

Article

Human Gingival Fibroblasts Response to Different Endodontic Sealers: An In Vitro Study

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Abstract: Endodontic treatment aims to eliminate infection of the root canals and fill the dental pulp space. The biocompatibility studies of the sealers used in root canals obturation are crucial since they are applied in direct contact with periradicular tissues. **Objective:** The aim of this study was to evaluate the cytotoxicity of three root canal sealers—AH Plus, Bio MTA+, and Bio C sealer—on immortalized human gingival fibroblasts. **Methods:** AH Plus, Bio MTA+, and Bio C sealers were evaluated through incubation in real-time and material-conditioned media. Cells were incubated for 24 h and 72 h, at three different concentrations (1, 10, and 100 mg/mL) of each sealer. The cytotoxic activity of the sealers was assessed by Methyl tetrazolium (MTT) and Sulforhodamine B (SRB) assays. Cell morphology and cytogenetic alterations were studied microscopically. **Results:** MTT and SRB assays revealed similar results within both approaches. Cell culture exposed to sealers through incubation in real-time revealed a cytotoxic effect of AH Plus at 100 mg/mL. Material-conditioned media study revealed a cytotoxic effect of Bio MTA+ and Bio C, increasing with higher compound concentration and reaching 50% with 100 mg/mL. Regarding the cell's morphology, Bio C sealer revealed a decrease in cell confluence and several morphological changes. AH Plus and Bio MTA+ did not seem to affect the cell confluence however morphology alterations were observed. In the cytogenetic study, a severe decrease of the mitotic index and a large number of chromosomal aberrations were observed. The present study represents an advance in the understanding of the biocompatibility of AH Plus, Bio MTA+, and Bio C sealers. These sealers demonstrated some cytotoxicity, depending on the concentration used. Although more validation studies are still needed, this study brings very relevant results in terms of cytotoxicity, cell morphology, and cytogenetic alterations. **Conclusions:** These results could help in the selection of the most appropriate compounds to be used in clinical practice as well as to determine the maximum recommended amounts of each sealer. **Clinical Relevance:** This study highlights the potential cytotoxic effects of three commonly used root canal sealers on human gingival fibroblasts, with varying degrees of impact depending on the concentration used. The results emphasize the importance of careful consideration when selecting and applying these materials in clinical practice.

Keywords: endodontic sealers; AH Plus; Bio MTA+; Bio C sealer; cytotoxicity; cell morphology; cytogenetic alterations; biocompatibility; human gingival fibroblasts; HGF-1 cell line



Citation: Noites, R.; Tavares, I.; Cardoso, M.; Carreira, I.M.; Bartolomeu, M.; Duarte, A.S.; Ribeiro, I.P. Human Gingival Fibroblasts Response to Different Endodontic Sealers: An In Vitro Study. *Appl. Sci.* **2023**, *13*, 10976. <https://doi.org/10.3390/app131910976>

Academic Editors: José João Mendes, Ricardo Castro Alves and Ana Cristina Mano Azul

Received: 8 September 2023
Revised: 30 September 2023
Accepted: 3 October 2023
Published: 5 October 2023



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1. Introduction

Endodontic treatment aims to prevent apical and coronal infiltration and microorganisms' proliferation by eliminating root canal infection and filling the dental pulp space [1–4]. Thus, the obturation of root canal systems is one of the most important steps of endodontic treatment [1]. Since endodontic sealers used to fill the root canals are applied in direct contact with the periapical tissues, such as the periodontal ligament, it is very important to evaluate the cellular effects of these materials [5,6]. In this context, minimal invasive endodontics enters as an approach to root canal treatment that emphasizes preserving the natural tooth structure as much as possible while effectively treating the infection or inflammation within the tooth. Thus, the removal of healthy tooth structure is minimized and consequently the long-term success of the treatment is enhanced while the patient discomfort is reduced [7]. The bioactivity, alongside with other properties of the sealers used in the endodontic treatment will imply the long-term success of the treatment.

There are several types of sealers, with different physical, chemical, and biological characteristics, which translate into different sealing abilities, adhesive properties, antimicrobial efficacy, radiodensity, and biocompatibility [8]. Sealers are categorized according to their main chemical constituents: zinc oxide eugenol, calcium hydroxide, glass ionomer, silicone, resin, and bioceramic-based sealers [9,10]. AH Plus (Dentsply DeTrey GmbH, Konstanz, Germany), an epoxy-resin-based sealer, is considered a gold standard due to its high bond strength to the dentin and physical properties, being one of the most used in clinical practice [6,11]. However, this endodontic sealer does not present bioactive properties [6]. Bio MTA+ (PPH CERKAMED Wojciech Pawłowski, Stalowa Wola, Poland), one of the most recent sealers, is a silico-calcium-based material that contributes to tissue regeneration after perforation of the canal wall. *In vitro* studies have shown that mineral trioxide aggregate (MTA), in general, has good biocompatibility, nonmutagenic sealing ability, and bioinductive properties [12]. Bio MTA+, a similar material to the conventional MTA, with decreased-sized grains and the insertion of nanoceramic particles and hydroxyapatite, provides higher penetration of calcium ions to the demineralized tissue, and increased sealing process [12,13].

Bio C sealer (Angelus Indústria de Produtos Odontológicos S/A, Londrina, PR, Brazil) is a novel bioceramic, nonresin sealer, composed of calcium silicates, calcium aluminate, calcium oxide, zirconium oxide, iron oxide and silicon oxide and its bioactivity is also related to the release of calcium ions that stimulates periapical healing [8,11]. This endodontic sealer has presented adequate flow and radiopacity, low volumetric change, and good biocompatibility, resulting in the allowance of the rapid regression of inflammatory reaction [6,14].

Our study aimed to compare the biocompatibility of Bio MTA+ and Bio C sealer with the most used sealer in clinical practice, AH Plus. Thus, a comprehensive evaluation of the biocompatibility of these three root canal sealers, AH Plus, Bio MTA+, and Bio C sealer, from cytotoxicity to genotoxicity, was performed on immortalized human gingival fibroblasts, at different concentrations and over 24 and 72 h, using two different methodologies. Cell viability and proliferation, as well as the cytotoxicity of compounds, may be assessed by the 3-(4,5-dimethylthiazol-2-yl) bromide-2,5-diphenyltetrazolium (MTT) assay [15], which measures the mitochondrial activity of cells, and the Sulforhodamine B (SRB) assay [16] that allows cell density determination, based on the quantification of cellular protein content. In this study, both methodologies were used as complementary methods.

2. Materials and Methods

2.1. Endodontic Sealers

Three endodontic sealers were used in this study, AH Plus (Dentsply DeTrey GmbH, Konstanz, Germany), Bio MTA+ (PPH CERKAMED Wojciech Pawłowski, Stalowa Wola, Poland), and Bio C sealer (Angelus Indústria de Produtos Odontológicos S/A, Londrina, PR, Brazil). The sealers were prepared according to the manufacturer's instructions and shaped

with 1.5-mm-thick polyvinyl chloride molds with a diameter of 3 mm. To allow complete setting, the sealers were stored at 37 °C. After setting, the disks were removed from the molds, weighed, and sterilized (on both surfaces) by ultraviolet irradiation for 20 min.

2.2. Cell Culture

Immortalized human gingival fibroblast-1, HGF-1 (ATCC CRL-2014) obtained from the American Type Culture Collection (ATCC[®], Manassas, VA, USA) were cultured in Ham's F-10 Nutrient Mix cell culture medium (Gibco, Life Technologies Corporation, New York, NY, USA) supplemented with 15% fetal bovine serum (FBS; Gibco, Life Technologies Corporation, New York, NY, USA), 1% L-Glutamine and 1% Penicillin-streptomycin (Gibco, Life Technologies Corporation, New York, NY, USA). Cells were incubated in a humidified atmosphere with 5% CO₂, at 37 °C.

2.3. Cells Exposure to Endodontic Sealers

HGF-1 cells were seeded in 24-well plates at 7.5×10^4 cells/well containing 1 mL of culture medium and allowed to attach overnight in a humidified atmosphere with 5% CO₂, at 37 °C. After attachment, the cells were exposed to 0, 1, 10, or 100 mg/mL of each sealer for 24 h and 72 h in a humidified atmosphere with 5% CO₂, at 37 °C. The sealers were placed over permeable cell culture inserts (VWR International), as previously described [17,18]. After 24 h or 72 h, the inserts were removed and transferred to a new plate and the experiment was carried out a second time with the same sealers. This experiment was repeated twice to guarantee four experiments for each assay.

2.4. Cell Exposure to Medium Conditioned with the Endodontic Sealers

Material-conditioned medium were prepared at 100 mg/mL of each sealer, as recommended by the American Society for Testing Material (ASTM, 1992). Cell culture medium was incubated with the sealers in a ratio of 100 mg of the material per mL of culture medium for 24 h at 4 °C. The sealers were removed before using the conditioned medium. The pH of the conditioned medium was evaluated before dilution. These conditioned medium was diluted in fresh culture medium, to obtain three final concentrations of the sealers extracts used to perform the cytotoxicity tests: undiluted, conditioned by 100 mg/mL; 1:10, corresponding to 10 mg/mL; 1:100, corresponding to 1 mg/mL.

2.5. Cytotoxic Effects of the Endodontic Sealers

Cytotoxicity of the three sealers was determined by MTT and SRB assays, according to ISO 10993-5 recommendations. For the MTT assay, the culture medium was removed, and cells were washed with Phosphate buffered saline (PBS). Afterwards, 200 µL of MTT solution was added to each well and the cells were incubated overnight at 37 °C. After incubation, 200 µL of isopropanol was added to each well to solubilize the formazan crystals resulting from the cleavage of MTT salt ring by viable cells. Aliquots (200 µL) of each well were transferred to a 96-well plate and the optical density of formazan dye was read at 570 nm and 620 nm using a multi-well plate reader (Synergy HT spectrophotometer; BioTek Instruments, Winooski, VT, USA). For the SRB assay, the culture medium was removed, cells were washed with PBS, and then allowed to dry. The cells were then fixed with 1% acetic acid in methanol for 1 h, at 4 °C. The fixation solution was removed, and the wells were allowed to dry. Two hundred µL of SRB was added and the plate was incubated for 1 h, at room temperature, protected from light. Then, the wells were washed with distilled water, several times, until the unbound dye was completely eliminated. Bounded SRB was solubilized with 200 µL of Tris base buffer and the spectrophotometric absorbance was read at 540 nm and 690 nm, using a multiwell plate reader (Synergy HT spectrophotometer; BioTek Instruments, Winooski, VT, USA).

2.6. Cell Morphology Evaluation

Cell morphology was analyzed before and after exposition to the three different sealers. Cells were cultured in 24-well plates with silicon wafers at 7.5×10^4 cell/well containing 1 mL of culture medium and allowed to attach overnight in a humidified atmosphere with 5% CO₂, at 37 °C. After attachment, cells were exposed to material-conditioned media at 100 mg/mL of each sealer for 24 h in a humidified atmosphere with 5% CO₂, at 37 °C. Cells incubated in normal culture medium were also analyzed as the control group. After 24 h of incubation, the silicon wafers were removed and washed with distilled water to remove medium residues and were placed on stubs with a carbon sticker. Observations were carried out using a variable-pressure Scanning Electron Microscope (FlexSEM 1000; Hitachi High-Tech Corporation, Hitachinaka, Japan).

2.7. Cytogenetic Studies

Cytogenetic analysis was performed using material-conditioned medium with AH Plus, as one of the most used sealers in the clinical practice. Cells were incubated for 24 h in material-conditioned medium with 10 mg/mL and 100 mg/mL of AH Plus. Cells incubated in normal culture medium were also studied as the control group. Metaphase chromosomes were prepared and analyzed by GTG-banding using standard protocols [19]. Metaphases were digitally imaged and karyotyped resorting to a microscope (Eclipse E400; Nikon, Tokyo, Japan) and CytoVision™ software version 3.93.2 (Applied Imaging System, San Jose, CA, USA).

2.8. Statistical Analysis

Statistical analysis was performed using the software GraphPad Prism software (GraphPad Software, San Diego, CA, USA). Normal distribution of quantitative variables was assessed using the Shapiro-Wilk test. Since not all the groups passed the normality test, data were analyzed using the Kruskal-Wallis test and Dunn's multiple comparisons test was used to compare the mean of each group with the mean of the control group. An alpha value of 0.05 was considered as the threshold for statistical significance.

3. Results

3.1. Cytotoxicity Evaluation of the Endodontic Sealers

3.1.1. Endodontic Sealers Exposure through Incubation in Real-Time

MTT assay revealed a cytotoxic effect of AH Plus, which determined a significant decrease in the metabolic activity to 29.85% and 8.39% after 24 h ($p < 0.001$; Figure 1A) and 72 h ($p < 0.0001$; Figure 1B), respectively, after exposure to 100 mg/mL. No cytotoxic effect was detected after exposure to Bio MTA+ and Bio C sealer for 24 h (Figure 1A) at the same final concentration of 100 mg/mL, while minor cytotoxicity was observed after exposure for 72 h, maintaining a metabolic activity of 76.35% and 83.09% (Figure 1B). The study of cell viability, by SRB assay, revealed similar results. Thus, a decrease in cell viability to 26.77% ($p < 0.001$; Figure 1C) and to 18.72% ($p < 0.0001$; Figure 1D) occurred when the cells were exposed to 100 mg/mL of AH Plus during 24 h and 72 h, respectively.

3.1.2. Endodontic Sealers Exposure through Conditioned Media

MTT assay, performed after exposure to material-conditioned media for 24 h, revealed a cytotoxic effect of Bio MTA+ and Bio C sealer with a growing decrease of metabolic activity with increasing compound concentration. After exposure to Bio MTA+ a decrease of metabolic activity to 50.79% after 24 h with 100 mg/mL ($p < 0.0001$; Figure 2) was observed. With Bio C sealer we verified a decrease in metabolic activity to 50.26% ($p < 0.0001$; Figure 2) after exposure to 100 mg/mL for 24 h. Interestingly, AH Plus did not seem to affect metabolic activity when exposure was through the material-conditioned medium. The pH of the Bio C sealer conditioned medium was significantly higher, pH 10, while AH Plus and the Bio MTA+ conditioned media did not vary significantly (pH 7.5).

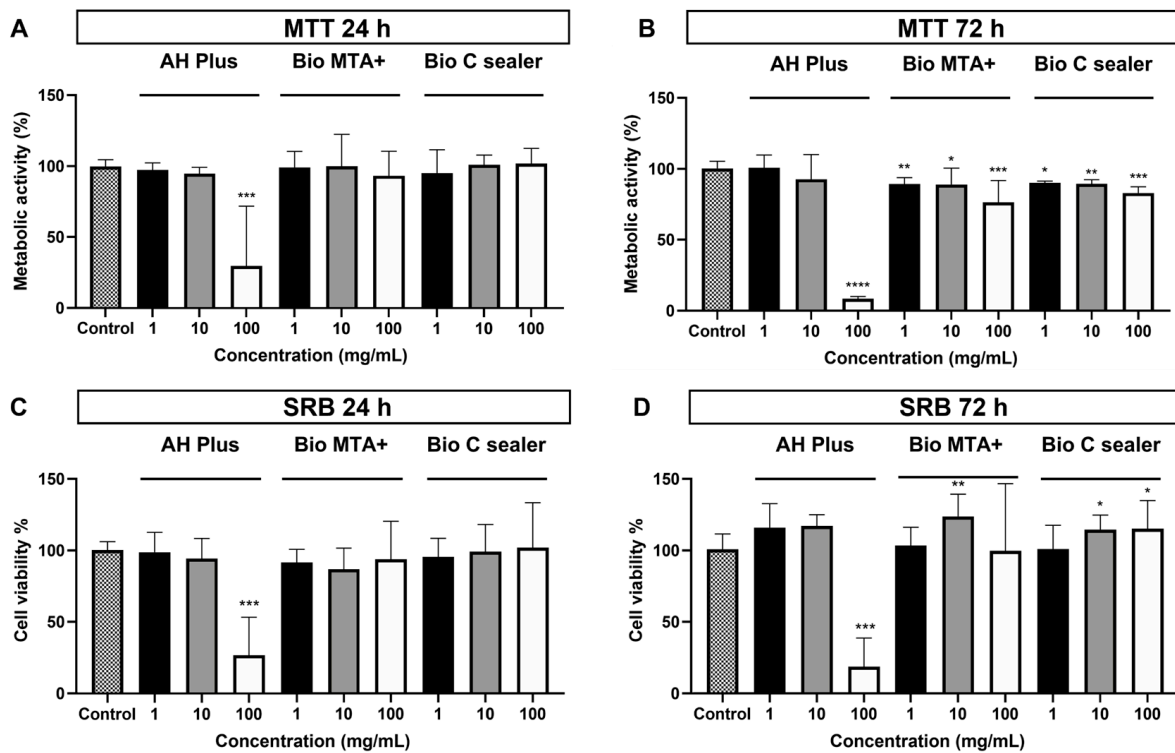


Figure 1. Cytotoxicity of AH Plus, Bio MTA+ and Bio C endodontic sealers to immortalized human gingival fibroblasts. Cells were exposed through incubation in real-time with three different concentrations (1 mg/mL, 10 mg/mL, and 100 mg/mL) using cell culture inserts. The results are presented as the average and standard error of four experiments. The asterisk indicates the cases where significant differences to the control group were observed: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$. Legend: (A) Metabolic activity of the HGF-1 cells after 24 h of incubation with the sealers; (B) Metabolic activity of the HGF-1 cells after 72 h of incubation with the sealers; (C) Viability of the HGF-1 cells after 24 h of incubation with the sealers; (D) Viability of the HGF-1 cells after 72 h of incubation with the sealers.

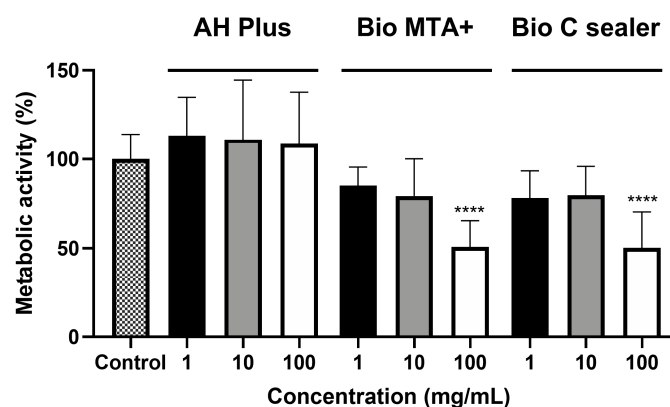


Figure 2. Cytotoxicity of AH Plus, Bio MTA+ and Bio C endodontic sealers to immortalized human gingival fibroblasts. Cells were exposed through material-conditioned media (1 mg/mL, 10 mg/mL, and 100 mg/mL). The results are presented as the average and standard error of three experiments. The asterisk indicates the cases where significant differences to the control group were observed: **** $p < 0.0001$.

3.2. Cell Morphology Evaluation

SEM evaluation revealed a normal morphology of control group cells, with a good confluence (Figure 3A), normal extensions, and an intact membrane (Figure 4A). With AH Plus, cell confluence was similar to the control group (Figure 3B), but the cells showed

a more irregular morphology, with compromised membranes and loss of cell content (Figure 4B). Regarding the treatment with Bio MTA+, the silicon wafer was covered with fibroblasts, close to the control group (Figure 3C), but the morphology of the cells presented some alterations, namely rounded cells and compromised cell membrane with loss of cell content (Figure 4C). The Bio C sealer was the compound that showed a more drastic effect on cell morphology, with a decrease in cell confluence and several morphological changes, including rounded cell shape, loss of membrane integrity, and leakage of cellular content (Figures 3D and 4D).

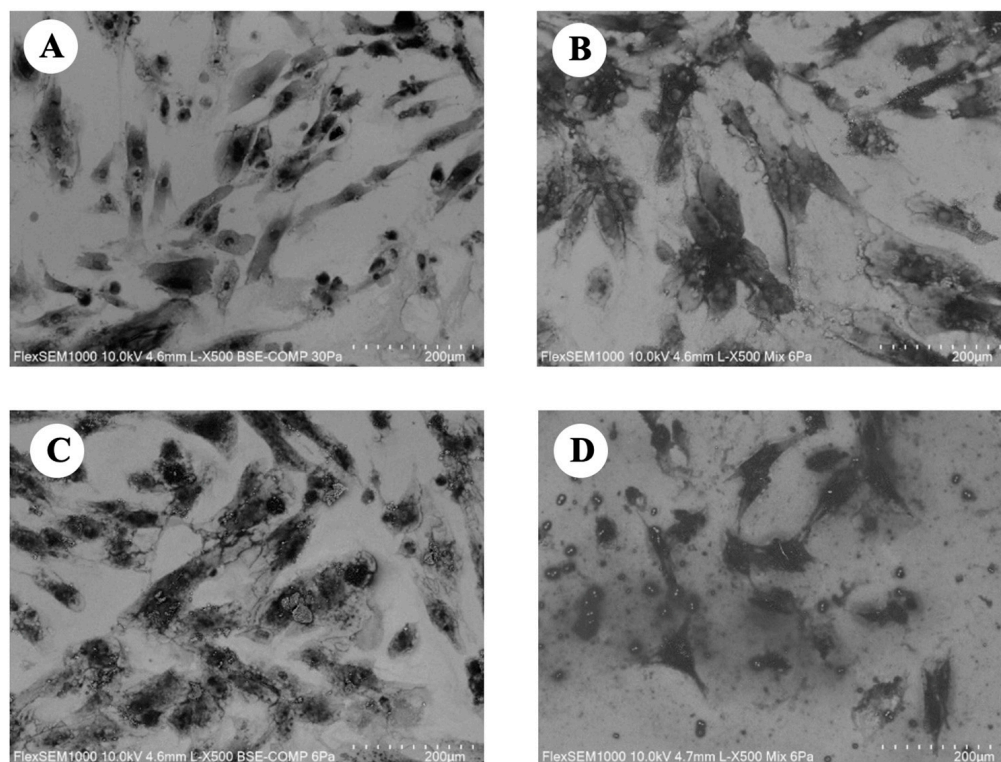


Figure 3. Morphology study after 24 h of HGF-1 fibroblasts incubation with material-conditioned media with 100 mg/mL of each sealer (500×). (A) Control group: cells organized in large number on the surface; (B) AH Plus group: the cells were distributed homogeneously on the glass substrate; (C) Bio MTA+ group: normal cell confluence was also observed; (D) Bio C group: presence of cell-free areas with a decrease in cell confluence.

3.3. Cytogenetic Studies

As a preliminary approach for the cytogenetic studies, we studied the effects of the most common sealer (AH Plus) used in routine dental consultations. The cytogenetic study was performed on the HGF-1 cells exposed to material-conditioned medium with AH Plus sealer and to a normal culture medium as the control group. Forty GTG metaphases of cells exposed to a normal culture medium and a total of 90 GTG metaphases, with both 10 mg/mL and 100 mg/mL of AH Plus concentration, were analyzed. Cells exposed exclusively to normal culture medium exhibited certain chromosomal abnormalities, with the majority of the cells remaining normal. However, after the incubation with AH Plus we observed a severe decrease in mitotic index and a five-fold increase in chromosomal aberrations, most structural associated with breakage, involving chromosomes 3, 4, 5, 6, 7, 8, 9, 11, 13, 14, 15, 16, 17, 19 and 22. Are examples of these chromosomal alterations several unbalanced translocations, marker chromosomes, and other complex rearrangements.

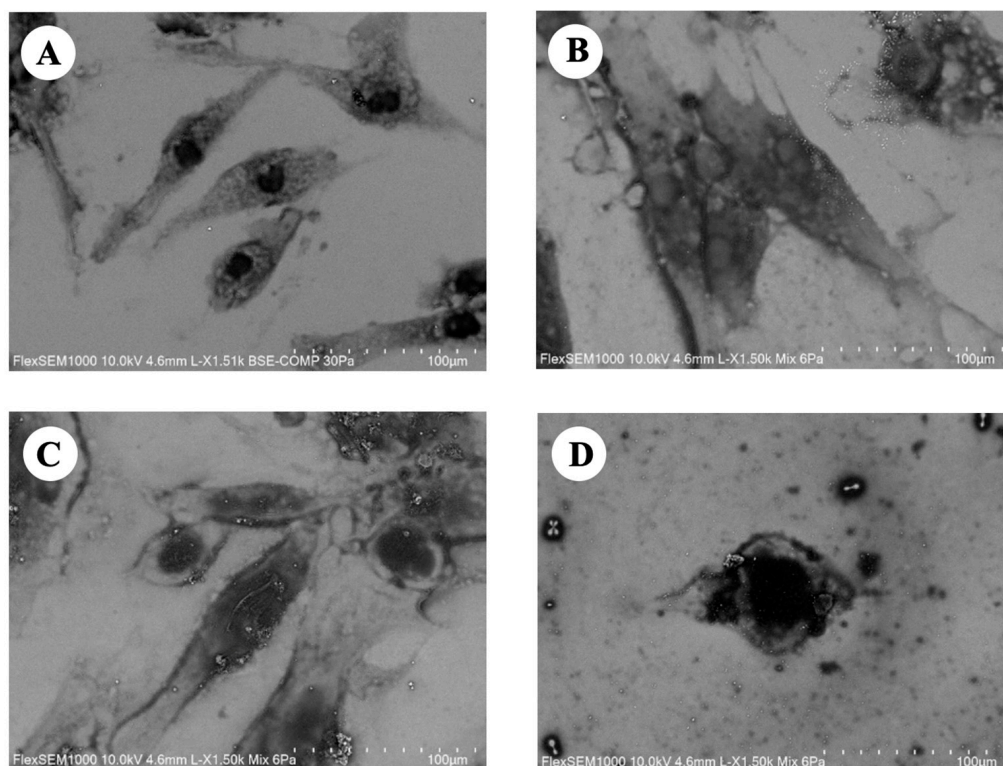


Figure 4. Morphology study after 24 h of HGF-1 fibroblasts incubation with each sealer with material-conditioned media with 100 mg/mL (500×). (A) Control group: cells with normal extensions and intact membranes; (B) AH Plus group: cells with irregular morphology, with compromised membranes and loss of cell content; (C) Bio MTA+ group: cells with some morphological alterations namely rounded cells and compromised cell membranes with loss of cell content; (D) Bio C group: several morphological changes including rounded cell shape, loss of membrane integrity and leakage of cellular content.

4. Discussion

Although endodontic sealers have a significant impact on endodontic treatment resolution, no extensive comparative studies have been made regarding the cytotoxicity and biocompatibility of the newly developed sealers [20]. The study of cytotoxicity and biocompatibility of the root canal sealers is crucial considering that they are applied in direct contact with periapical tissue [5,21]. Therefore, the selection of the best sealer to be used in clinical practice—particularly in a minimal invasive endodontic approach since the goal is to preserve more of the natural tooth structure—must be taken into account. The chemical and physical properties, as well as the sealing ability of endodontic sealers, should ensure the effective sealing of the remaining root canal space to prevent reinfections or further damages. Also, their biocompatibility determines the interaction with the surrounding tissues, by releasing antimicrobial substances and tissue regeneration molecules that promote the healing process [5,7,8,21].

In this context, a cell line of immortalized human gingival fibroblast (HGF-1) was considered as a cellular model since fibroblasts are predominant cells in connective tissue, being highly predominant in periodontal and periimplant connective tissues, responsible for the maintenance and production of the extracellular matrix [22].

Several methods have been used to evaluate the toxicity of root canal sealers *in vitro* [3,4]. Two different methods were applied: incubation in real-time (using a cell culture system with inserts) and material-conditioned media. Thereby, we were able to evaluate both the effect of the sealers through incubation in real-time with the cells and the effect of the root canal sealers diffusing substances on the culture medium. Regarding the incubation in real-time exposure, both MTT and SRB assays revealed a cytotoxic effect of AH Plus at 100 mg/mL

after 24 h (p -value < 0.001), making this effect even more pronounced after 72 h of incubation. No cytotoxic effects were detected by using Bio MTA+ and Bio C sealer for 24 h, while slight cytotoxicity was observed by testing these compounds for 72 h. Previous studies revealed similar results regarding AH Plus toxicity [3,11,23,24]. This cytotoxicity has been attributed to the release of formaldehyde from amines added to accelerate the polymerization of epoxy resin, as well as to the presence of bisphenol-A (BPA) in its composition that had been identified as a mutagenic component [11,25,26]. BPA, commercially used in the manufacture of epoxy resins, was identified as one of the first produced synthetic estrogens, and has endorsed the recent discussion about endocrine-disrupting chemicals regarding their presence and potential leakage from the products whose composition they integrate, as dental sealants. Although the data concerning the effective leakage of BPA from resin-based dental sealants has shown to be contradictory, this possibility must remain a concern [27].

Moreover, other studies also demonstrated that Bio C sealer, along with another bioceramic premixed root canal sealer, showed higher cell viability and biocompatibility than AH Plus [11,14], also attributed to the calcium ions, present in their composition, elution into the medium [11]. Also, it was previously suggested that calcium silicate-based sealers, such as Bio C sealer, have more pronounced biological properties than resin-based sealers, such as AH Plus [28]. Although some primary studies have been made to investigate the effect of the mineral trioxide aggregate (MTA) based bioceramic material revealing an expected high bioactivity and biocompatibility [29,30], as far as we know, no cytotoxicity studies have been performed until now using Bio MTA+.

On the other hand, AH Plus did not seem to cause any cytotoxic effect on cells when applied in the material-conditioned medium, while Bio MTA+ and Bio C sealers revealed growing cytotoxicity with increasing compound concentration, reaching a decrease in the metabolic activity of HGF-1 cells of 50% with 100 mg/mL. The material-conditioned medium with Bio C sealer revealed a significantly higher pH than the control medium. This indicates that Bio C sealer has a strong capacity to release hydroxyl ions and the alkaline pH is a possible cause of high cytotoxicity, which could explain the results shown by this material [31]. In addition, previous studies have shown that AH Plus has low solubility while Bio C sealer has high solubility [32]. This high solubility of Bio C sealer may suggest an increased solute degradation in the culture medium, possibly responsible for the cytotoxic effects demonstrated by Bio C sealer conditioned-medium tests. Despite the fact that, as mentioned above, some studies have revealed a cytotoxic effect of AH Plus, some other studies reported similar results to those that we obtained in the material-conditioned medium, where no cytotoxic effect of AH Plus was detected [1,5,33,34]. These discrepant results may be due to the lack of standardized methodologies. Thus, further studies are needed to clarify this effect of AH Plus.

In general, it has been reported that there is a possible association between the antimicrobial properties of the endodontic sealers and their cytotoxic effects, regarded as the lack of selective toxicity towards the microorganisms [35,36].

The antimicrobial properties of the endodontic sealers are an important aspect of the success of the endodontic treatment since an efficient antimicrobial behaviour from the sealers will reduce the residual amount of microorganisms left by the root canal's cleaning procedures. Regarding the sealers' antimicrobial behaviour, an increase in the pH of the environment may be translated into higher antimicrobial efficiency, namely against *Enterococcus faecalis*, the most prevalent bacteria in (re)infected root canals. [37]. The pH increase of the medium during the Bio C sealer incubation will most likely result in an efficient antimicrobial behaviour by this sealer. Previous studies have demonstrated good antimicrobial activity (against both Gram-positive and Gram-negative bacteria) by calcium silicate-based sealers also containing oxide compounds [38]. Additionally, Shin et al. have also demonstrated that the antimicrobial activity of the endodontic sealers is higher for the freshly mixed sealers, and tends to decrease after sealers setting [38].

Related to AH Plus, its antimicrobial activity was previously demonstrated [39], and more recently Mak et al. have shown that although this sealer has a strong antimicrobial

activity when freshly prepared (from the formaldehyde released during the polymerization process), it decreases after time [40], which could be related with the low solubility of this sealer.

Although no antimicrobial studies have been made specifically using Bio MTA+ sealer, studies have been made using other Mineral Trioxide Aggregate (MTA) based sealers, demonstrating that the high amounts of oxide compounds, such as iron oxide, aluminum oxide, sodium oxide, and magnesium oxide, are capable of cell wall's damaging [38].

Beyond the study of cytotoxicity, we also performed the analysis of the cell morphology after incubation with the material-conditioned media. SEM analysis was in line with the results of the cytotoxicity study by material-conditioned media. With AH Plus a cellular confluence similar to the control group was observed, despite some minor changes in morphology. Similar results with the use of AH Plus have already been observed in a previous study [5]. Bio C sealer was the sealer that showed a more drastic effect on cells morphology with a decrease in cell confluence and several morphological changes. Unlike Bio C sealer, Bio MTA+ did not display a sudden decline in cell confluence, but many changes in cell morphology were observed.

The cytogenetic study was performed using the most common sealer used in dental treatments, AH Plus. The analysis of 40 GTG metaphases revealed that, although the majority of the cells were normal, some chromosomal aberrations were identified. The occurrence of these rearrangements could be due to the cultivation of these cells over different passages, which might induce some chromosomal changes. On the other hand, after the addition of AH Plus, several cytogenetic harvesting procedures were needed, and a larger number of metaphases had to be observed because of the severe decrease in mitotic index, and the high number of chromosomal aberrations. Most of the cytogenetic alterations were structural but some numerical changes were also observed. The study of a total of 90 GTG metaphases, with both the 10 mg/mL and the 100 mg/mL of AH Plus concentration, revealed several unbalanced translocations, aneuploidies, marker chromosomes, and other complex rearrangements. These results could be due to a directly induced chromosomal instability by the sealer but also due to the selection in the culture of cells with previous specific aberrations- However, some previous studies suggested that the epoxy resin in the AH Plus sealer has mutagenic behaviour, which can be translated in breaks in cellular DNA [41]. One of the most frequently observed chromosomal changes was the duplication of almost the whole long arm of chromosome 11 and the loss of the Y chromosome, which were always observed simultaneously, in a total of 31 metaphases (20 metaphases of 100 mg/mL and 11 metaphases of 10 mg/mL). This chromosomal alteration was also observed in metaphase cells exposed to the normal culture medium. Therefore, the high number of metaphases with this alteration after incubation with the compound is suggestive of a selection of the cells showing this specific alteration.

5. Conclusions

In conclusion, this study allowed us to verify that these root canal sealers exhibit some cytotoxicity, regarding cell viability, morphology of cells, and chromosomal stability, depending on the concentration used, and also contributes to the importance' confirmation of the endodontic sealers composition in the assurance of their biocompatibility towards the periapical cells/tissue. Although more studies are still needed, this work evaluates and compares these three sealers reaching important results that could help in the selection of the most appropriate compounds to be used in clinical practice and to determine the maximum recommended amounts of each sealer, as well as the importance of the sealers composition, and their antimicrobial behaviour. Our research into the biocompatibility of endodontic sealers, specifically Bio C sealer and Bio MTA+, has enhanced our comprehension of their impact on cells and provided valuable insights to guide and support future research. Ultimately, this knowledge aims to ensure the safe and effective application of these sealers in clinical practice.

Author Contributions: R.N., I.T., M.C., I.M.C., A.S.D. and I.P.R. participated in the conception and design of the study. All the authors participated in the acquisition, analysis, or interpretation of the data. All the authors participated in the writing of the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: The Center for Innovative Biomedicine and Biotechnology (CIBB) was funded by the Foundation for Science and Technology, I.P. (FCT, Portugal) through the Strategic Project UIDB/04539/2020 and UIDP/04539/2020. The Center for Interdisciplinary Research in Health (CIIS) was funded by FCT through the Strategic Project UIDB/04279/2020 and UIDP/04279/2020.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The authors confirm that the data supporting the findings of this study are available within the article.

Acknowledgments: The authors thank to Foundation for Science and Technology (FCT, Portugal) for funding Center for Innovative Biomedicine and Biotechnology (CIBB) through the Strategic Project UIDB/04539/2020 and UIDP/04539/2020; and also for the financial support of Center for Interdisciplinary Research in Health (CIIS) under the project UIDB/04279/2020 and UIDP/04279/2020. Thanks are also due to FCT and UCP for the CEEC institutional financing of Ana Sofia Duarte (CEECINST/00137/2018/CP1520/CT0013). Maria Bartolomeu and thanks to the UCP for the Junior Researcher position project InDig (POCH-02-5312-FSE-000025) supported by National Funds through Programa Operacional Capital Humano e Fundo Social Europeu (FSE).

Conflicts of Interest: The authors declare no conflict of interest.

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