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Biocompatibility of Four Root Canal Sealers: A Histopathological Evaluation in Rat Subcutaneous Connective Tissue

Camila Maggi Maia SILVEIRA
Shelon Cristina Souza PINTO
Rosário de Arruda Moura ZEDEBSKI
Fábio André SANTOS
Gibson Luiz PILATTI

Department of Dentistry, Ponta Grossa State University, Ponta Grossa, PR, Brazil

The aim of this study was to evaluate the subcutaneous biocompatibility of: Epiphany, AH Plus, Pulp Canal Sealer and Sealapex root canal sealers. Sixty rats were randomly assigned to 4 groups, according to the sealer. Polyethylene tubes containing the tested materials were inserted into the connective tissue. The implants were removed after 7, 15 and 30 days, and the tissue samples were processed, stained and examined by light microscopy. The descriptive analysis considered: thickness of the fibrous capsule, severity of the inflammatory reaction, and presence of giant cells. After 7 days, all sealers induced moderate to severe inflammatory reaction. After 15 days, Epiphany and AH Plus sealers showed a moderate inflammatory reaction, while Pulp Canal Sealer and Sealapex induced severe and mild inflammatory reactions, respectively. After 30 days, mild inflammatory reactions were observed for Epiphany, Sealapex and AH Plus. Sealapex induced the lowest inflammatory response at all evaluation periods, and only Pulp Canal Sealer did not show a decreased in the inflammatory reaction over time.

Key Words: biocompatibility testing, connective tissue cells, rats.

INTRODUCTION

One of the goals of endodontic therapy is to induce periapical repair with the formation of a cementum-like tissue. After obturation of the root canals, the filling material may get in contact with the periapical connective tissues. The chemical composition of the endodontic sealer may influence positively or negatively the final result of the endodontic therapy (1,2). Therefore, it must be inert, nonirritating and biocompatible with the living connective tissues (3).

Several methods have been used to evaluate the biocompatibility of endodontic sealers. One of the most practical and widely used methods is the implantation of the material into the subcutaneous connective tissue of rats. The irritating effect of the materials can be evaluated by the histopathological examination of tissue response around the implants (4,5).

Biological compatibility of root canal sealers is of key importance because under clinical conditions these materials are placed in direct contact with vital tissues. Tissue response to these materials may influence the outcome of the endodontic treatment. Pulp Canal Sealer EWT, a zinc oxide-eugenol-based sealer, has been widely used as a comparative sealer in several studies on biocompatibility (6,7), while the biological properties of the resin-based AH Plus and Epiphany sealers have been poorly investigated (8). The manufacturer of Sealapex, a calcium hydroxide-based sealer, has recently modified its formulation by adding bismuth trioxide to improve its radiopacity and increase its shelf life (9). The alterations in the original formulation could have affected negatively the tissue compatibility of this material.

The aim of this study was to evaluate the biocompatibility of 4 root canal sealers with different chemical compositions (Epiphany, AH Plus, Pulp Canal Sealer, Sealapex) after implantation into the subcutaneous connective tissue of rats.

Correspondence: Dr. Gibson Luiz Pilatti, Departamento de Odontologia, Universidade Estadual de Ponta Grossa, Avenida Carlos Cavalcanti, 4748, Uvaranas, 84030-900 Ponta Grossa, PR, Brasil. Tel: +55-42-3220-3741. Fax: +55-42-3220-3101. e-mail: gibsonpilatti@gmail.com

MATERIAL AND METHODS

The experimental procedures performed in this study followed the protocol reviewed and approved by the Ethics in Animal Research Committee of the Ponta Grossa State University and were in accordance with the international guiding principles for biomedical research involving animals.

Sixty 2-3 month-old male rats (*Rattus novergicus albinus*, Wistar) weighing 200 to 300 g were randomly selected and divided into 4 experimental groups (n=15), according to the sealer: Group 1: Epiphany (Pentron Clinical Technologies, Wallingford, CT, USA) a methacrylate resin-based sealer (urethane dimethacrylate); Group 2: AH Plus (Dentsply, De Trey GmbH, Konstanz, Germany), an epoxy resin-based sealer (epoxy-amine); Group 3: Pulp Canal Sealer EWT (Kerr; Sybron Dental Specialties, Romulus, MI, USA), a zinc oxide eugenol-based sealer; Group 4: Sealapex, new formulation (Kerr; Sybron Dental Specialties), a calcium hydroxide-based sealer. All groups were subdivided into 3 evaluation periods (n=5): 7, 15 and 30 days.

The animals were anesthetized by intraperitoneal administration of ketamine hydrochloride (75 mg/kg) and xylazine hydrochloride (10 mg/kg). The dorsal skin was shaved and disinfected with 5% iodine in alcohol. A 15 mm-long incision was made through the skin with a scalpel and 2 separated subcutaneous pockets were prepared by blunt dissection at each side of the incision. The endodontic sealers were freshly mixed according to the manufacturer instructions. Two sterile polyethylene tubes (10-mm long and 1.5 mm inner diameter) - one empty (control) and one filled with freshly mixed endodontic sealer - were carefully placed into the pockets to a depth of 20 mm in order to prevent smearing of the test material on the outer tube areas. After material implantation, the margins of the wound were joined and closed with interrupted suture (4-0 silk sutures, Ethicon; Johnson & Johnson S/A, São José dos Campos, SP, Brazil). All animals received normal diet and water *ad libitum* during the entire study period.

After 7, 15 and 30 days, the rats were anesthetized, shaved and had their skin disinfected as described. The implants were removed together with the surrounding tissues and immersed in 10% phosphate-buffered formalin solution. The animals were killed by cervical dislocation, according to the guidelines of the Brazilian College of Animal Experimentation. After fixing for 48 h, the tissue samples were processed for paraffin embedding and 4 μm longitudinal serial sections (Manual Microtome 820 Spencer; Lupe, São Paulo, SP, Brasil), parallel to the tube, were obtained and stained with hematoxylin and eosin. Each specimen was analyzed at ×40, 160, 400 magnifications with a light microscope.

A previously trained blind pathologist performed the histopathological analysis at three different moments. Tissue reaction was classified according to the following criteria: a) thickness of the fibrous capsule: thin - 3 to 5 cell layers; intermediate - 5 to 8 cell layers; thick - more than 8 cell layers; b) Inflammatory reaction: mild, moderate and severe, according to the number of inflammatory cells; c) presence of giant cells. The material was considered as biocompatible if the severity of the connective tissue reaction decreased with time.

RESULTS

Figure 1 shows the inflammatory reaction at 7, 15, and 30 days for all groups. Figures 2 to 6 illustrate the histopathological findings of all experimental groups.

7 Days

An intermediate fibrous capsule was found for Epiphany (Fig. 3A), Pulp Canal Sealer (Fig. 5A) and Sealapex (Fig. 6A) groups, while AH Plus and the control group showed a thin fibrous capsule around the polyethylene tube (Figs. 2A to 6A). A moderate to severe inflammatory reaction was observed for Epiphany (Fig. 3A), a moderate infiltrate was found for AH Plus (Fig. 4A) while Pulp Canal Sealer and Sealapex displayed a mild to moderate inflammatory infiltrate (Figs. 5A and 6A), mainly composed by lymphocytes, plasma cells and macrophages. A thin fibrous capsule and a mild

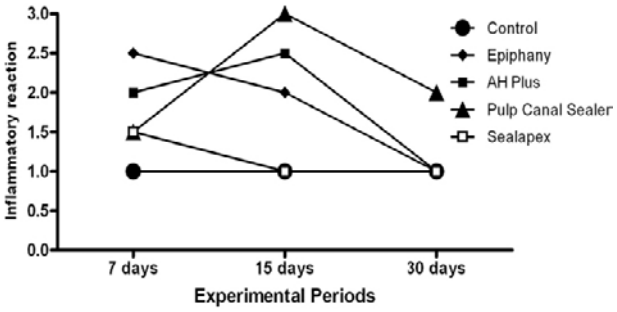

Figure 1. Inflammatory reaction after 7, 15 and 30 days (Scores: 0 =none; 1 = mild; 2 = moderate; 3 = severe).

inflammatory infiltrate were observed for the control group (Fig. 2A).

15 Days

All groups displayed a intermediate fibrous capsule around the tubes (Figs. 3B, 4B, and 5B), except for Sealapex (Fig. 6B) and the control group (Fig. 2B), which presented a thin fibrous capsule. The severity of the inflammatory reaction increased for Pulp Canal Sealer (Fig. 5B). Moderate and moderate to severe reactions were observed for Epiphany (Fig. 3B) and AH Plus (Fig. 4B), respectively. Sealapex (Fig. 6B) and the control group (Fig. 2B) exhibited a mild inflammatory reaction close to the implanted material.

30 Days

AH Plus (Fig. 4C) and Pulp Canal Sealer (Fig.

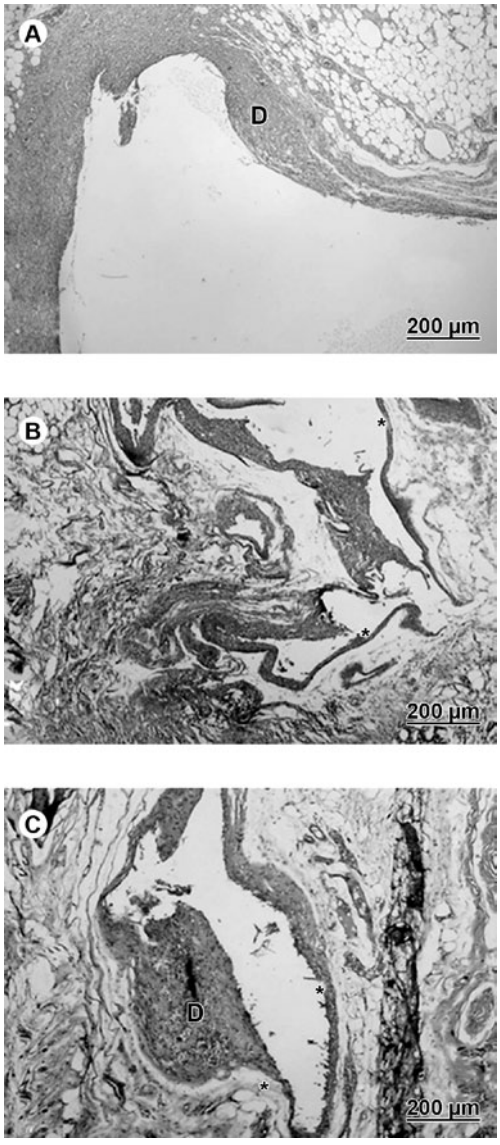

Figure 2. Control group. (*) Thin fibrous capsule and mild inflammatory reaction (D) at all the experimental periods (A = 7 days; B = 15 days; C = 30 days) (HE, original magnification ×40).

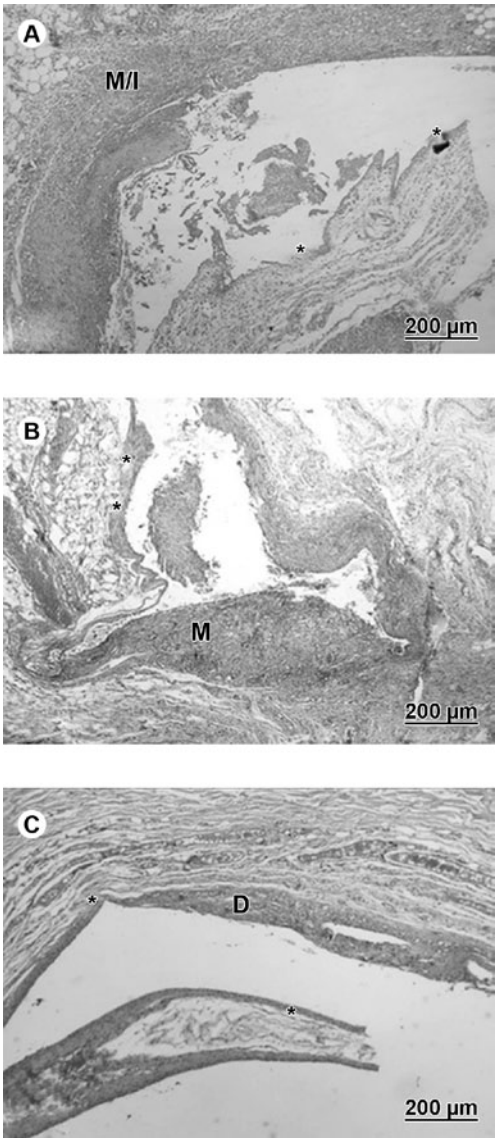

Figure 3. Epiphany. A = 7 days: (*) intermediate fibrous capsule and moderate/severe inflammatory reaction (M/I); B = 15 days: (*)intermediate fibrous capsule and moderate inflammatory reaction (M); C = 30 days: (*) thin fibrous capsule and mild inflammatory reaction (D) (HE, original magnification ×10 - A, and ×40 - B and C).

5C) were involved by an intermediate fibrous capsule, while Epiphany (Fig. 3C), Sealapex (Fig. 6C) and the control group (Fig. 2C) exhibited a thin fibrous capsule. All groups developed a mild inflammatory reaction close to the implanted material (Figs. 2C, 3C, 4C and 6C), except for Pulp Canal Sealer, which was surrounded by a moderate inflammatory infiltrate (Fig. 5C), mainly composed by plasma cells and macrophages.

DISCUSSION

The implantation of polyethylene tubes into the subcutaneous connective tissue of rats has been widely accepted as an important secondary test to access the biocompatibility of endodontic sealers (10). The polyethylene tube itself does not elicit a persistent irritation and the tested material tends not to spread to the surrounding area, leading to a precise control of the amount of material in direct contact to the tissues.

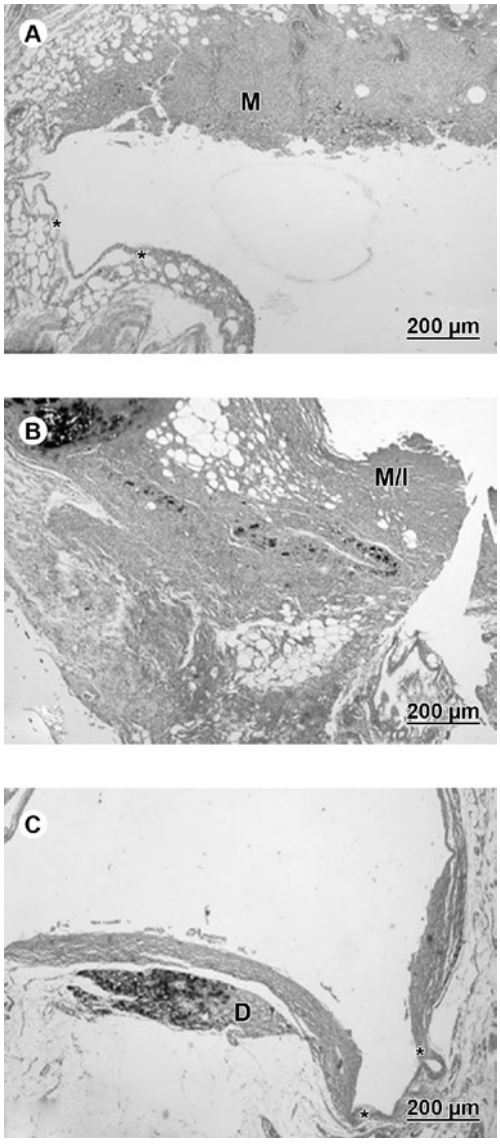

Figure 4. AH Plus group. A = 7 days: (*) thin fibrous capsule (FC) and moderate inflammatory reaction (IR); B= 15 days: (*) intermediate FC and moderate/severe (M/I) IR; C = 30 days: (*) intermediate FC and mild (D) IR (HE, original magnification ×10 - A, and ×40 - B and C).

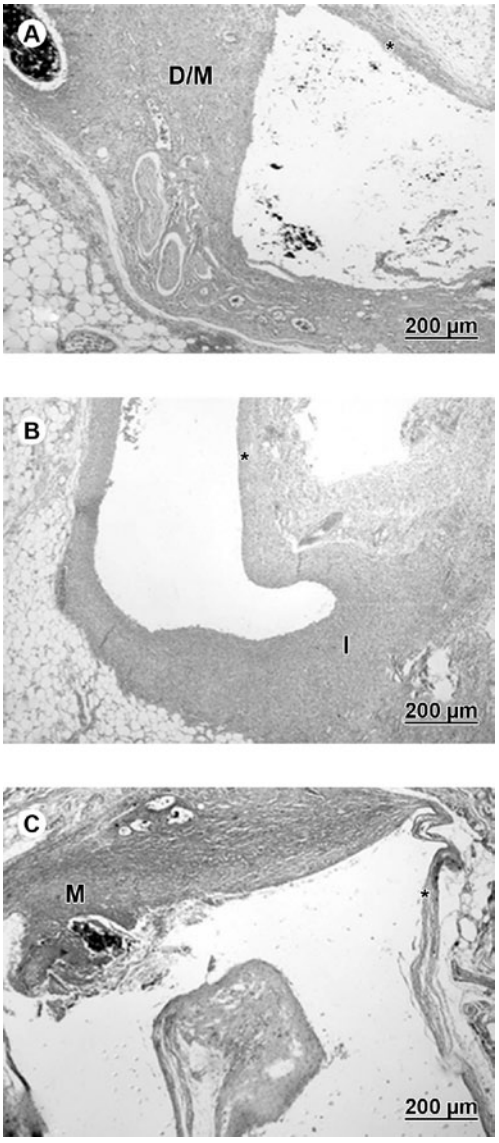

Figure 5. Pulp Canal Sealer group. A = 7 days: moderate fibrous capsule (FC) and a mild/moderate inflammatory reaction (IR); B = 15 days: moderate FC and a severe IR; C = 30 days: moderate FC and a mild IR (HE, original magnification ×10).

The implantation of a contralateral empty tube served as a control in the same animal, eliminating possible individual variability (3-5,11).

The use of resin-based sealers has demonstrated good sealing property and an excellent adhesion to the root canal walls (12,13). In relation to biocompatibility of a resin-based material, there are many factors related, particularly the amount and nature of leachable components (14). Conventional composite resins contain a polymerizable organic matrix that consist of several comonomers, such as Bis-GMA, UDMA, EGDMA and TEGDMA, in addition to several additives that act as coinitiators, stabilizers or inhibitors. These materials can show irritating effect due to unreacted monomers that remain after polymerization and induce a persistent local inflammatory reaction. There is little information, however, on the biological properties of these sealers.

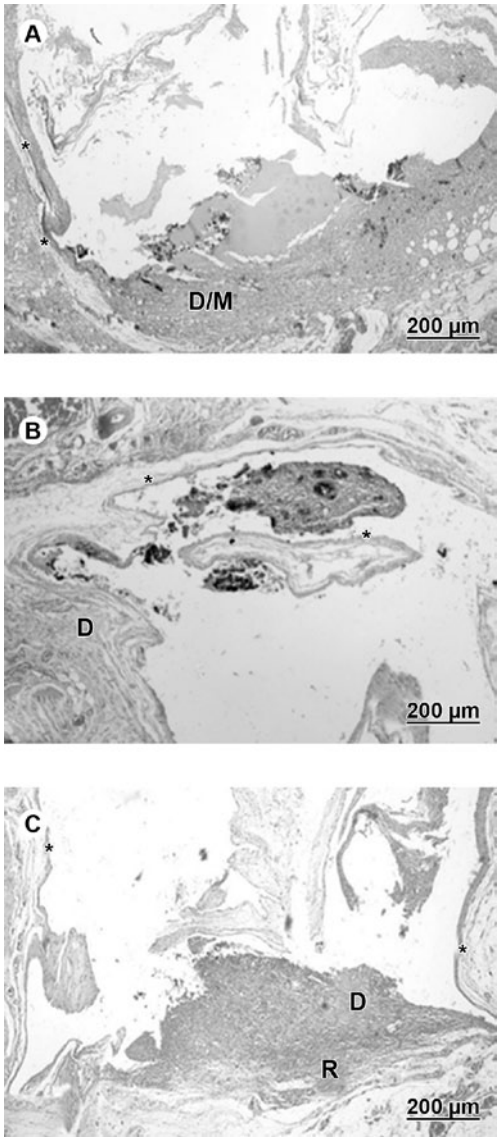

Figure 6. Sealapex group. A = 7 days:*intermediate fibrous capsule (FC) and a (D/M) mild/moderate inflammatory reaction (IR); B = 15 days:*thin FC and a (D) mild IR; C= 30 days:*thin FC and a (D) mild IR (HE, original magnification ×40 - A-C).

Epiphany also contains a small percentage of BisGMA, ethoxylated BisGMA, UDMA and hydrophilic dysfunctional methacrylates. The presence of these monomers can explain a mild to severe inflammatory reaction found in this study at the 7 days, 15 days and 30 days periods after implantation of this sealer. The results agree with those of Onay et al. (14), who found mild to severe inflammatory reaction at the 1-week period. Sousa et al. (8) reported no mild reaction in 80% of the Epiphany intraosseous implants after 4 weeks in comparison to AH Plus and EndoRez sealers. Although EndoRez and Epiphany sealers share some ingredients (e.g.: UDMA and barium sulfate), those authors (8) found a severe reaction 4 weeks after intraosseous implantation when EndoRez was used. This was attributed to the presence of amines in its composition, which accelerates the sealer polymerization, and to the release of formaldehyde, which can also induce non-neoplastic responses such as epithelial degeneration and a mixed inflammatory cell infiltration (5,15,16). In the present study, however, Epiphany showed a mild inflammatory reaction after 30 days, demonstrating its compatibility with the subcutaneous connective tissue.

Campos-Pinto et al. (17) evaluated Epiphany associated or not with self-etch primer. Epiphany showed a mild inflammatory reaction. However, when photoactivated Epiphany was evaluated, in which the primer was not used, severe necrosis and moderate to intense inflammatory reaction were found. The good biological response induced by Epiphany, in the other groups can be explained by its high calcium release, which promotes a more alkaline pH at the tissue site and favors tissue repair (18).

AH Plus is another resin-based sealer tested in this study; its composition is based on epoxy resin. Scarparo et al. (19) found that AH Plus promotes a mild inflammatory reaction, mainly after longer periods (60 days), less intense than that produced by EndoRez and EndoFill. In the present study, AH Plus showed a thin fibrous capsule at 7 days, similar to control group, and mild inflammatory reaction at the other periods (15 and 30 days). The results presented are similar to previous

studies (20,21).

Pulp Canal Sealer has been previously tested for toxicity *in vitro* (22) and *in vivo* (23). Zmener et al. (24) observed a severe tissue reaction to Pulp Canal Sealer after 10, 30 and 90 days of implantation. In the present study, Pulp Canal Searler showed a mild inflammatory reaction 7 days after implantation of the tubes. After 15 days the inflammatory reaction increased and became moderate at 30 days, being composed mainly by plasma cells and macrophages.

Sealapex is based on calcium hydroxide. Sealers containing calcium hydroxide will be biologically active when calcium and hydroxide ions are released (13). The diffusion of hydroxyl ions from the root canal sealers increases the pH at the root surface adjacent to the periodontal tissues, favoring the repair. Sealers based on calcium hydroxide are used to enhance healing process. Gomes-Filho et al. (25) studied the biocompatibility of two calcium hydroxide-based sealers (Sealapex and Acroseal) and found mild to moderate inflammatory infiltration after 7 and 30 days of tube implantation. These results agree with those of the present study in which mild to moderate inflammatory reaction to Sealapex was elicited at 7 days, decreasing the subsequent evaluation periods.

The outcomes of the present study showed that the sealers presented acceptable biological compatibility after 30 days, except for Pulp Canal Sealer, which induced mild inflammatory response at 7 days and moderate response at 30 days. Based on the parameters established for evaluation, the material was considered as biocompatible if the severity of the connective tissue reaction decreased with time, and thus Pulp Canal Sealer was not considered a biocompatible sealer. Further research is necessary to determine the clinical behavior of these sealers in patients undergoing endodontic therapy.

RESUMO

O objetivo deste estudo foi avaliar a biocompatibilidade subcutânea de cimentos endodônticos radiculares: Epiphany, AH Plus, Pulp Canal Sealer e Sealapex. Sessenta ratos foram divididos aleatoriamente em 4 grupos, de acordo com o cimento. Tubos de polietileno contendo os materiais testados foram inseridos no tecido conjuntivo. Os implantes foram removidos após 7, 15 e 30 dias, e as amostras de tecido foram processadas, coradas e examinadas por microscopia de luz. A análise descritiva considerou: espessura da cápsula fibrosa, severidade da reação inflamatória e presença de células gigantes. Após 7 dias, todos os cimentos induziram moderada e severa reação inflamatória. Após 15 dias, os cimentos Epiphany e AH Plus apresentaram uma reação inflamatória moderada, enquanto Pulp Canal Sealer e Sealapex induziram severa e leve reações inflamatórias, respectivamente. Após 30 dias, leve reação inflamatória foi observada para o Epiphany, Sealapex e AH Plus. Sealapex induziu a menor resposta inflamatória em todos os períodos de avaliação, e somente Pulp Canal Sealer não apresentou uma diminuição da reação inflamatória ao longo do tempo.

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