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In vitro evaluation of cytotoxicity in human osteoblastic cells and antimicrobial activity in the biofilm of different root canal sealers

Avaliação *in vitro* da citotoxicidade e atividade antimicrobiana em biofilme de diferentes cimentos endodônticos obturadores

Evaluación *in vitro* de la citotoxicidad y actividad antimicrobiana en biofilm de diferentes cementos endodónticos

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Abstract

Objective: Evaluate the cytotoxicity in human osteoblastic cells and antimicrobial activity in different root canal sealers in vitro. Methods: BioRoot RCS, TotalFill BC Sealer, and Bio-C Sealer were used in experimental groups, and AH Plus was used as a control. Human osteoblast-like cells and MTT quantitative colorimetric assay were used to evaluate cytotoxicity. Saos-2 cells were exposed to undiluted sealer extracts for 24 h. The supernatant was then collected and the formazan crystals resulting from MTT reduction were dissolved in pure dimethyl sulfoxide. Absorbance was measured in an automated spectrophotometer at a wavelength of 540 nm. Antimicrobial activity was analyzed by the direct contact test using a polymicrobial biofilm composed of Enterococcus faecalis, Candida albicans, and Streptococcus mutans. At 24, 48, and 72 h, colony-forming units were counted on agar plates. A nonparametric Kruskal-Wallis test was used for the statistical analysis. The level of significance was set at 5%. Results: AH Plus showed the lowest cytotoxicity after 24 h, with a significant difference in relation to BioRoot RCS and Bio-C Sealer ($p \leq 0.01$). There was no significant difference in cytotoxicity between TotalFill BC Sealer and Bio-C Sealer ($p > 0.05$). At 24 h, TotalFill BC Sealer and AH Plus showed the least microbial growth compared to Bio-C Sealer ($p < 0.05$). At 48 and 72 h, there were no significant differences between sealers ($p > 0.05$). Conclusions: AH Plus had the lowest cytotoxicity. TotalFill BC Sealer and AH Plus yielded greater reductions in microbial counts in the first 24 h compared to Bio-C Sealer. Clinical Relevance: 2c.

Keywords: Biofilms; Cell survival; Endodontics. *In Vitro* techniques; Osteoblasts.

Resumo

Objetivo: Avaliar a citotoxicidade em células osteoblásticas humanas e a atividade antimicrobiana em diferentes cimentos endodônticos in vitro. Métodos: BioRoot RCS, TotalFill BC Sealer e Bio-C Sealer foram usados nos grupos experimentais e o AH Plus foi usado como controle. Células semelhantes a osteoblastos humanos e ensaio colorimétrico quantitativo de MTT foram usados para avaliar a citotoxicidade. As células Saos-2 foram expostas a extratos de cimento não diluídos por 24 h. O sobrenadante foi então recolhido e os cristais de formazan resultantes da redução do MTT foram dissolvidos em dimetilsulfóxido puro. A absorbância foi medida em um espectrofotômetro automatizado em um comprimento de onda de 540 nm. A atividade antimicrobiana foi analisada pelo teste de contato direto utilizando um biofilme polimicrobiano composto por Enterococcus faecalis, Candida albicans e Streptococcus mutans. Em 24, 48 e 72 h, as unidades formadoras de colônias foram contadas em placas de ágar. O teste não paramétrico de Kruskal-Wallis foi utilizado para a análise estatística. O nível de significância foi estabelecido em 5%. Resultados: AH Plus apresentou a menor citotoxicidade após 24 h, com diferença significativa em relação ao BioRoot RCS e Bio-C Sealer ($p \leq 0,01$). Não houve diferença significativa na citotoxicidade entre TotalFill BC Sealer e Bio-C Sealer ($p > 0,05$). Às 24 h, TotalFill BC Sealer e AH Plus apresentaram o menor crescimento microbiano em comparação com Bio-C Sealer ($p < 0,05$). Em 48 e 72 h, não houve diferenças significativas entre os cimentos ($p > 0,05$). Conclusões: AH Plus apresentou a menor citotoxicidade. TotalFill BC Sealer e AH Plus produziram maiores reduções na contagem microbiana nas primeiras 24 h em comparação com o Bio-C Sealer. Relevância Clínica: 2c.

Palavras-chave: Biofilmes; Endodontia; Osteoblastos; Sobrevivência celular; Técnicas *In Vitro*.

Resumen

Objetivo: Evaluar in vitro la citotoxicidad en células osteoblásticas humanas y la actividad antimicrobiana de diferentes cementos obturadores de conductos radiculares. Materiales y métodos: BioRoot RCS, TotalFill BC Sealer y Bio-C Sealer se utilizaron como grupos experimentales y AH Plus se utilizó como control. Se usaron células similares a osteoblastos humanos y ensayo colorimétrico cuantitativo MTT para evaluar la citotoxicidad. Las células Saos-2 se expusieron a extractos de cementos sin diluir durante 24 h. A continuación, se recogió el sobrenadante y los cristales de formazán resultantes de la reducción de MTT se disolvieron en sulfóxido de dimetilo puro. La absorbancia se midió en un espectrofotómetro automático con una longitud de onda de 540 nm. La actividad antimicrobiana se analizó mediante la prueba de contacto directo, utilizando una biopelícula polimicrobiana compuesta por Enterococcus faecalis, Candida albicans y Streptococcus mutans. A las 24, 48 y 72 h, se contabilizaron las unidades formadoras de colonias en placas de agar. Para el análisis estadístico se utilizó la prueba no paramétrica de Kruskal-Wallis. El nivel de significación se fijó en el 5%. Resultados: AH Plus mostró la menor citotoxicidad después de 24 h, con una diferencia significativa en relación con BioRoot RCS y Bio-C Sealer ($p \leq 0.01$). No hubo diferencias significativas en la citotoxicidad entre TotalFill BC Sealer y Bio-C Sealer ($p > 0.05$). A las 24 h, TotalFill BC Sealer y AH Plus mostraron el menor crecimiento microbiano en comparación con Bio-C Sealer ($p < 0.05$). A las 48 y 72 h no hubo diferencias significativas entre los cementos obturadores ($p > 0.05$). Conclusiones: AH Plus presentó la menor citotoxicidad. TotalFill BC Sealer y AH Plus produjeron mayores reducciones en los recuentos microbianos en las primeras 24 h en comparación con Bio-C Sealer. Relevancia Clínica: 2c.

Palabras clave: Biopelículas; Endodoncia; Osteoblastos; Sobrevivência celular; Técnicas *In Vitro*.

1. Introduction

Obturation of the root canal system aims to seal off all spaces, making the environment inhospitable to the survival of microorganisms and promoting repair of periradicular tissues. In combination with gutta-percha cones, root canal sealers play a key role in obturation techniques (Schilder, 2006; Mann, et al., 2022). These substances are expected to have minimal contact with periradicular tissues when sealing the apex. However, when extruded into the periradicular region, root canal sealers are not always reabsorbed and can cause a range of tissue reactions, depending especially on their composition (Rodriguez-Lozano, et al., 2017).

In vitro cytotoxicity studies are the first step in evaluating the clinical applicability of root canal sealers (Peters, 2013; Sanz, et al., 2021). Several such studies have evaluated root canal sealers for their cytotoxic potential on human osteoblast cells (Willershausen, et al., 2013; Bortoluzzi, et al., 2015; Zordan-Bronzel, et al., 2019b) and their antimicrobial activity by methods such as the agar diffusion test (ADT) and direct contact test (DCT) (Candeiro, et al., 2016; Singh, et al., 2016). The DCT is a quantitative, reproducible assay which allows testing of insoluble materials and can be used in standardized configurations (Weiss, et al., 1996; Zhang, et al., 2009).

Calcium silicate-based materials are recognized as bioactive products due to their ability to induce formation of

mineralized tissue. Their advent has defined a new treatment approach for dentin remineralization, vital pulp therapy, and bone regeneration in endodontics (Koch & Brave, 2012; Prati & Gandolfi, 2015). Two main advantages are associated with the use of bioceramic materials as root canal sealers. The first is their high biocompatibility, which reduces inflammatory reaction even in event of apical extrusion (Koch & Brave, 2009). Second, calcium silicate-based root canal sealers contain calcium phosphate, which results in a chemical composition and crystal structure similar to that of natural tooth and bone hydroxyapatite (Ginebra, et al., 1997).

Few studies have evaluated the antimicrobial activity of different root canal sealers against a polymicrobial biofilm. The present investigation was designed to address this research gap. The null hypothesis was that the tested root canal sealers would have equivalent antimicrobial and cytotoxic properties in *in vitro* laboratory studies.

2. Methodology

Materials

Three calcium silicate-based root canal sealers were evaluated: BioRoot RCS (Septodont, Saint-Maur-des-Fossés, France); TotalFill BC Sealer (Brasseler, Savannah, Georgia, USA); and Bio-C Sealer (Angelus Indústria de Produtos Odontológicos S/A, Londrina, Paraná, Brazil). AH Plus (Dentsply, Konstanz, Germany) was used as control (Table 1). All materials were prepared as per manufacturer instructions, under aseptic conditions, in a laminar flow hood (Filtracom, Valinhos, São Paulo, Brazil).

Table 1. Chemical composition, manufacturer, and lot number of the tested root canal sealers.

Group	Material	Composition	Manufacturer	Lot
BR	BioRoot RCS	Powder: tricalcium silicate, zirconium oxide; Solution: calcium chloride.	Septodont, Saint-Maur-des-Fossés, France	B19555 (powder) B18640 (solution)
TF	TotalFill BC Sealer	Zirconium oxide, calcium silicates, monocalcium phosphate, calcium hydroxide.	Brasseler, Savannah, Georgia, USA	16002SP
BS	Bio–C Sealer	Calcium silicate, calcium oxide, zirconium oxide, iron oxide, silicon dioxide, dispersant.	Angelus Indústria de Produtos Odontológicos S/A, Londrina, Paraná, Brazil	43980
AP	AH Plus	Paste A: bisphenol-A epoxy resin, bisphenol-F epoxy resin, calcium tungstate, zirconium oxide, silica, iron oxide.	Dentsply, Konstanz, Germany	1610000519 (A)
		Paste B: dibenzyldiamine, aminoadamantane, tricyclodecane diamine, calcium tungstate, zirconium oxide, silica, silicone oil.		1610000519 (B)

AP, AH Plus; BR, BioRoot RCS; BS, Bio-C Sealer; TF, TotalFill BC Sealer. Source: Authors.

Sample size calculation was performed by ANOVA after a pilot experiment. For a minimum difference between treatment means = 0.02, standard error = 0.0001, number of treatments = 3, statistical power = 0.80, and alpha = 0.05, a sample size of 3 specimens per group was required for cytotoxicity and antimicrobial activity tests.

Cytotoxicity

The sealers were placed into Teflon rings (Centerflon Ind Com Ltda, São Paulo, São Paulo, Brazil) (diameter 5 mm,

height 2 mm), as recommended in ISO standard 10993-5. The procedure was done in triplicate. The rings were stored for 24 h in an incubator at 37°C to allow cure. After 24 h, once the initial cure period was completed, the Teflon rings were removed and each mold placed in 48-well plates. A 1.5-mL aliquot of Dulbecco's Modified Eagle Medium (DMEM) (Gibco, Grand Island, NY, USA) was added to each well to produce an extract of each sealer. These extracts were kept in an incubator at 37°C for 24 h, and then collected and stored at -20°C.

Cell culture

Human osteoblastic cells from the Saos-2 osteosarcoma cell line were obtained from ATCC (American Type Culture Collection) and cultured in DMEM (Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco, Grand Island, NY, USA), 100 µg/mL streptomycin, and 100 mg/mL penicillin. The cells were maintained in an incubator at 37°C in a 5% CO2 atmosphere. Confluent cells were trypsinized with 0.25% trypsin and 0.05% ethylenediamine tetraacetic acid (EDTA) (Gibco, Grand Island, NY, USA) for 5 min; aliquots were then replated.

For the experimental assay, cells were plated at a concentration of 5×10^4 per well in 96-well plates (TPP, Trasadingen, Switzerland) until approximately 80% confluency was reached. After 24 h of culture, once cells were adherent, they were exposed directly to the undiluted extracts of the experimental sealers for 24 h.

Cytotoxicity assay

Cytotoxicity was tested by the MTT quantitative colorimetric assay. Saos-2 cells were exposed to extracts of the undiluted sealers for 24 h. After 24 h, the supernatant was removed and 0.1 mL of MTT solution (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Sigma, St Louis, MO, USA), prepared at a concentration of 0.5 mg/mL, was added to each cell well. The samples were stored at 37°C for 2 h. After the incubation period, the supernatant was collected and the formazan crystals resulting from reduction of MTT were dissolved in 0.1 mL of pure dimethyl sulfoxide (DMSO). The plates were shaken for 5 min and incubated for 5 min for color stabilization. Absorbance was measured in an automatic spectrophotometer (EPOCH, Biosystems, Curitiba, Paraná, Brazil) at a wavelength of 540 nm to assess cell viability.

Antimicrobial activity

All microbiological assays were performed under aseptic conditions in a laminar flow hood (Filtracom, Valinhos, São Paulo, Brazil).

Standard strains of Enterococcus faecalis (ATCC 29212), Candida albicans (ATCC 10231), and Streptococcus mutans (ATCC 25175) were simultaneously introduced into 100 mL of brain heart infusion (BHI) and cultured for 7 days for biofilm formation in a 10% CO2 atmosphere (TE-399, Tecnal, Piracicaba, São Paulo, Brazil). BHI broth was renewed every 24 h. Antimicrobial activity was evaluated using a 7-day biofilm.

Direct contact test (DCT)

The DCT was used to evaluate the antimicrobial properties of root canal sealers by counting the number of bacterial colonies after plating on agar.

The sealers were placed into Teflon rings (Centerflon Ind Com Ltda, São Paulo, São Paulo, Brazil) (diameter 5 mm, width 2 mm), using the manufacturer-provided applicator tips or, if necessary, a calcium hydroxide applicator (Golgran, São Caetano do Sul, São Paulo, Brazil). The resulting specimens were placed in glass petri dishes (Precision, São Paulo, São Paulo, Brazil) lined with sterilization wrap (Master Polymers, São Paulo, São Paulo, Brazil) and incubated in an aerobic incubator

(Fiales Científica ME, São Paulo, São Paulo, Brazil) at 37°C for 24 h.

After 24 h, the sealers were removed from the Teflon rings (on average, 180 mg of sealer was recovered from in each ring) and placed into the wells of a 24-well microtiter plate containing 1 mL of brain heart infusion (BHI) broth.

After 20 min, 50 µL of biofilm suspension (3×108 CFU mL^{-1}, corresponding to McFarland turbidity standard 1.0) was placed on the surface of the specimens. In the positive-control DCT group, 50 µL of biofilm suspension alone was placed into wells containing 1 mL BHI, without any root canal sealer. In the negative-control DCT group, each well was filled with 1 mL sterile BHI broth to check for absence of contamination in the culture medium.

All procedures were performed in triplicate. The biofilm that remained in contact with the endodontic sealers was collected after 24, 48, and 72 h and homogenized in a solution shaker (AP 56, Phoenix Luferco, Araraquara, São Paulo, Brazil).

A 100-µL aliquot of the sample was collected and placed into a sterile Eppendorf tube (Eppendorf Brazil, São Paulo, São Paulo, Brazil) containing 900 µL saline solution. This procedure was repeated for five serial dilutions (10-1, 10-2, 10-3, 10-4, and 10-5).

All Eppendorf tubes were stirred for 30 s. Three 25-µL drops of each dilution were collected (drop method) and placed into petri dishes containing BHI agar (Difco Laboratories Incorporated, Detroit, Michigan, USA). After incubation at 37°C for 24, 48, and 72 h in a 10% CO2 atmosphere (TE-399, Tecnal, Piracicaba, São Paulo, Brazil), the colonies that formed on the plates were counted and the bacterial burden in CFU mL^{-1} was determined. The microtiter plate was stored at 37°C for up to 3 days, according to each measurement time point (24, 48, and 72 h). Once daily, 250 µL of fresh sterile BHI broth (25%) was placed into each well and 250 µL of contaminated broth removed to maintain the mean volume constant.

Statistical analysis

Results were analyzed with BioStat 4.0. The Shapiro-Wilk test for normality revealed that the sample was nonparametric. Descriptive statistics were calculated and the nonparametric Kruskal-Wallis test (with Student-Newman-Keuls post-hoc test) used for quantitative analysis. The significance level was set at 5%.

3. Results

Cytotoxicity

The lowest cytotoxicity, after 24 h, was found for AH Plus, with a significant difference in relation to BioRoot RCS and Bio-C Sealer ($p \leq 0.01$). There was no significant difference in cytotoxicity between TotalFill BC Sealer and Bio-C Sealer ($p > 0.05$, Table 2).

Table 2. Arithmetic Mean (AM), Median, Interquartile Range (IQR), and statistical analysis by the Kruskal–Wallis (Student–Newman–Keuls) test of cytotoxicity assays of BioRoot RCS, TotalFill BC Sealer, Bio-C Sealer, and AH Plus

	BR	TF	BS	AP	(p)
AM	0.29	0.39	0.33	0.56	
Median	0.29	0.39	0.33	0.55	0.0095
IQR	$(0.02)^{A}$	$(0.01)^{B}$	$(0.01)^{AB,1}$	$(0.07)^{B,2}$	

Different letters or numbers in the same row denote statistically significant differences. AM, Arithmetic Mean; AP, AH Plus; BR, BioRoot RCS; BS, Bio-C Sealer; IQR, Interquartile Range; TF, TotalFill BC Sealer. Source: Authors.

Antimicrobial activity

At 24 h, TotalFill BC Sealer and AH Plus showed the least microbial growth compared to Bio-C Sealer ($p \leq 0.05$). There was no significant difference between BioRoot RCS and the other sealers ($p > 0.05$).

At 48 and 72 h, there was no significant difference among sealers ($p > 0.05$, Table 3). Within-group comparisons at different time points (24, 48, and 72 h) showed no statistically significant difference ($p > 0.05$, Table 3).

Table 3. Arithmetic Mean (AM), Median, Interquartile Range (IQR), and statistical analysis by the Kruskal–Wallis (Student–Newman–Keuls) method of direct contact tests of BioRoot RCS, TotalFill BC Sealer, Bio-C Sealer, and AH Plus at 24, 48, and 72 hours (×106 CFU·ml-1).

	BR	**TF**	**BS**	**AP**	**(p)**
24 h					
AM	0.05	0.03	0.15	0.03	
Median	0.04	0.04	0.16	0.04	0.0373
IQR	$(0.03)^{AB,1}$	$(0.02)^{B,1}$	$(0.01)^{A,1}$	$(0.00)^{B,1}$	
48 h					
AM	0.52	0.20	0.60	0.00	
Median	0.17	0.22	0.61	0.01	0.0873
IQR	$(0.66)^{A,1}$	$(0.09)^{A,1}$	$(0.30)^{A,1}$	$(0.00)^{A,1}$	
72 h					
AM	0.54	0.20	0.53	0.22	
Median	0.26	0.20	0.53	0.22	0.2040
IQR	$(0.47)^{A,1}$	$(0.05)^{A,1}$	$(0.18)^{A,1}$	$(0.21)^{A,1}$	
(p)	0.1203	0.0665	0.0650	0.1031	

Different letters in the same row denote statistically significant differences. The same number in the same column denotes absence of a statistically significant difference.
AM, Arithmetic Mean; AP, AH Plus; BR, BioRoot RCS; BS, Bio-C Sealer; IQR, Interquartile Range; TF, TotalFill BC Sealer. Source: Authors.

4. Discussion

Based on the results obtained, the null hypothesis was rejected, as the tested root canal sealers did not exhibit equivalent cytotoxicity and antimicrobial activity.

Florid inflammatory reactions caused by periapical extrusion of root-canal obturating materials are a common complication of endodontic treatment (Poggio, et al., 2017). The biocompatibility of root canal sealers is important for the success of endodontic therapy. Therefore, a biocompatible sealer should not delay or hinder the tissue repair process (Poggio, et al., 2014). In the present study, the choice of osteoblasts as the cell line for cytotoxicity testing is based on the hypothesis of mineralization of the periradicular milieu in the presence of the calcium silicate component of bioceramic sealers, as well as on their viability and capacity for osteogenic differentiation (Bortoluzzi, et al., 2015; Zordan-Bronzel, et al., 2019b). Articles evaluating the cytotoxicity of calcium silicate-based root canal sealers on fibroblasts have not observed the reaction of osteoblastic cells in contact with sealers, a clinical situation that happens routinely after obturation, and the possible contact of sealer with bone tissue (Jafari, et al., 2017; Jagtap, et al., 2018; Nair, et al., 2018).

Under our experimental conditions, BioRoot RCS and Bio-C Sealer exhibited significantly greater cytotoxicity than

AH Plus. This may be associated with a greater release of calcium ions; basic pH, greater Ca2+ ion release, and formation of hydroxyapatite have all been described as factors in the cytotoxicity of calcium silicate-based root canal sealers (Loushine, et al., 2011; Candeiro, et al., 2012; Zordan-Bronzel, et al., 2019a). AH Plus and bioceramic endodontics sealers were tested after the initial setting of 24 h to simulate what happens clinically one day after root canal system obturation.

The initial cytotoxicity of AH Plus was first described by Brackett et al. (2008) and Huang et al. (2011) The genotoxic effects of formaldehyde are detected at high concentrations and decrease over time (Leonardo, et al., 1999; Eldeniz, et al., 2007; Camargo, et al., 2009). The lower cytotoxicity of AH Plus (i.e., the high viability of cells exposed to it) observed in the present study is consistent with the findings of Oztan et al. (2003), Camargo et al. (2009), and Poggio et al. (2017) This may also be attributable to the fact that cell viability was measured after 24 h of exposure of Saos-2 osteoblasts to the sealer extracts. According to Cotti et al. (2014), testing the cytotoxicity of freshly prepared sealers is clinically relevant because these compounds are in an undefined state when they are first introduced into the root canal system and may thus come into contact with periapical tissues.

It is known that, even after canal preparation, the antimicrobial properties of root canal sealers may act on microorganisms resistant to chemical irrigation and mechanical preparation, as well as prevent the ingress of fluids into the root canal system that could provide nutrients for microorganisms (Baumgartner, et al., 2007). Hence the importance of this study in ascertaining whether endodontic sealers have low cytotoxicity and satisfactory antimicrobial activity. Some microorganisms express virulence factors that contribute to their ability to survive the effects of conventional root canal therapy (Sundqvist, et al., 1998; Gomes, et al., 2006). Therefore, Enterococcus faecalis, Candida albicans, and Streptococcus mutans were the microorganisms selected to form the biofilm against which the antimicrobial properties of endodontic sealers were tested in this study.

The antibacterial effect of TotalFill BC Sealer observed in this study may be due to a combination of high pH and active calcium hydroxide diffusion (Zhang, et al., 2009; Candeiro, et al., 2012; Zordan-Bronzel, et al., 2021). Zhang et al. (2009) noted that the antimicrobial effect decreased 7 days after mixing, as in the present study. Furthermore, at 24 h, TotalFill BC Sealer exhibited the best antimicrobial activity among the tested sealers. This is probably attributable to monocalcium phosphate, a compound present only in this sealer. Upon contact with tissue fluids, this component probably contributed to additional antimicrobial activity, more prolonged and significant alkalinization of the culture medium, and greater release of calcium ions (Bienek, et al., 2018; Vouzara, et al., 2018). Khashaba et al. (2009) also evaluated the importance of monocalcium phosphate and its biological properties in endodontic applications. Several chemical compounds with alkalinizing activity included in the composition of bioceramic endodontic sealers may play a role in antimicrobial effect (Colombo, et al., 2018).

Pizzo et al. (2006) reported that only AH Plus had antibacterial activity as measured by the DCT. Similar findings were reported by Kayaoglu et al. (2005) The antimicrobial effect of epoxy-based root canal sealers may be due to formaldehyde release during the polymerization process (Poggio, et al., 2017). The present study also found that AH Plus had a significant antimicrobial effect (including microbicidal effect in the first 24 h), which can prevent recontamination and reinfection of root canals by residual microorganisms (Kishen, et al., 2008). However, it bears noting that AH Plus had lower long-term efficacy, perhaps because it releases paraformaldehyde only during the initial cure period (Poggio, et al., 2017).

5. Conclusion

In conclusion, AH Plus had the lowest cytotoxicity, while TotalFill BC Sealer and AH Plus yielded greater reductions in microbial counts in the first 24 h compared to Bio-C Sealer. These results provide additional evidence on the cytotoxicity and antimicrobial activity of calcium silicate-based root canal sealers.

The results of this study contribute to the creation of scientific evidence on the cytotoxicity and antimicrobial activity of endodontic sealers. However, further studies in this line of research using diversified cell lineage and biofilm should be conducted.

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