ISSN: 2754-4893

Journal of Dental Science Research Reviews & Reports

SCIENTIFIC Research and Community

Research Article

Open Access

Biological Tissue Response of Endodontic Sealers after Subcutaneous Implantation

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ABSTRACT

This study evaluated the tissue reaction and inflammatory response after implantation of endodontic sealers in the subcutaneous connective tissue of isogenic mice. 120 mice were divided into 4 groups: BioRoot RCS, AH-Plus jet, ZOE and Sham group (negative control), which were evaluated in the experimental periods of 7, 21 and 63 days (n= 10). A macroscopic analysis was performed through digitized photograph to measure the thickness of the reaction tissue. Microscopic analysis were performed to count the number of blood vessels present in the reaction tissue and to evaluate collagen fiber networks. Data were analyzed using the non-parametric Kruskal-Wallis test and Dunn´s post test (α=5%). The reaction tissue showed greater thickness and amount of vessels and blood cells in the initial period of 7 days and was reduced over time, except the group AH Plus Jet which presented similar values of blood cells and vessels in all experimental periods. The BioRoot RCS at 7 days, presented the values of thickness and blood cells in the reaction tissue significantly higher ($p< 0.001$). Regarding collagen fiber formation, there was a greater amount of collagen fibers in the Sham group (7, 21 and 63 days), BioRoot at 63 days and AH Plus Jet at 21 and 63 days. In conclusion, endodontic cements presented greater thickness and amount of blood cells in the initial period, being reduced with time. On the other, collagen fiber formation were more pronounced at final periods.

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Received: July 01, 2025; **Accepted:** July 11, 2025; **Published:** July 19, 2025

Keywords: Endodontic Cement, Macroscopic Analysis, Reaction Tissue, Microscopic Analysis, Blood Vessels

Introduction

All steps of endodontic treatment have a specific objective and are guided by the principle of infection control. Biomechanical preparation is a fundamental step that promotes cleaning and modeling of the root canal, providing an ideal three-dimensional conformation for the delivery and action of irrigating solutions, intracanal dressings and filling materials [1].

The obturation of root canals consists of the use of two main materials: gutta- percha and endodontic sealer. Gutta-percha, a polyisoprene trans-isomer, is considered the gold standard material for filling root canals [2]. Root canal sealers, in turn, aim to fill in three-dimensionally the irregularities of the root canals, as well as dentinal ramifications and tubules, promoting a hermetic seal [3].

Ideally, these materials should have tissue compatibility and bioactivity, considering that they will be in close contact with apical and periapical living tissues [4, 5].

Endodontic treatment of root canals should promote repair in cases of apical periodontitis and the endodontic sealers should have a reparative potential in periapical lesions [6].

Cements based on zinc oxide and eugenol (ZOE), were used for a long time in root canal treatment due to their low cost, good sealing capacity and antibacterial property. However, ZOE has relevant disadvantages, such as high cytotoxicity, genotoxicity and damage to adjacent tissues [7-9]. Furthermore, this material is considered an inflammation-inducing material, characterized by areas of tissue destruction and necrosis [10].

BioRoot RCS (Septodont, Saint - Maur - des Fosses, France) is an endodontic sealer based on tricalcium silicate introduced in 2015, which is composed of a powder containing tricalcium silicate, povidone and zirconium oxide, and the aqueous solution based on calcium chloride and polycarboxylate [11].

This root canal searler has shown satisfactory results in different studies, such as the capacity for prolonged release of calcium ions in the medium and an alkaline pH, favorable properties for the formation of apatite and antimicrobial activity, evidencing its bioactivity [12, 6].

Additionally, this material showed low cytotoxic potential, and biological compatibility in different cell lines [13, 14].

At the same time, other beneficial properties such as induction of proliferation of periodontal ligament fibroblasts and the synthesis of angiogenic and osteogenic growth factors, as well as inhibition of the recruitment of inflammatory cells and of the secretion of pro-inflammatory cytokines, such as interleukin IL-6, were demonstrated by the BioRoot RCS [15, 16].

However, in a recent in vivo study published by our research group, BioRoot endodontic sealer triggered an intense initial inflammatory tissue response, despite intense calcium precipitation in all periods [6].

AH Plus Jet was launched as an innovation based on the conventional AH Plus, featuring the same composition, but supplied in syringes with self-mixing disposable tips. The use of these tips allows the cement to be injected directly into the canal orifices, ensuring a balanced and homogeneous mixture of the components with less porosity when compared to the conventional AH Plus, making its use more effective with control of possible contaminations [17].

In addition, it has biological compatibility, lower cytotoxicity when compared to MTA Fillapex, ability to inactivate microorganisms in the root canals and induce repair of inflamed periapical tissues due to its alkaline pH [18-20].

Recently, AH Plus Jet showed a greater inflammatory response cells in comparison to calcium silicate–based sealers after 7 and 30 days of subcutaneous tissue implantation [21].

Also, this material exhibited an intermediate and more constant inflammatory response pattern over time in subcutaneous tissue [6].

The action of endodontic sealers is of fundamental importance in the modulation of the inflammatory response in all stages of pulpal and periapical disease, considering the direct contact with the apical living tissues and the indirect effects resulting from the release of substances and ions. Inflammatory and anti-inflammatory molecular interactions of endodontic sealers can change and influence the state of the pathology, justifying the relevance of studies in different parameters and levels, including evaluation of biological compatibility and bioactivity. The subcutaneous implant model reproduces a micro-environment of living connective tissue, making it possible to evaluate the tissue response triggered after close contact with the endodontic sealers through macroscopic and microscopic evaluation, elucidating the profile of the inflammatory and reparatory potential of the evaluated materials. Animal models more closely replicate the clinical scenario and may serve as a reference approach for biological assessment. This method helps maintain consistency in host conditions [22].

Thus, despite the literature presenting studies regarding the physical-chemical and biological properties of AH Plus jet and BioRoot RCS endodontic sealers, most of these were performed through in vitro assays in cell culture. Thus, the relevance of the present study is justified, which evaluated in vivo the tissue response of resinous and bioceramic endodontic sealers, elucidating their biological behavior.

Therefore, the objective of the present study was to evaluate the tissue response after implantation of different endodontic filling materials (ZOE, BioRoot RCS and AH-Plus jet), in subcutaneous connective tissue of isogenic mice.

Material and Methods

The study design followed the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines, and care for the welfare of the animals followed the standards and ethical principles adopted by the Ethics Committee for the Use of Animals of the Ribeirão Preto School of Dentistry, University of São Paulo (CEUA/FORP-USP) and by the Normative Resolutions of the National Council for the Control of Animal Experimentation (CONCEA), regulated by the Brazilian Federal Constitution in Law No. 11,794 of October 8, 2008 [23]. In addition, tests were conducted as required by ISO 10993-6:2007. The project was submitted to the CEUA/FORP-USP, having been approved (0022/2021R2).

Preparation of Materials

The BioRoot RCS, AH-Plus jet and zinc oxide and eugenol – ZOE materials (Biodinamica Química e Farmacêutica LTDA., Ibiporã, PR – Brazil), were manipulated according to the recommendations of their respective manufacturers in laminar flow so that the aseptic chain was maintained. Thirty specimens of each material were prepared, with dimensions of 5 mm in height and 1.5 mm in diameter by introducing the material into Teflon matrices, previously sterilized, which were made in the Precision Workshop of the Ribeirão Preto Campus at the University of São Paulo (USP). A setting time of up to 3 times the recommended by the manufacturer of each material was expected.

Obtaining the Animals

A total of 120 isogenic male mice of the BALB/c strain, 6 to 8 weeks old, weighing an average of 20 grams, which were purchased from the central animal facility of the Ribeirão Preto Campus (USP) were used. All animals were kept at the animal facility I at FORP/USP in polypropylene cages (5 animals per cage), with constant temperature (22 ± 2ºC), and relative humidity (55 ± 10%), in a light-dark cycle of 12:12 hours with standard ration and free access to water.

In vivo study of the response in subcutaneous tissue in isogenic mice

After a week of adaptation, the animals were anesthetized in the inner part of the left hind paw with an intramuscular injection of 10% Ketamine (Agener União Química Farmacêutica Nacional S/A, Embu-Guaçu, SP, Brazil), and 2% Xylazine (Dopaser, Laboratories Calier, SA, Barcelona, Spain), in the proportion of 0.2 mg/kg and 0.8 mg/kg, respectively, immediately before surgery. Next, trichotomy was performed on the animal back, and antisepsis was performed in the region with 1% chlorhexidine digluconate. The incision of 1 cm was made with surgical scissors in the dorsal region, followed by divulsion with blunt scissors. After positioning the specimen inside the tissue, it was sutured using silk thread (Vicryl 4-0, Ethicon, Johnson & Johnson). The animals were kept at the animal facility I of the Ribeirão Preto School of Dentistry – USP during the experimental periods with food and water ad libitum. Negative control group (Sham) consisted of animals submitted to surgical procedure only without receiving any material.

The experimental groups were divided according to the material inserted in the subcuaneous tissue of each animal. The distribution of groups, materials used, number of animals and experimental times are shown in Table 1.

Table 1: Distribution of Experimental Groups, Materials used, Number of Animals and Experimental Periods.

Group	Material	Animals per period	Experimental periods
Experimental	BioRoot RCS	n=10	7, 21 and 63 days
Experimental	AH-Plus jet	n=10	7, 21 and 63 days
Experimental	Zinc oxide and eugenol	n=10	7, 21 and 63 days
Negative control	Surgical procedure only (Sham)	n=10	7, 21 and 63 days

At the end of each of the experimental periods (7, 21 and 63 days), the animals were euthanized with an anesthetic overdose of 1mg/kg, followed by a carbon dioxide chamber. The tissue block containing the specimen of the tested material, the surrounding portion of the subcutaneous connective tissue and the skin was removed with sterile surgical scissors and fixed by immersion in 10% buffered formalin for 24 hours at room temperature.

Macroscopic Evaluation

The pieces were then washed for approximately 4 hours in running water and stored in 70% alcohol. In this step, a quantitative macroscopic analysis of the tissue inflammatory response was performed using standardized photographic images of the tissue. The images were digitized and analyzed using the Image J software (National Institutes of Health - NIH), in order to quantify the intensity of the inflammatory response triggered by each material by measuring the thickness of the reaction tissue.

Data were expressed in millimeters (mm). For scale calibration of the software measurement tool, it was necessary to determine the size of the clamp used to fix the samples obtained (12mm). All analyses were performed by a single experienced and calibrated evaluator. Proceeding to the Image J software, the "analyze" and "set scale" tools were selected to standardize the actual size and carry out the measurements. Next, the total area of the specimen was delimited, as well as the surrounding reaction tissue area. Subsequently, the thickness of this reaction tissue was measured in mm at four representative points around the material, using the "measure" tool, obtaining the average per specimen (Figure 1).

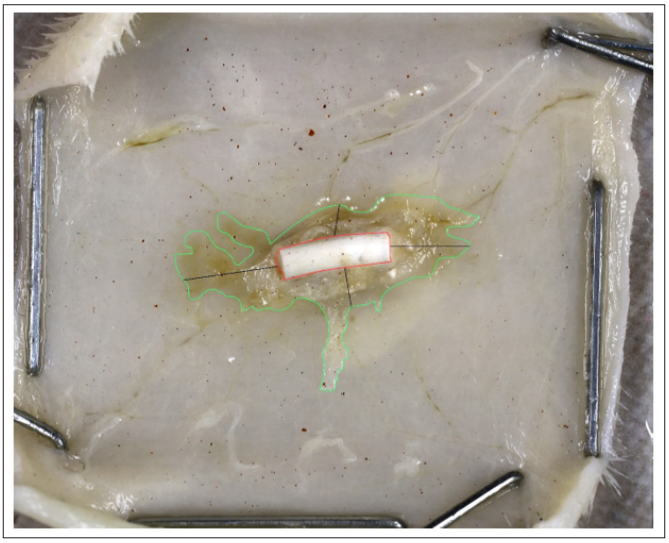

Figure 1: Representative Image of a Subcutaneous Connective Tissue Sample Containing the Specimen of the Tested Material (ZOE, 7 days), Surrounded by reaction Tissue. The Thickness of the Reaction Tissue was measured at Four Predefined Points within the Selected Area

Histotechnical Processing and Microscopic Evaluation

Subsequently, the pieces were submitted to routine histotechnical processing, being washed in running water for 2 hours, dehydrated in alcohol of increasing concentrations (70% and 95% for 30 minutes each; 2 changes of 100% for 20 minutes each and 2 changes of 100% alcohol for 40 minutes each), cleared in xylol (2 baths of 20 minutes and 1 of 40 minutes), and embedded in paraffin. Semi-serial sections of 5µm (10 to 15 slides with 2 slices per slide), with intervals of 15µm were obtained throughout the tissue extension.

Blood Cell Counting

The sections were stained with hematoxylin and eosin (HE), and submitted to analysis under conventional light microscopy (brightfield), and under fluorescent mode using the Alexa Fluor 488 filter (AF488), on the Axio Imager.M1 microscope (Carl Zeiss, Jena, Germany), coupled to an AxioCam MRc5 camera (Carl Zeiss). The quantification of blood vessels and cells, identified by strong light green fluorescence under fluorescent light, present in representative areas of the reaction tissue around the material (granulation tissue), at 40X magnification in an area of approximately 0.085mm 2 was performed. The images were submitted to the Image J software for quantification of vessels and blood cells through color segmentation, and the data were expressed in percentage (%) of area. Initially, it was necessary to install the additional Plugging IHC Toolbox in ImageJ in order to perform the color segmentation. Next, in the Set Measurements tool, the information area, min and max gray value, mean gray value and fraction area were selected. Subsequently, the color of interest was determined and, when applying the Training tool, the software provided a new image with only the selected color, being able to modify its intensity with the color chooser tool, including or excluding areas. The image obtained was transformed into grayscale with the RGB stack scale with three new images provided with black and white intensities depending on the predominance of red, green and blue before. The scale in green was selected and, applying the Threshold tool, the area covered by vessels and blood cells was highlighted and their quantity measured through the percentage obtained in relation to the total area.

Figure 2 illustrates a representative image stained with HE in conventional light microscopy (A), and its respective image in fluorescence microscopy (B), showing blood vessels and cells. Figure C illustrates the grayscale image obtained by the ImageJ software after selecting the color of interest to determine the % area occupied by cells and blood vessels.

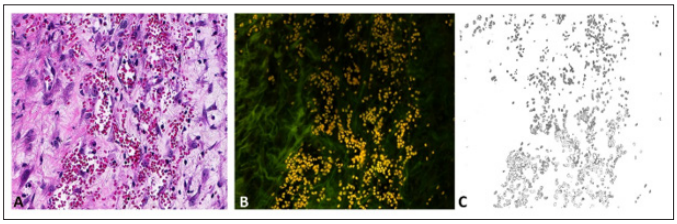

Figure 2: Representative Image (AH Plus Jet group at 63 days) Stained with Hematoxylin and Eosin, Observed Under Conventional Light Microscopy (a) and Fluorescence Microscopy (b), Highlighting Blood Cells and Blood Vessels. Selection of Blood Cells in the Representative Image (AH Plus Jet group, 63 days), Displayed in Grayscale using Image Analysis Software (C).

Collagen Fiber Evaluation

In parallel, subsequent specimens were submitted to Picrosirius red staining and conventional light microscopy to evaluate collagen fiber networks. This analysis allowed for quantitative characterization of fibrillar collagen networks (in red color) in tissue sections. Images from representative areas (approximately 0.085mm 2) of the reaction tissue were taken at 40X magnification and submitted to the Image J software for quantification of collagen fiber formation, expressed in percentage (%) of area. Using the color deconvolution tool, the red image was converted to grayscale and the quantity of collagen fiber networks was determined through the percentage obtained in relation to the total area. All microscopic analysis were performed by a single experienced and calibrated evaluator without prior knowledge of the group to be analyzed.

Statistical Analysis

For the statistical analysis of the data obtained in the macroscopic evaluation, 5 specimens of each group and experimental period were included, choosing the images that contained the specimen or that had the place of its occupation evidenced in the sample. All data were analyzed using the GraphPad Prism 7 software (GraphPad Software Inc., San Diego, CA, USA), using the non-parametric Kruskal-Wallis test and Dunn´s post test;s post-test. The significance level adopted was 5%.

Results

Macroscopic Evaluation

After comparing the thickness values of the reaction tissue formed around the implanted materials in the periods of 7, 21 and 63 days, it was possible to observe a statistically significant difference between the groups (endodontic cements and experimental periods) ($p< 0.0001$) (Figure 3).

In general, it was possible to observe that the reaction tissue presented greater thickness in the initial period of 7 days, and it was reduced over time, with the lowest value at 63 days. BioRoot presented the highest values in all periods (Figures 3 and 4).

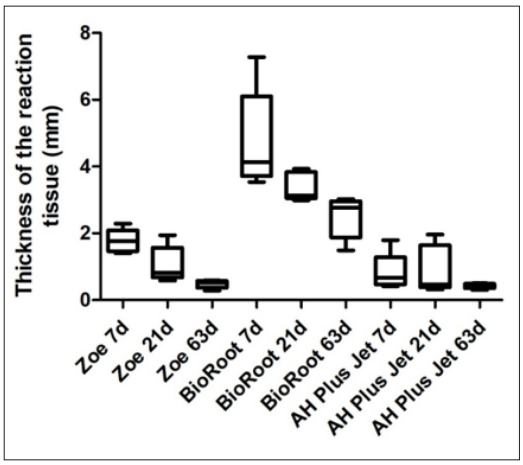

Figure 3: Box Plot illustrating the Minimum, First Quartile, Median, Third Quartile, and Maximum Values of the Thickness (mm) of the Reaction Tissue Formed Around the Endodontic Sealers and Sham Group at the Experimental Time Points of 7, 21, and 63 days

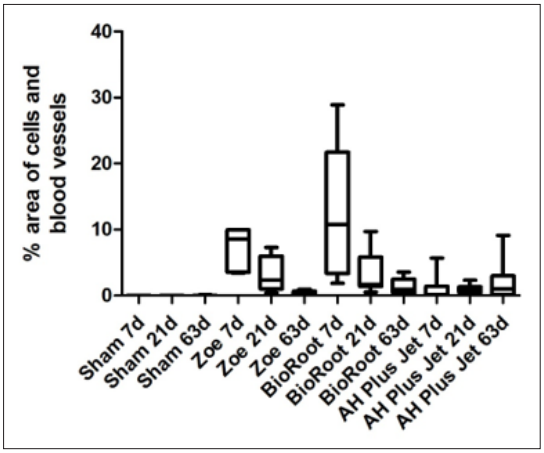

Figure 4: Box plot illustrating the Minimum, First Quartile, Median, Third Quartile, and Maximum Values of the Amount of Cells and Blood Vessels Present in the Reaction Tissue Formed Around the Endodontic Sealers and Sham Group at the Experimental Time Points of 7, 21, and 63 days

The Dunns post-test revealed that the BioRoot at 7 days had the highest value of reaction tissue thickness, and was statistically different from the ZOE at 63 days and the AH Plus Jet at 7, 21 and 63 days ($p< 0.01$), with the ZOE and AH Plus Jet at 63 days having the lowest values. Additionally, the BioRoot at 21 days presented a statistically higher value of the thickness of the reaction tissue in relation to the ZOE and the AH Plus Jet at 63 days ($p< 0.01$).

In the separate analysis within the ZOE and BioRoot groups, when comparing the different experimental periods, a statistically significant difference was observed between 7 and 63 days ($p=0.0059$ and 0.0049, respectively), with the period of 7 days showing the greatest thickness.

Regarding the analysis within the AH Plus Jet group, it was not possible to observe a significant difference between 7, 21 and 63 days ($p=0.2299$). When comparing the three cements with each other, in each experimental period, a statistically significant difference was observed between the BioRoot and AH Plus Jet at 7, 21 and 63 days ($p= 0.0037$; 0.0081; 0.0075, respectively), with the BioRoot presenting the highest tissue thickness values. It was not possible to show statistical differences between the other groups of materials ($p>0.05$).

Microscopic Evaluation

Blood Cell Counting

After comparing the percentage (%) of area relative to cells and blood vessels present in the reaction tissue of the implanted materials, in the periods of 7, 21 and 63 days, it was possible to observe a statistically significant difference between the control and experimental groups ($p< 0.0001$) (Figure 4).

In general, it can be seen that the reaction tissue showed a greater amount of vessels and blood cells (expressed in percentage of area), in the initial period of 7 days, and it was reduced over time, with the lowest value at 63 days, except for the AH Plus Jet sealer, which maintained a similar amount throughout the experimental times. The BioRoot RCS cement at 7 days showed the highest values of cells and blood vessels. The Sham Group (negative control), presented minimum values (0 or close to 0), in all periods.

The Dunn post-test revealed that the BioRoot RCS at 7 days had the highest value of blood vessels and blood cells and was statistically different from all groups ($p< 0.001$), except for the ZOE at 7 and 21 days and the AH Plus Jet at 21 and 63 days. The Sham Group (negative control), had the lowest values, being statistically different from all groups ($p< 0.001$), except for the ZOE and BioRoot at 63 days and the AH Plus Jet at 7, 21 and 63 days ($p>0.05$).

In the separate analysis within the Sham and the AH Plus Jet groups, it was not possible to observe a statistically significant difference between the periods of 7, 21 and 63 days ($p=0.8744$ and $p=0.2948$, respectively). For the ZOE cement group, there was a statistically significant difference between the periods of 7 and 21 days compared to 63 days ($p=0.0002$), which presented the lowest values. Regarding the BioRoot, when comparing the different experimental periods, a statistically significant difference was observed between 7 and 63 days ($p=0.0008$), with the 7-day period presenting the highest number of vessels and blood cells.

When comparing the Sham group and the three cements among themselves, over a period of 7 days, it was observed that the BioRoot, which presented the highest values in the area of vessels and blood cells, was statistically different from the Sham and the

AH Plus Jet. In addition, the Sham at 7 days, which presented the lowest values, was statistically different from the ZOE in the same period ($p < 0.0001$). At 21 days, there was a statistically significant difference between the ZOE and BioRoot compared to the Sham ($p = 0.0005$). In the period of 63 days, there was a statistically significant difference between the BioRoot and AH Plus Jet compared to the Sham ($p = 0.0005$).

Figure 5 illustrates representative microscopic images of the control and experimental groups in the different evaluation periods, showing the histopathological tissue response in relation to the amount of blood vessels and cells.

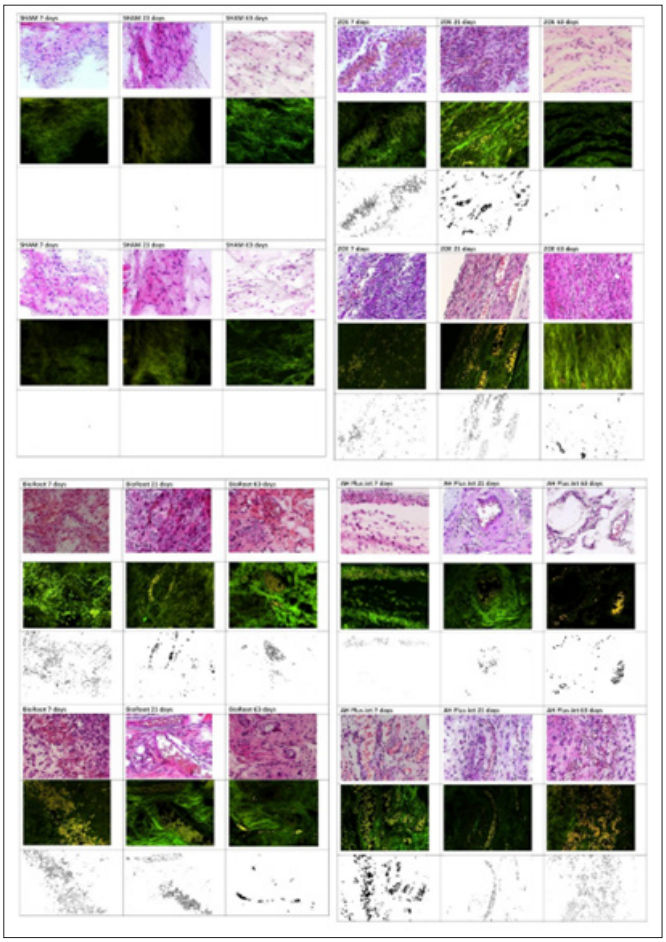

Figure 5: Representative Microscopic Images of the Control Group (Sham) and Experimental Groups in the Periods of 7, 21 and 63 Days Stained in HE and Analyzed in Bright Field Microscopy and in Fluorescence Microscopy, and Corresponding Image in Grayscale Obtained by the Color Selection Tool in the ImageJ Software for Quantification of Blood Cells and Vessels in % of Area

Collagen Fiber Evaluation

After comparing the percentage (%) of area relative to collagen fiber networks present in the reaction tissue of the implanted materials, in the periods of 7, 21 and 63 days, it was possible to observe a statistically significant difference between the control and experimental groups ($p < 0.0001$) (Figure 6).

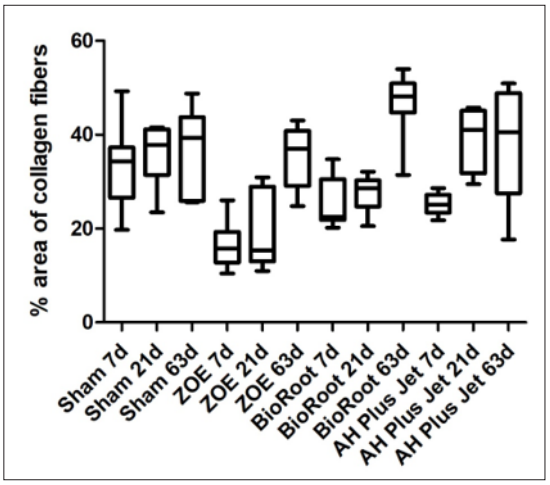

Figure 6: Box Plot illustrating the Minimum, First Quartile, Median, Third Quartile, and Maximum values % of Area of Collagen Fiber Networks in the reaction Tissue Formed Around the Endodontic Sealers and Sham Group at the Experimental Time Points of 7, 21, and 63 days

In general, it can be seen that there was a greater amount of collagen fibers in the Sham group (7, 21 and 63 days), BioRoot at 63 days and AH Plus Jet at 21 and 63 days. ZOE at 7 and 21 days presented the lowest values of % collagen fiber area.

The Dunn post-test revealed that the ZOE at 7 days had the lowest values of collagen fiber networks and was statistically different from Sham at 7, 21 and 63 days; ZOE and BioRoot at 63 days and; AH Plus Jet at 21 and 63 days. ZOE at 21 days also presented low fiber formation and statistical difference of BioRoot at 63 days and AH Plus Jet at 21 and 63 days. BioRoot at 63 days had the highest value of collagen fibers and was statistically different from its respective group in the 7 and 21-day periods and from AH Plus Jet at 7 days.

In the separate analysis within the Sham group, it was not possible to observe a statistically significant difference between the periods of 7, 21 and 63 days ($p = 0.5632$). For the ZOE and BioRoot groups, there was a statistically significant difference between the periods of 7 and 21 days compared to 63 days ($p=0.0003$ and $p=0.0001$, respectively). Regarding the analysis within the AH Plus jet group, the 7- and 21-day periods were statistically different from the 63-day period ($p=0.0016$).

When comparing the Sham group and the three cements among themselves, over a period of 7 days, it was observed that the ZOE group that had the lowest values was statistically different from the Sham and the AH Plus Jet ($p < 0.0001$). At 21 days, there was a statistically significant difference between the ZOE and Sham and the AH Plus Jet ($p < 0.0001$), which presented greater amounts of fiber. In addition, AH Plus Jet showed significantly higher value compared to BioRoot ($p < 0.01$). In the period of 63 days, there was a statistically significant difference between the ZOE and BioRoot ($p = 0.0116$), that had the highest values.

Figure 7 illustrates representative microscopic images after Picrosirius red staining showing
formation and intensity of collagen fiber networks in the control and experimental groups at the different evaluation periods.

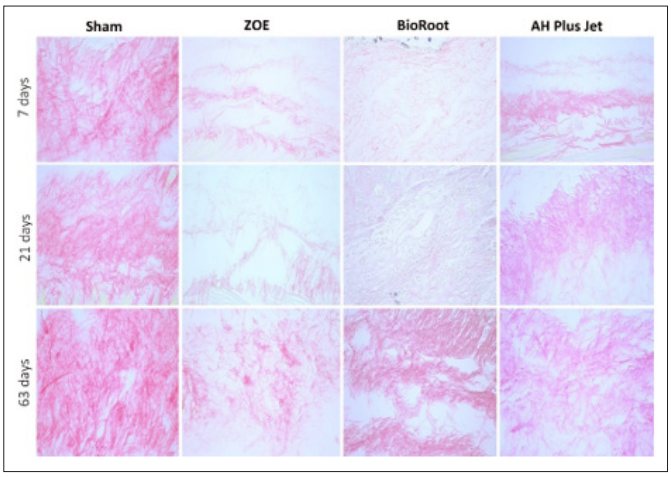

Figure 7: Representative Microscopic Images of the Control Group (Sham) and Experimental Groups in the Periods of 7, 21 and 63 Days Stained with Picrosirius Red, Evidencing Collagen Fiber Networks in % of Area

Discussion

An ideal endodontic sealer must present adequate physical, chemical and biological properties to fulfill the objective of hermetically sealing the three-dimensional space of the root canal and to stimulate the apical and periapical repair process after endodontic treatment. Thus, the type and composition of root canal sealer may interfere with therapy success rates, since substances present in these materials can be released causing different reactions in the apical and periapical tissues [24]. These materials will remain in contact with periapical living tissues for a prolonged period, justifying the clinical relevance of their biological compatibility, which can be understood as the ability of the material to promote an adequate and favorable host response in specific applications [14, 25]. The present study aimed to evaluate in vivo the tissue response triggered by endodontic cements of different compositions over time according to macroscopic analysis of the thickness of the reaction tissue formed and microscopic analysis of the intensity of vessels and blood cells and collagen fiber networks present in this tissue. Thus, the biological behavior of cement based on zinc oxide and eugenol (ZOE), which is known to have inflammatory properties, the BioRoot RCS, a bioceramic cement based on tricalcium silicate, and the AH-Plus jet, an innovation of conventional AH Plus based on epoxy-amine resin was evaluated in the reactionary tissue after subcutaneous implantation in mice. The data obtained by the macroscopic analysis demonstrated that, in general, the reaction tissue presented greater thickness in the initial period of 7 days, and it reduced with time, presenting the lowest value at 63 days. Additionally, the BioRoot RCS experimental group presented more intense and significant tissue alterations when compared to the other groups. At the same time, microscopically, in agreement with the macroscopic findings, the reaction tissue presented a greater amount of vessels and blood cells in the initial period of 7 days, being reduced in the subsequent periods. This response pattern was not observed in the Sham group (negative control), which as expected, presented values of 0 or close to 0 in all periods, and in the AH Plus Jet group, which maintained a similar and intermediate amount of red blood cells throughout the experimental times of 7, 21 and 63 days. Regarding collagen fiber evaluation, a higher percentage of collagen fiber deposition was observed in the Sham group across all evaluated time points (7, 21, and 63 days), as well as in the BioRoot group at 63 days and in the AH Plus Jet group at 21 and 63 days. Conversely, the ZOE group exhibited the lowest collagen fiber density at 7 and 21 days, as expected due to the lack of reparative capacity of this material. The BioRoot RCS at 7 days presented thicker reaction tissue with a greater amount of cells and blood vessels compared to the other groups, demonstrating an intense and exacerbated initial reaction. This response can be justified by the composition and mechanism of action of the material, which has a high alkaline pH (around 12), in the evaluation periods of 3 hours, 1, 3, 7 and 14 days, with this value reduced to approximately 8.7 in the period of 28 days [11].

The literature presents satisfactory results in relation to the BioRoot cement, such as antimicrobial activity, low cytotoxic potential, and biological compatibility with periodontal ligament cells (PDL) [14,16,26].

Gingival Fibroblasts, PDL stem cells, and pulp- derived stem cells [13, 27]. Additionally, it was shown that the BioRoot RCS promoted the induction of proliferation of periodontal ligament fibroblasts and the synthesis of angiogenic and osteogenic growth factors, showed osteogenic potential in undifferentiated mesenchymal cells, mineralizing activity in undifferentiated cells of the dental pulp, in addition to the prolonged release of calcium ions in the medium and an alkaline pH [11,15,26,27].

Thus, the alkalinity of the material and its proliferative and formative properties, including its angiogenic capacity, may explain the thicker reaction tissue with a greater amount of blood cells observed in the present study for the BioRoot group, although this exacerbated response may reflect negative biological behavior. Recently, the BioRoot RCS showed moderate cytotoxicity when compared to other cements in an in vitro study with fibroblasts [28].

Additionally, it was demonstrated that this cement promoted an increase in the proliferation of periodontal ligament cells on the first day, and this effect was significantly reduced over time [25]. In line with this finding, a recent in vivo study conducted by our research group using the subcutaneous tissue model in mice demonstrated that, despite the intense amount of inflammatory cells and the highly thickened reaction tissue in the initial period, the BioRoot RCS endodontic sealer promoted an improvement in histopathological inflammatory parameters over time. It was evidenced a reduction in inflammatory cell infiltration and a more organized arrangement of collagen fibers within the granulomatous reaction tissue after 63 days. Notably, this sealer was the only material that consistently induced marked calcium precipitation in the subcutaneous tissue across all experimental periods, reinforcing its bioactive potential [6].

These histopathological findings may support the outcomes observed in the present study, explaining the greater thickness of reaction tissue with a greater number of cells and blood vessels in the first 7 days, with its reduction in the subsequent periods, and a more pronounced collagen fiber network values at 63 days. It should be noted that cellular toxicity/compatibility does not present a static behavior whose effect is directly influenced by the release of cement components, which is dynamic over time, and this profile is confirmed by the evaluated parameters in the present study [29].

However, although the BioRoot RCS exhibited this pattern, its macroscopic and microscopic tissue response was intense, which needs to be clarified and elucidated in future studies so that this material can be used clinically with safety and efficacy. ZOE is described by many authors as a cement that has relevant

disadvantages, being cytotoxic, and irritating to living tissues [7,10,30].

These properties can be indirectly confirmed in the present study since the material induced considerable macroscopic and microscopic alterations superior to the negative control group (Sham). The ZOE showed values of thickness of reaction tissue and intensity of cells and blood vessels significantly higher in the first 7 days, followed by 21 and 63 days, although it showed less intense macroscopic tissue alterations in relation to the BioRoot. In addition, among all groups, ZOE demonstrated the lowest collagen fiber area percentages at 7 and 21 days.

The group of mice that received the AH Plus Jet endodontic sealer specimens demonstrated a more homogeneous and stable behavior during the experiment, inducing the formation of a less thick reaction tissue in relation to the BioRoot, but equivalent to the ZOE, showing a slight variation between the periods. Additionally, this cement had a lower amount of blood cells in the initial period compared to the BioRoot, and this level remained similar over the periods of 7, 21 and 63 days, whose profile was different from the other materials, which showed a reduction over time. Regarding collagen fiber evaluation, greater amount was observed in the AH Plus Jet group at 21 and 63 days. In the study of Elias et al. (2024), this sealer demonstrated an intermediate quantity of inflammatory cells in a constant manner throughout the experimental periods, and an increase in the thickness of the fibrous capsule, microscopically, after 63 days.

Taking the findings together, it can be hypothesized that the resinous composition of the AH Plus Jet cement could induce a continuous and long-term inflammatory response as well. Furthermore, the low solubility of epoxy resin-based sealers directly reflects on their biological behaviour [6].

According to some studies the literature, the AH Plus Jet demonstrated adequate biological properties, antibacterial activity and alkaline pH [18-20]. However, studies in culture of human periodontal ligament fibroblasts (HPdLF), have shown unsatisfactory results from this cement, such as reduced viability and cell proliferation, with induction of cytotoxic reaction and cellular apoptosis [31,32].

The cytotoxic potential may partially justify the results of the present study since the AH Plus Jet showed a satisfactory and stable biological behavior throughout the experiment, however, the permanence of microscopic reactionary changes at 63 days may suggest an unfavorable effect and be detrimental to the occurrence of the repair process.

Conclusion

Based on the methodology employed and the results obtained in the present study, it can be concluded that endodontic sealers present a more exacerbated inflammatory tissue reaction in the initial period, which is reduced with time, with the exception of the AH Plus jet, which showed a more constant behavior over the experimental periods. BioRoot caused a more intense initial response, presenting a thicker reaction tissue with a greater number of cells and blood vessels. Additionally, greater collagen fiber networks were evidenced at 63 days for all groups.

Acknowledgements

Undergraduate Scholarship by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (grant no. 2022-1016).

References

1. Peters OA (2004) Current challenges and concepts in the preparation of root canal systems: a review. J Endod 30: 559-567.
2. Vishwanath V, Rao HM (2019) Gutta-percha in endodontics - a comprehensive review of material science. J Conserv Dent 22: 216-222.
3. Roizenblit RN, Soares FO, Lopes RT, Dos Santos BC, Gusman H (2020) Root canal filling quality of mandibular molars with EndoSequence BC and AH Plus sealers: a micro-CT study. Aust Endod J 46: 82-87.
4. Kapralos V, Valen H, Koutroulis A, Camilleri J, Ørstavik D (2022) The dentine-sealer interface: modulation of antimicrobial effects by irrigation. Int Endod J 55: 544-560.
5. Khandelwal A, Janani K, Teja K, Jose J, Battineni G (2022) Periapical healing following root canal treatment using different endodontic sealers: a systematic review. Biomed Res Int 8: 3569281.
6. Elias VV, Lima RB, Lucisano MP, Araujo LDC, Pucinelli CM (2024) Inflammatory response to bioceramic and epoxy resin-based endodontic sealers implanted in mice subcutaneous tissue: an in vivo study. Microsc Res Tech 87: 2447-2458.
7. Kwon JS, Illeperuma RP, Kim J, Kim KM, Kim KN (2014) Cytotoxicity evaluation of zinc oxide-eugenol and non-eugenol cements using different fibroblast cell lines. Acta Odontol Scand 72: 64-70.
8. Dos Santos Costa FM, Fernandes MH, Batistuzzo de Medeiros SR (2020) Genotoxicity of root canal sealers: a literature review. Clin Oral Investig 24: 3347-3362.
9. Mutoh N, Tani-Ishii N (2011) A biocompatible model for evaluation of the responses of rat periapical tissue to a new zinc oxide-eugenol sealer. Dent Mater J 30: 176-182.
10. Mori GG, Teixeira LM, de Oliveira DL, Jacomini LM, da Silva SR (2014) Biocompatibility evaluation of biodentine in subcutaneous tissue of rats. J Endod 40: 1485-1488.
11. Siboni F, Taddei P, Zamparini F, Prati C, Gandolfi MG (2017) Properties of BioRoot RCS, a tricalcium silicate endodontic sealer modified with povidone and polycarboxylate. Int Endod J 2: e120-e136.
12. Dong X, Xu X (2023) Bioceramics in endodontics: updates and future perspectives. Bioengineering (Basel) 10: 354.
13. Collado-González M, García-Bernal D, Oñate-Sánchez RE, Ortolani-Seltenerich PS, Lozano A, et al. (2017) Biocompatibility of three new calcium silicate-based endodontic sealers on human periodontal ligament stem cells. Int Endod J 50: 875-884.
14. Jung S, Libricht V, Sielker S, Hanisch MR, Schäfer E (2019) Evaluation of the biocompatibility of root canal sealers on human periodontal ligament cells ex vivo. Odontology 107: 54-63.
15. Camps J, Jeanneau C, El Ayachi I, Laurent P, About I. (2015) Bioactivity of a calcium silicate-based endodontic cement (BioRoot RCS): interactions with human periodontal ligament cells in vitro. J Endod 41: 1469-1473.
16. Jeanneau C, Giraud T, Laurent P, About I (2019) BioRoot RCS extracts modulate the early mechanisms of periodontal inflammation and regeneration. J Endod 45: 1016-1023.
17. De-Deus G, Scelza MZ, Neelakantan P, Sharma S, Neves Ade A, et al. (2015) Three-dimensional quantitative porosity characterization of syringe- versus hand-mixed set epoxy resin root canal sealer. Braz Dent J 26: 607-611.
18. Mutoh N, Satoh T, Watabe H, Tani-Ishii N (2013) Evaluation of the biocompatibility of resin-based root canal sealers in rat periapical tissue. Dent Mater J 32: 413-419.
19. Güven EP, Yalvaç ME, Kayahan MB, Sunay H, Şahın F, et

al. (2013) Human tooth germ stem cell response to calcium-silicate based endodontic cements. J Appl Oral Sci 21: 351-357.
20. Pawińska M, Szczurko G, Kierklo A, Sidun J (2017) A laboratory study evaluating the pH of various modern root canal filling materials. Adv Clin Exp Med 26: 387-392.
21. Janini ACP, Moraes BF, Pelepenko LE, Dos Santos VAB, Barros-Costa M, et al. (2025) physicochemical properties and biological interaction of calcium silicate-based sealers - in vivo model. Clin Oral Investig 29: 86.
22. Yang X, Zheng T, Yang N, Yin Z, Wang W, et al. (2023) A review of the research methods and progress of biocompatibility evaluation of root canal sealers. Aust Endod J 1: 508-514.
23. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG (2012) Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. Osteoarthritis Cartilage 20: 256-260.
24. Saxena P, Gupta SK, Newaskar V (2013) Biocompatibility of root-end filling materials: recent update. Restor Dent Endod 38: 119-127.
25. Rebolledo S, Alcántara-Duffey R, Luengo Machuca L, Ferrada L, Alejandra G, (2023) Real-time evaluation of the biocompatibility of calcium silicate- based endodontic cements: an in vitro study. Clin Exp Dent Res 2: 10.
26. Jing Y, Gong T, Duan C, Wang H, Zhang C, et al. (2020) In vitro cytocompatibility and osteogenic potential of calcium silicate-based dental cements in a root canal-filling model. J Int Med Re 48: 300060519894801.
27. Seo DG, Lee D, Kim YM, Song D, Kim SY (2019) Biocompatibility and mineralization activity of three calcium silicate-based root canal sealers compared to conventional resin-based sealer in human dental pulp stem cells. Materials (Basel) 12: 2482.
28. Tolosa-Monfà A, Veroni A, Blasi-Cabús J, Ballester-Palacios ML, Berástegui- Jimeno E (2023) Cytotoxicity comparison of Bio C Sealer against multiple root canal sealers. J Clin Exp Dent 15: e110-e117.
29. Brackett MG, Messer RL, Lockwood PE, Bryan TE, Lewis JB, et al. (2010). Cytotoxic response of three cell lines exposed in vitro to dental endodontic sealers. J Biomed Mater Res B Appl Biomater 95: 380- 386.
30. Mittal M, Chandra S, Chandra S (1995) Comparative tissue toxicity evaluation of four endodontic sealers. J Endod 21: 622-624.
31. Accardo C, Himel VT, Lallier TE (2014) A novel GuttaFlow sealer supports cell survival and attachment. J Endod 40: 231-324.
32. Szczurko G, Pawińska M, Łuczaj-Cepowicz E, Kierklo A, Marczuk-Kolada G, et al. (2018) Effect of root canal sealers on human periodontal ligament fibroblast viability: ex vivo study. Odontology 106: 245-256.