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ORIGINAL RESEARCH

Evaluation of Inflammatory Response to Endodontic Sealers in a Bone Defect Animal Model

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ABSTRACT

Aim: The aim of this study was to evaluate the inflammatory response to MTA Fillapex, AH Plus, and Pulp Canal Sealer Extensive Work Time (EWT), in a murine bone defect grafting model.

Materials and methods: Bilateral mandibular critical defects were produced in 45 Wistar rats with a trephine bur#2 and filled with the endodontic sealers. After 7, 14, and 28 days, the rats were euthanized and their jaws were histologically prepared.

Results: For the 7-day group, no statistical significance was observed among all studied groups ($p > 0.05$), and high levels of inflammatory infiltrate were detected. After 14 and 28 days, Pulp Canal Sealer EWT showed statistically lower inflammatory response in comparison to other sealers ($p < 0.05$) except for the control group (no sealers).

Conclusion: Pulp Canal Sealer EWT presented the lowest levels of inflammatory response. The critical defect grafting model was an effective method to detect differences among differences on the biological response to endodontic sealers.

Clinical significance: Knowing the biocompatibility of endodontics sealers that will be used in filling the root canal.

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INTRODUCTION

Since gutta-percha cones do not adhere directly to the root canal wall, their application on endodontic filling is often combined to the use of an endodontic sealer.[1] Nowadays, several different compositions for, such sealers have been already developed and employed with the purpose of achieving more desirable physicochemical properties, as well as improved biocompatibility with periapical tissues.[2] In this context, root canal sealers may be ordered based on chemical composition in several different groups, such as calcium hydroxides, bioceramics, zinc oxide/eugenol, or epoxy resins.

Endodontic filling materials may be considered true implants, as they remain in direct and intimate contact with vital tissues for considerably longer period of time.[3] Therefore, regardless of their chemical composition, endodontic sealers should be associated with adequate biological responses, in order to ensure the safety of their clinical use.

The International Organization for Standardization (ISO) states that tissue compatibility evaluation, both *in vitro* and *in vivo*, when appropriate, should be carried out as important steps prior to the clinical employment of any material or device.[4-6]

A considerable amount of research effort has been dedicated to the understanding of the biocompatibility

of root canal sealers, mostly by *in vitro* approaches and cytotoxicity assays. Several studies were focused on the comparison of cytotoxicity of different sealer groups. Al-Hiyasat et al[2] evaluated, through a murine fibroblast model, the materials Epiphany, EndoREZ, Metaseal, and AH Plus, which showed different levels of toxicity. Zhang et al[7] reported that the bioceramic sealer EndoSequence BC Sealer was less toxic to L929 mouse fibroblasts when compared to the resin-based AH Plus. In a recent *in vitro* study, Scelza et al[8] described strong levels of cytotoxicity in primary human osteoblasts for several representatives of different sealer groups. Other parameters have also been assessed, such as the capacity of differentiation and mineralization of osteogenic cell lineages in the presence of endodontic sealers.[9]

However, *in vitro* cytotoxicity assays are usually not able to access all long-term effects of some materials, which might cause a persistent inflammation or foreign body reaction in the periapical tissues and may delay the wound-healing process.[10,11] Consequently, international standards for material testing recommend the subsequent use of *in vivo* assays, such as subcutaneous tissue tests.[12] Nevertheless, data obtained through the subcutaneous approach constitute a model of limited value to represent the periapical region, because there is a lack of mineralization potential and it does not access events, such as movements, which might contribute to local irritation. Other *in vivo* animal models employing bone tissues may represent promising tools for a better understanding of the initial and long-term biocompatibility of endodontic materials.[13]

Therefore, the aim of this study was to compare the biocompatibility of different endodontic sealers: The bioceramic MTA Fillapex (Angelus, Curitiba, PR, Brazil), the epoxy resin-based AH Plus (Dentsply/Maillefer, Konstanz, Germany), and the zinc oxide/eugenol-based Pulp Canal Sealer Extensive Work Time (EWT) (SybronEndo, Orange, CA, USA) employing an animal grafting model in critical-sized mandibular defects.

MATERIALS AND METHODS

The present study has been independently reviewed and approved by the Ethical Committee for Animal Experiments of the Fluminense Federal University (00198/09), and followed national guidelines for animal welfare and the care/use of animals for experimental procedures.

Sixty adult Wistar rats (Rattus novergicus Albinus) were included in this study, weighting 180 to 200 gm. For surgical procedures, all animals were anesthetized intraperitoneally with ketamine hydrochloride (Ketalar; Pfizer, São Paulo, Brazil) at a dosage of 0.2 mL/100 gm of body weight, associated with dihydrothiazine hydrochloride (Rompum; Bayer, Rio de Janeiro, Brazil), at a dosage of 0.05 mL/100 gm. To prevent local discomfort, 0.6 mL of 2% xylocaine with epinephrine (1:100,000) was injected in the mucobuccal fold of the mandibular incisors region.

Bone Defect Creation

The bone defect was induced according to the previous work.[13] A 20 mm incision was made in both the right and left sides of the jaw region of each animal after shaving and washing the skin with povidine iodine. A trephine bur #2 (Incol; Instrumentos Cirúrgicos Oftalmológicos Ltda, São Paulo, Brazil), was used to create a standardized, round, through-and-through osseous defect (5 mm in diameter) on both sides of the jaw. The defects size is consistent with a so-called critical size defect implying that the defect does not heal spontaneously during the animal's lifetime.[14]

The animals were randomly distributed into three experimental groups (n = 15 animal per group, total = 45). The bone defects were filled with MTA Fillapex, Pulp Canal Sealer EWT, and AH Plus on one side of the lower jaw. All materials were prepared according to the manufacturers' recommendation for their clinical use (Table 1). The sealers were implanted in a freshly mixed, unset state and the opposite side incision was used as a control (blood clot) without the endodontic sealer.

Histological Preparation

The animals were euthanized with tripled doses of the same anesthetics employed on surgery, on the 7th, 14th, and 28th day (n = 5 per time). The lower jaw was excised, and any excessive tissue was removed. The hemijaws were fixed in 10% formaldehyde and subsequently decalcified in 20% formic acid for 21 days. Afterwards, the samples were

Table 1: Constituents of the investigated endodontic sealers

Product and manufacturer	*Composition*	*Brief preparation mode*
Pulp Canal Sealer EWT (Sybron Endo, Orange, CA, USA)	Powder: Zinc oxide, silver, resin, liquid, eugenol, Canada balsam	The components are combined trough a mixing the powder into liquid.
AH Plus (Dentsply/ Maillefer, Konstanz, Germany)	Epoxy resins, Calcium tungstate, Zirconium oxide, aerosol, Iron oxide, Adamantane amine, N,N-Dibenzyl-5-oxanonane, TCD-Diamine, Calcium tungstate, Zirconium oxide, Aerosil	The components are combined trough a mixing of equal portions by length of base and catalyst paste.
MTA Fillapex (Angelus, Curitiba, PR Brazil)	Salicylate resin, diluting resin, natural resin, bismuth trioxide, nanoparticulated silica, MTA, pigments	The components are combined trough a self-mixing tip attached to a syringe.

embedded in paraffin, and frontal semi-serial sections of the hemijaws were cut at a thickness of 7 µm. The sections were then processed for hematoxylin-eosin staining.

Histological Evaluation

The inflammatory responses were measured by two experienced pathologists, as blind observers previously calibrated to the research setting. Tissue inflammatory response was graded according to previous references,[15,16] following the score of 0 = (absence of inflammatory cells), 1 = light (few inflammatory cells), 2 = moderate (presence of macrophages and/or plasma cells), and 3 = severe (focal areas of necrosis; tissue densely infiltrated by inflammatory cells). The histological evaluation and description of the observed tissue response were performed with a light binocular microscope (Zeiss Axioskop 2, Carl Zeiss MicroImaging GmbH, Jena, Germany).

Statistical Analysis

Comparisons between scores obtained during the histological evaluation were performed using nonparametrical analysis of variance (ANOVA), through the Kruskal-Wallis test, setting the alpha error to 5%. Two-way ANOVA was also applied followed by Bonferroni test in order to analyze the intragroup statistical differences.

RESULTS

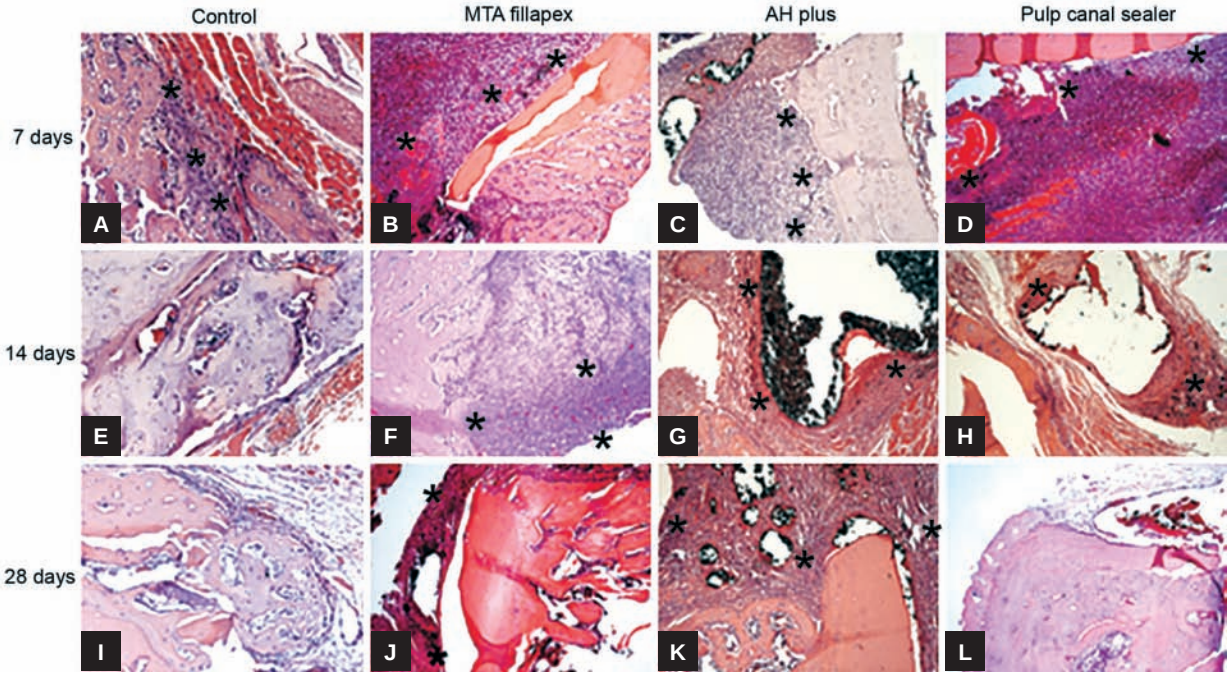
Figure 1 shows the representative histological section for each test group. It is possible to observe that all groups present some level of inflammatory infiltrate by 7 days (Figs 1A to D), but it has a tendency to disappear on the control group after 14 and 28 days (Figs 1E to L). MTA Fillapex and Pulp Canal Sealer EWT were completely removed during histological processing. It was possible to observe residues of AH Plus on all samples, usually associated with high levels of inflammatory infiltrate.

Graphs 1A to C shows the result of the score-based analysis of the histological images for each group. Over a period of 7 days, all endodontic sealers and the control group showed similar high levels of inflammatory response, without significant statistical difference ($p > 0.05$) (Graph 1A). The inflammatory reaction remained intense for both AH Plus and MTA Fillapex after 14 and 28 days. On the other hand, the Pulp Canal Sealer EWT and control groups showed a significant decrease on inflammatory reaction after 7 days (Graphs 1B and C). Twenty-eight days after surgery, samples from the control group presented virtually no inflammatory response.

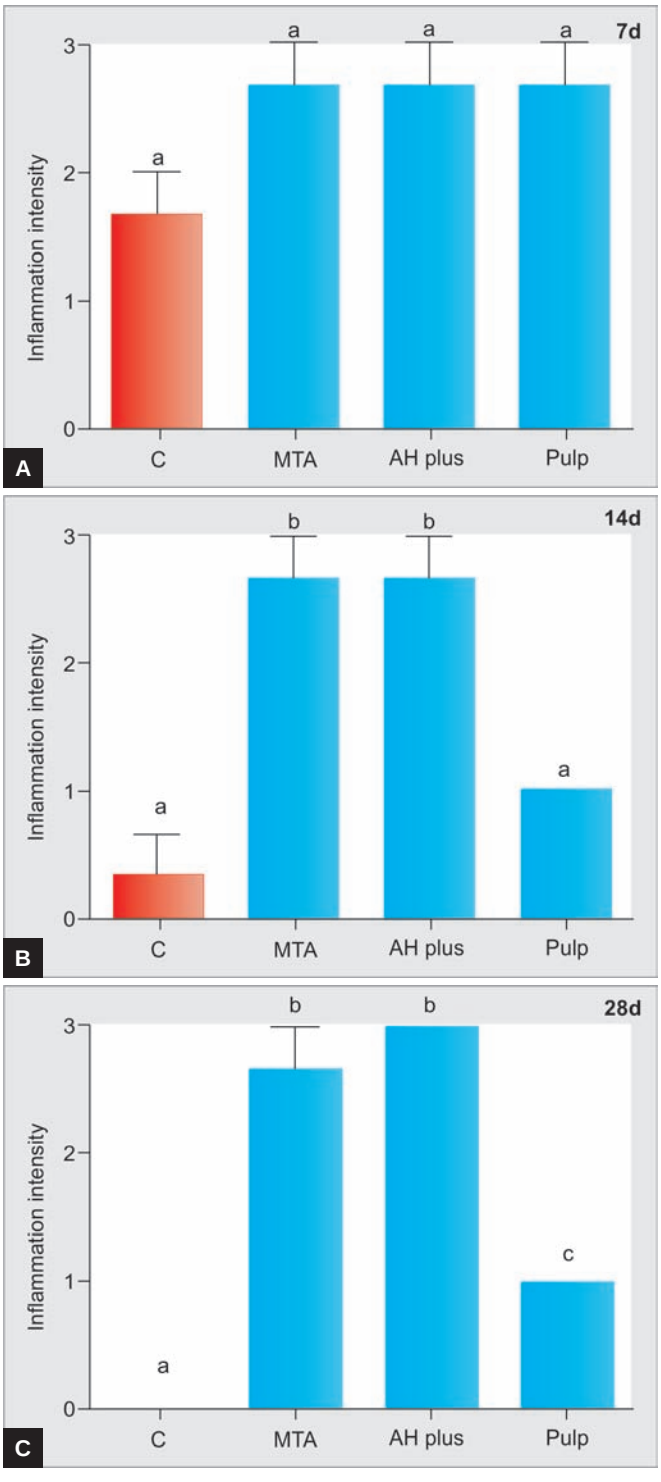
Graph 2 presents an intragroup analysis of the inflammatory response over time for each endodontic sealer. The control group statistically decreases the inflammatory response over time ($p < 0.05$) as observed also with the Pulp Canal Sealer ($p < 0.05$). Both AH Plus and MTA Fillapex maintained elevated inflammatory response during all time periods evaluated ($p > 0.05$).

DISCUSSION

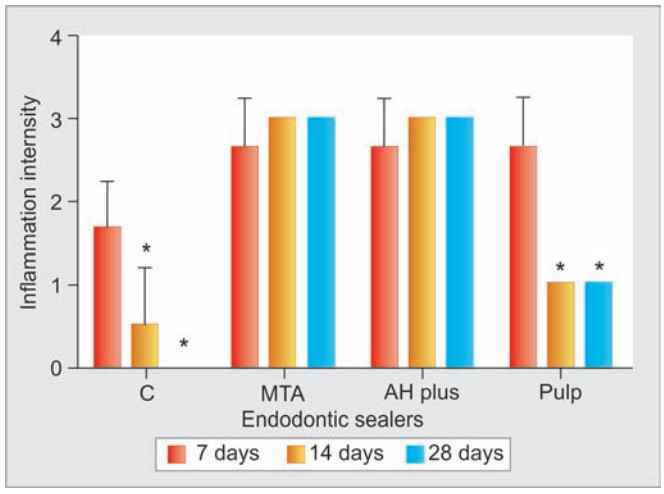
The development of novel endodontic materials should be accompanied by adequate methodologies for the assessment of their biological responses. The method employed in the present study was previously described



Figs 1A to L: Histological images showing the inflammatory infiltrate around the bone defects (indicated by asterisks). Day 7 (A) Control; (B) MTA Fillapex; (C) AH Plus; (D) Pulp Canal Sealer; 14 days (E) Control; (F) MTA Fillapex; (G) AH Plus; (H) Pulp Canal Sealer; 28 days (I) Control; (J) MTA Fillapex; (K) AH Plus; and (L) Pulp Canal Sealer; (H&E, original magnification ×100)



Graphs 1A to C: Results of the histological score analysis of the inflammatory reaction to the materials tested, as compared with the control (bone defects without material), 7 days (A) 14 days; (B) 28 days; and (C) after surgery. Same letters indicate no significant statistical difference ($p > 0.05$) between the tested sealers and the control



Graph 2: Results of the intragroup histological score analysis of the inflammatory reaction to the materials tested. The symbol * indicates statistical significance in comparison to day 7 in the group analyzed ($p < 0.05$)

as adequate for the evaluation of tissue reactions in animal models, which is an indispensable step to complete material examination.[1,2,5] In the present study, it was demonstrated that this method is also able to identify differences on compatibility between members of diverse groups of sealers. The bone tissue response model can simulate to the clinical application of those materials.[8]

Regarding studies of biocompatibility, it is also important to consider the inherent characteristics and possible interpretations of the results associated with the experimental model employed. The diffusion of substances through live periapical tissues, for instance, may be rather different than the release and diffusion on the cell culture media employed on *in vitro* assays, and often cells are more susceptible to noxious effects.[8] In addition, protein molecules that participate in the fluid phase and extracellular matrix of tissues, as well as phagocyte cells, blood, and lymphatic systems, may decrease the cytotoxic effect of some released substances.[17] In this context, a bone defect model might be adequate to assess the inflammatory response expected on this kind of tissue, considering even the effects of material movement.

In the 1st week after grafting, both control and test groups presented considerable levels of inflammatory response (Figs 1A to D), a phenomenon which could be already observed 24 hours after grafting (data not shown). It is very possible, therefore, that this response can be associated with surgical trauma, rather than caused by the material toxicity.

The cytotoxic behavior that seemed to remain in longer period of time for AH Plus, which is an epoxy-based resin material,[18] is coherent with the reactivity of silorane resins that have been used in the formulation of some dental composites and have a similar resin chemical composition.[19] The comparable cytotoxic behavior of AH Plus and epoxies suggests that a parallel corrosion processes may be occurring when the materials are placed in biological contexts.[20] On the other hand, the short-term cytotoxicity of AH Plus has also been attributed to a minute release of formaldehyde, which decreases after setting.[21] Probably, it was this reason that previous *in vivo* studies have report severe reactions to this material.[22]

In the present study, MTA Fillapex, a bioceramic sealer, showed high levels of inflammatory response 28 days after grafting. This material was created based on a calcium silicate composition, in an attempt to combine the physicochemical properties of a root canal sealer with the biological properties of MTA. The setting and hardening of this sealer occurs by a complex reaction between the calcium ions present in the catalyst paste and the disalicylate present in the base paste. The literature has demonstrated that MTA Fillapex strongly affects primary human cell viability up to 7 days after setting.[8] In addition, Bin et al[23] have identified a strong genotoxicity for this material.

Currently, there are two other MTA-based or bioceramic sealers commercially available: Endo-CPM-Sealer (EGEO S.R.L., Buenos Aires, Argentina) and IRoot SP (Innovative BioCeramix, Inc., Vancouver, CA). Both sealers had presented good preliminary results on biocompatibility tests and differ chemically from MTA Fillapex, including the absence of salicylate in their composition.[24,25] In fact, there are evidences indicating a potential toxicity for salicylate-containing materials. A previous study evaluating the effect of resin salicylate on human fibrosarcoma cell line (HT-1080) showed a direct correlation between salicylate concentration and cell death rate.[26]

Pulp Canal Sealer EWT showed the lowest inflammatory response when compared with MTA Fillapex and AH Plus, as it decreased from 14 to 28 days. This favorable result for Pulp Canal Sealer does not agree with a previous *in vitro* report, showing that standard Pulp Canal Sealer remained severely cytotoxic throughout a 6-weeks conditioned-media assay.[9] Such cytotoxicity could be due to the continuous elution of eugenol, which could also act as a potential source of irritation when this sealer is inadvertently extruded through the apical foramen into the periapical tissues.[27] On the other hand, a recent study employing primary human osteoblasts conducted by Scelza et al[8] demonstrated that even in longer setting period (7 days), Pulp Canal Sealer EWT can show high levels of cytocompatibility. It is relevant to note that standard Pulp Canal Sealer and Pulp Canal Sealer EWT have differences in their composition: Pulp Canal Sealer EWT does not contain thymol iodine, which may provide a possible explanation for the lower cytotoxicity observed for the EWT. Further studies are required to evaluate the influence of thymol iodine over cytotoxicity.

The present study has shown that the bone defect model is adequate to identify differences on the tissue response to endodontic sealers. However, further *in vivo* research efforts are required to completely validate the potential and predictability of clinical outcome provided by the method.

CONCLUSION

MTA Fillapex, Pulp Canal Sealer EWT, and AH Plus elicited different levels of inflammatory response when their biocompatibility was tested through an *in vivo* murine bone defect grafting model, after a 28-day period. Pulp Canal Sealer EWT demonstrated high levels of biocompatibility and the parameters for inflammatory response decreased with time. The critical defect grafting model was an effective method to detect differences on the biological response to endodontic sealers.

CLINICAL SIGNIFICANCE

Knowing the biocompatibility of endodontics sealers that will be used in filling the root canal.

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