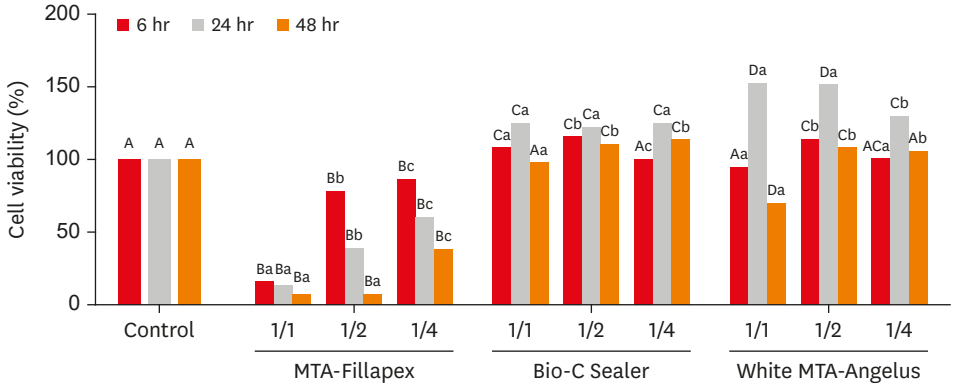

Figure 1. Cell viability for MTA-Fillapex, Bio-C Sealer, and white MTA-Angelus.
[A-D]The same uppercase letters indicate a lack of statistically significant differences among the materials at the same period and in the same dilution. [a-c]The same lowercase letters indicate a lack of statistically significant differences among extract dilutions of the same material at the same time point.

RESULTS

In vitro study: cell viability assay

Data regarding cell viability are shown in **Figure 1**. MTA-Fillapex decreased the cell viability (undiluted, one-half, and one-fourth dilutions) at all time points compared to the control group ($p < 0.05$). In contrast, Bio-C Sealer was associated with increased cell viability relative to the control at most time points ($p < 0.05$). At 24 hours, all white MTA-Angelus extracts increased the cell viability compared to the control ($p < 0.05$); similar results were obtained at 6 hours and 48 hours for the one-half dilution ($p < 0.05$).

At the same dilutions of the material, MTA-Fillapex exhibited greater cytotoxicity than Bio-C Sealer and white MTA-Angelus at all time points ($p < 0.05$). The undiluted white MTA-Angelus displayed lower cell viability than Bio-C Sealer ($p < 0.05$), except at 24 hours. Regarding the one-half and one-fourth dilutions of white MTA-Angelus, the overall outcome was similar to that observed with Bio-C Sealer at most time points ($p > 0.05$).

Comparing the different dilutions of the same material, we observed a greater decrease in cell viability with more concentrated extracts of MTA-Fillapex ($p < 0.05$). Lower cell viability was also observed for the undiluted Bio-C Sealer compared to its one-half dilution at 6 hours and 48 hours and its one-fourth dilution at 48 hours ($p < 0.05$). In addition, the one-half dilution of white MTA-Angelus exhibited higher cell viability at 6 hours and 48 hours than the undiluted extract; similar findings were observed when the one-half dilution was compared to the one-fourth dilution at 6 hours and 24 hours ($p < 0.05$).

In vivo study

1. Biocompatibility analysis

Images of tissue responses are shown in **Figure 2A-H and M-T**, and the histological analysis is summarized in **Table 2**. After 7 days, moderate infiltration of polymorphonuclear cells, macrophages, and multinucleated giant cells was observed in the thick fibrous capsules in all groups ($p > 0.05$).

After 30 days, most specimens of the groups exhibited mild inflammation with the presence of polymorphonuclear cells and a small number of lymphocytes. Some specimens from the

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control and Bio-C Sealer groups displayed no inflammation, whereas some MTA-Fillapex specimens displayed moderate inflammation. However, no significant differences were present among the groups ($p > 0.05$), and the fibrous capsules were thin in this period in all specimens.

2. Immunohistochemical analysis

The images of immunolabeled tenascin are depicted in **Figure 2I-L and U-X**, and the data are summarized in **Table 2**. The immunolabeling was observed in the cytoplasm of fibroblastic cells and principally in the extracellular matrix. After 7 days, most specimens in the control group displayed strong immunolabeling, whereas those in the other groups exhibited

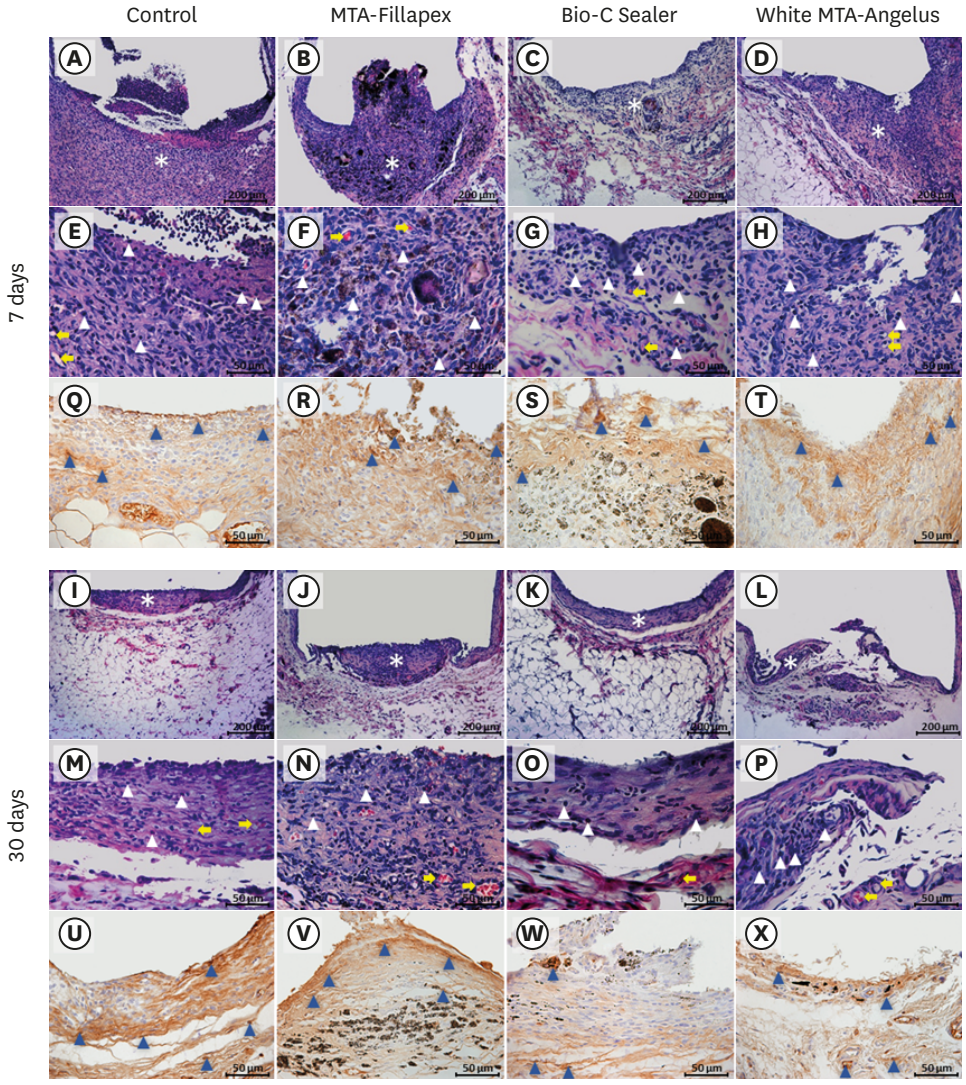

Figure 2. Representative images of the tissue response in terms of inflammatory infiltration and immunolabeling of tenascin. (A-L) Under the highest magnification, the white arrowheads indicate inflammatory cells, the yellow arrows indicate blood vessels, and asterisks indicate the fibrous capsule. The blue arrowheads indicate immunolabeling in the cytoplasm of the cells and in the extracellular matrix. At 7 days: (A, E) control, (B, F) MTA-Fillapex, (C, G) Bio-C Sealer, and (D, H) white MTA-Angelus displaying mild inflammatory cell infiltration and a thick fibrous capsule; (I) the control group with strong immunolabeling and (J) MTA-Fillapex, (K) Bio-C Sealer, and (L) the white MTA-Angelus groups with moderate immunolabeling. (M-X) At 30 days: (M, Q) the control, (N, R) MTA-Fillapex, (O, S) Bio-C Sealer, and (P, T) white MTA-Angelus, with mild inflammatory infiltration and a thin fibrous capsule; (U) control and (V) MTA-Fillapex groups with strong immunolabeling, (W) Bio-C Sealer with low immunolabeling, and (X) white MTA-Angelus with moderate immunolabeling. (A-D, M-P) The histological sections were stained using hematoxylin and eosin staining (×100 magnification). (E-H, Q-T) The histological sections were stained using hematoxylin and eosin staining (×400 magnification). (I-L, U-X) Results of the immunohistochemical analysis with regard to tenascin (×400 magnification).

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Table 2. Scores for inflammation and tenascin expression, as well as thickness of the fibrous capsule

Time/*p*-value	Group	Scores for inflammation				Median	Scores for tenascin					Median	Fibrous capsule thickness	*n*
		1	2	3	4		1	2	3	4	5			
7 days	Control^{Aa}	0	1	4	3	3	0	2	2	4	0	4	Thick	8
$^{*}p = 0.521$	MTA-Fillapex^{Aa}	0	2	4	2	3	0	0	4	2	2	3	Thick	
$^{\dagger}p = 0.553$	Bio-C Sealer^{Aa}	0	3	4	1	3	0	1	4	3	0	3	Thick	
	White MTA-Angelus^{Aa}	0	2	5	1	3	0	2	4	1	1	3	Thick	
30 days	Control^{Aa}	2	4	2	0	2	0	0	3	5	0	4	Thin	8
$^{*}p = 0.082$	MTA-Fillapex^{Aab}	0	6	2	0	2	0	1	3	4	0	4	Thin	
$^{\dagger}p = 0.017$	Bio-C Sealer^{Ab}	2	6	0	0	2	0	4	4	0	0	2.5	Thin	
	White MTA-Angelus^{Aab}	0	4	4	0	2.5	0	2	4	2	0	3	Thin	

^{A}The same uppercase letters indicate a lack of a statistically significant difference among the groups with regard to inflammation. ^{a,b}The same lowercase letters indicate a lack of a statistically significant difference among the groups with regard to the immunolabeling of tenascin ($p > 0.05$).
$^{*}p$ value obtained in the statistical analysis of inflammation, in each analysis period; $^{\dagger}p$ value obtained in the statistical analysis of tenascin immunolabeling, in each analysis period.

moderate immunolabeling. However, no significant differences were present among the groups ($p > 0.05$).

After 30 days, the control and MTA-Fillapex specimens displayed strong immunolabeling, whereas the white MTA-Angelus specimens exhibited moderate immunolabeling. However, the specimens in the Bio-C Sealer group exhibited low-to-moderate immunolabeling, with a significant difference between the control and Bio-C Sealer groups ($p < 0.05$).

DISCUSSION

The clinical use of hydraulic sealers has become increasingly common. The present study involved the analysis of the cell viability and biocompatibility of MTA-Fillapex, as well as those of a new formulation of a calcium silicate sealer, the Bio-C Sealer. White MTA-Angelus was used as a reference due to its known cytocompatibility and biocompatibility. In this study, MTA-Fillapex showed similar cytotoxicity to that displayed by the control group and the other materials assessed. In general, Bio-C Sealer and white MTA-Angelus exhibited excellent cytocompatibility. No significant difference was present between the materials in the histological analysis, and all of the materials were considered to be biocompatible. Nevertheless, compared with the control group, the Bio-C Sealer group displayed lower tenascin immunolabeling after 30 days.

The cytotoxicity of MTA-Fillapex has been previously evaluated using different cell types, including primary human osteoblasts [25], cells of the human periodontal ligament [8], NIH/3T3 cells [1], and human dental pulp stem cells [26]. In the present study, L929 fibroblasts were used. The undiluted and different dilutions of the material extracts were used in a similar fashion to previous studies (ISO 10993-5:2009) [13,22,23]. After the insertion of the material in the tissue, the extracellular fluids gradually eliminate leachable compounds, and their local concentrations progressively decrease as represented by the distinct dilutions [13,27].

In a previous study, the cytotoxicity of MTA-Fillapex was detected in the form of a defective organization of the F-actin cytoskeleton. This structure controls cell surface motion, which is important for cell proliferation and differentiation [28]. However, the formation of a slower-growing mineralized matrix with functional activity was observed [11]. According to the authors of that study, cytotoxicity occurred most prominently during the adjustment

period; however, recovery of the cells and mineralization of the matrix were observed later. In contrast, in the present study, cytotoxicity was observed after adjustment of the material, as the extracts were obtained after this stage.

Moreover, when in contact with osteoblast cells in a previous study, MTA-Fillapex exhibited cytotoxicity in a fresh as well as in a set state [25], which corroborates our *in vitro* results, but contradicts our *in vivo* results, in which the material was used while still fresh. We must consider that the negative results obtained with cell studies can be influenced by the conditions of *in vitro* studies. In this case, the analysis was performed in cellular monolayers, whereas in *in vivo* studies, it was conducted on tissues with a continuous flow of fluid, which may favor the biocompatibility of the material [11]. In addition, a 3-dimensional system can be more appropriated than the traditional 2-dimensional system for *in vitro* analysis [29], which is a limitation of this study.

For the *in vivo* analysis, we implanted tubes filled with the evaluated materials (as well as empty tubes) into the subcutaneous tissue of rats, in the method recommended by ISO 10993 [30]. This experimental condition simulated the reactions of the periapical tissues when in contact with a still-fresh sealer. A previous study reported that MTA-Fillapex resulted in reduced irritation of the subcutaneous tissue of rats after 60 days, with the fibrous capsule containing some fibroblasts and a few inflammatory cells [31]. These results are consistent with those of the present study, in which we observed some inflammatory cells, most notably after 7 days. Consequently, the fibrous capsule was observed to be thin after 30 days, in contrast to the thick capsule observed at the 7-day mark.

Thus, MTA-Fillapex has a limited toxic effect on living tissues, while other endodontic sealers are capable of releasing toxic components for a prolonged period. Similarly, a zinc oxide-eugenol–based sealer has been shown to provoke strong irritation in the living tissues over time [13,31], while MTA-Fillapex was associated with lower immunoexpression of interleukin-6 in an *in vivo* analysis relative to other sealers, such as AH Plus [31].

The presence of resins has been associated with the cytotoxicity of MTA-Fillapex [10,11], which has been attributed to the release of arsenic [10]. Arsenic is a heavy metal that is capable of inducing genotoxicity [32]. Furthermore, aluminum was found to be present in the circulation of rats after the use of MTA-Fillapex, and this increased the levels of oxidative stress in the erythrocytes and livers of these animals [33,34]. However, aluminum release was observed with MTA-Angelus [34], even though this material generally has high cytocompatibility. Based on these results, the ability of a material to release aluminum cannot be considered to be related to its level of cytotoxicity, and further studies are needed to reach a firm conclusion.

In addition, in the present study, MTA-Fillapex allowed the expression of tenascin, which is associated with tissue repair as well as cellular motility [21]. Studies have indicated that tenascin is necessary for mineralization [18,20]. Although the present study was conducted in connective tissue with an absence of cells suitable for biomineralization, such as odontoblasts or osteoblasts, the immunolabeling of tenascin indicated not only a repair process, but also the induction of biomineralization [18,21].

In alignment with the results of this study, previous studies have reported good cell viability with MTA-Angelus in L929 fibroblasts [22] as well as in other cell types, such as dental pulp

stem cells [26], human dermal fibroblasts [35], and odontoblast-like cells [36]. Moreover, the tissue biocompatibility of white MTA-Angelus has been previously demonstrated [22]. However, no study has evaluated the biological properties of the new hydraulic Bio-C Sealer, which makes comparison difficult. Regarding the physicochemical properties of this sealer, a recent study revealed a short setting time and satisfactory flow in compliance with the ISO 6876 standard; in contrast to the AH Plus sealer, which has been shown to be associated with a relatively low pH, it exhibits a low volumetric change and alkalinization capacity due to the release of OH^- and Ca^{2+} ions [37].

In addition to calcium silicate, the Bio-C Sealer contains aluminate and calcium oxide, along with zirconium oxide, iron oxide, and silicon dioxide. Calcium aluminate supports the acquisition of osteogenic cell phenotypes *in vitro* [38], and calcium aluminate materials exhibit good biocompatibility in the subcutaneous tissue of rats [39], which can explain our results. Moreover, calcium oxide reacts with tissue fluids, stimulating the deposition of hard tissue through the release of calcium ions [40].

The radiopacity of the Bio-C Sealer material was considered adequate and met the American National Standards Institute/American Dental Association criteria [37]. Unlike MTA-Fillapex, the Bio-C Sealer includes zirconium oxide as a radiopacifier, and this substance is associated with the proliferation of fibroblasts and the acceleration of the regression of inflammatory reactions [41]. Other studies reported the cytotoxic effects of bismuth oxide in different cell types [42,43]. This validates the results found in the present study and could be a reason for studying a new formulation of MTA-Fillapex, in which calcium tungstate is used instead of bismuth oxide [44].

In contrast to the abovementioned studies, the present study demonstrated that the immunolabeling of tenascin increased after 30 days in all groups except the Bio-C Sealer group. Although tenascin immunolabeling has already been shown to generally decrease over time, especially after mineralization [18], the results of the present study suggested relatively less intense induction of biomineralization associated with the Bio-C Sealer. Therefore, further studies are needed to gain a better understanding of the properties of this sealer, such as the transplantation of tricalcium phosphate/hydroxyapatite–containing extract-treated cells.

Bio-C Sealer is a new formulation hydraulic sealer with potential applications in root canal fillings. Based on the findings of this study, this new material exhibited higher cell viability at most evaluation time points and dilutions relative to MTA-Fillapex and white MTA-Angelus. In addition to being biocompatible, Bio-C Sealer could serve as an alternative for endodontic sealers. However, additional investigations are required to verify its physical, chemical, and bioactive properties prior to its indication for clinical use.

CONCLUSIONS

Endodontic sealers are materials that remain in close contact with living tissues. Thus, the development of new, biologically acceptable materials with more appropriate physiochemical properties is a priority. In this study, the Bio-C Sealer material displayed cytocompatibility, biocompatibility, and tenascin expression similar to those exhibited by white MTA-Angelus.

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