

# EVALUATION OF THE BIOCOMPATIBILITY OF ROOT CANAL SEALERS USING SUBCUTANEOUS IMPLANTS

João E. GOMES-FILHO<sup>1</sup>, Brenda P. F. A. GOMES<sup>2</sup>, Alexandre A. ZAIA<sup>2</sup>, Caio R. FERRAZ<sup>2</sup>, Francisco J. SOUZA-FILHO<sup>2</sup>

1- DDS, MSc, PhD Associate Professor, Department of Endodontics, Dental School of Araçatuba, State University of São Paulo, Araçatuba, SP, Brazil.

2- DDS, MSc, PhD Associate Professor, Department of Endodontics, Dental School of Piracicaba, Campinas State University, Piracicaba, SP, Brazil.

**Corresponding address:** Prof. Dr. João Eduardo Gomes Filho - R. José Bonifácio, 1193, Araçatuba-SP, 16015-050, Brasil - Faculdade de Odontologia de Araçatuba, FOA-UNESP Endodontia - Phone: 55 18 3636-3252 - Fax: 55 18 3636-3279 - e-mail: joao@foa.unesp.br

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## ABSTRACT

The purpose of this study was to evaluate *in vivo* the biocompatibility of Endométhasone, Pulp Canal Sealer EWT and AH-Plus root canal sealers after implantation in rat connective tissue. Twenty-four Wistar-Furth rats were used. Polyethylene tubes were filled with the sealers and implanted into specific dorsal subdermal tissue sites of the rats. Implants were removed after 3, 7 and 30 days, fixed and processed for glycol methacrylate-embedding technique to be examined microscopically. On the 3rd day, there was a mild inflammatory reaction to Pulp Canal Sealer EWT implants, but a severe response to the other sealers with presence of acute inflammatory cells. On the 7th day, tissue organization was more evident with attenuation of the inflammatory reaction, especially for the AH-Plus implants. On the 30th day, connective tissue with few inflammatory cells was observed in contact with all sealer implants. In this time interval, the tissue in contact with Pulp Canal Sealer EWT implants was more organized, while the tissue close to Endométhasone and AH-Plus implants showed a mild persistent inflammatory reaction and had similar results to each other. In conclusion, the sealers had a similar pattern of irritation, which was more severe in the beginning and milder with time, in such a way that all sealers showed a persistent mild reaction. Pulp Canal Sealer EWT yielded better tissue organization than Endométhasone and AH-Plus, which, in turn, showed similar results to each other.

**Uniterms:** Endodontics; Root canal; Sealer; Subcutaneous implant; Biocompatibility; Glycol methacrylate.

## INTRODUCTION

The main goal of endodontic therapy is the proper cleaning and shaping of the root canal system, as well as obturation with an inert, dimensionally stable and biologically compatible material. Root canal sealers and gutta-percha are the materials that have been used with this purpose, which should have certain physical, chemical and biological properties<sup>11</sup>. Root canal sealers and their diffusible components, therefore, need to have their biocompatibility critically evaluated before clinical use.

Several root canal sealers have been developed and it is mandatory to know the response induced in the tissue prior to their use in humans. Most currently used root canal sealers are zinc-and-eugenol-based materials, but some resin-based sealers are available as well. Spangberg, et al.<sup>30</sup> (1993) reported that AH26, a resin-based sealer, emanates formaldehyde from the curing material and this substance may be the main causative factor of its high cytotoxicity, especially during the early setting period. Formaldehyde release from curing endodontic materials has been

recognized for many years<sup>30</sup>, formaldehyde being reputed to act as a disinfectant<sup>32</sup>. The disinfecting agent in AH26 is methenamin<sup>27</sup>, which is hydrolyzed to ammonia and formaldehyde<sup>14</sup>. The efficacy of long-term root canal disinfection by formaldehyde released from an endodontic sealer has previously been shown to be low<sup>30</sup>. There are published case reports of adverse reactions, such as paraesthesia of the inferior alveolar nerve, attributed to formaldehyde released from root canal sealers<sup>6,12</sup>, though no nerve cell reaction to formaldehyde released from a sealer has yet been reported.

Endométhasone is another controversial sealer, which also releases formaldehyde and is claimed to have a neurotoxic effect causing paresthesia<sup>4,13,18</sup>. AH-Plus resin-based sealer has been recently introduced and some authors have shown a satisfactory periapical healing in dogs<sup>10,19-21</sup>. However, this material still needs *in vivo* studies to improve the understanding of its biocompatibility in order to justify its clinical use.

Several different methods have been described for assessing tissue toxicity. One of them evaluates the

biocompatibility of endodontic sealers in subcutaneous tissue of rats using by implantation of polyethylene tubes filled with the material to be tested. This method is simple and easy to be reproduced and standardized<sup>23,24</sup>. However, the histological processing of the tissue samples surrounding the tube, often embedded in paraffin, was a challenging step of the technique<sup>29</sup>, especially because the cutting process created distortion and artifacts<sup>1,7</sup>. These problems were overcome by Gomes-Filho, et al.<sup>9</sup> (2001) who aggregated a method of inclusion of rat subcutaneous tissue in glycol methacrylate. This improvement increased the final quality of the histological samples and optimized the analysis.

This study evaluated the biocompatibility of three root canal sealers, namely AH-Plus, Endométhasone and Pulp Canal Sealer EWT, using subcutaneous implants in rats according to the method described by Gomes-Filho, et al.<sup>9</sup> (2001).

## MATERIAL AND METHODS

Animal care was performed according to the Ethics in Research Committee of the School of Dentistry of Piracicaba - UNICAMP, which approved the research protocol before the experiments began.

Twenty-four white albino female Wistar-Furth rats were used in this study and received 96 subcutaneous implants. The rats were kept in groups of 3 or 4 *per* cage and fed standard pellet food and water *ad libitum*. The animals were selected on a weight basis of about 180 to 200 g at the start of each experiment.

Polyethylene tubes with 1.5-mm inner diameter, 2.0-mm outer diameter and 10.0 mm long were sterilized in autoclave prior to use. The root canal sealers to be tested, namely Endométhasone (Spécialités Septodont, Saint-Maur-des-Fossés, Cedex, France), AH Plus (Dentsply De Trey GmbH, Konstanz, Germany) and Pulp Canal Sealer EWT (Kerr, Sybron Dental Specialties, Romulus, MI, USA), were mixed according to the manufacturers' instructions, except for Endométhasone, which was mixed using a ratio of 0.19 g of powder *per* drop of eugenol<sup>3</sup>. The freshly mixed materials were placed into the polyethylene tubes and implanted into the subcutaneous tissue of the rats. Empty tubes were used as control.

The animals were anesthetized with an intraperitoneal injection of 65 mg/mL sodium pentobarbital at a dose of 5.1 mg/100 g body weight. The skin on the animals' back was shaved, the implants were placed subcutaneously into a pocket created by a blunt dissection through 10 mm incision of the skin, and the wounds were sutured. Four pockets were created on the back, being 2 in the cranial portion, both receiving tubes containing the sealers, and other 2 in the caudal portion, one receiving a sealer tube and the other receiving an empty tube (control). Eight rats were assigned to each time interval (3, 7 and 30 days), sizing up 24 animals. Each animal received 3 tubes filled with the sealers and 1 empty tube. It was performed a rotation on the position of

the implantation of the tubes filled with sealer.

At the end of the experimental periods (3, 7 and 30 days), the animals were killed by anesthetic overdose. The skin overlaying the implants was shaved and tubes with surrounding tissue were removed from the rats, immersed in Bouin's solution and fixed for 24 hours. The tubes were then bisected transversely and both halves were cut again longitudinally using a sharp blade. This was made to allow the surfaces being readily kept in contact with the processing solutions. The specimens were processed for glycol methacrylate (GMA) embedding, serially sectioned into 3- $\mu$ m cuts and stained with hematoxylin and eosin according to Gomes-Filho, et al.<sup>9</sup> (2001).

The conditions of the tissue surrounding the implants, the occurrence and location of fibrous tissue, the types of inflammatory cells present, and the vascular changes were examined. Tissue inflammatory response was graded as mild, moderate or severe<sup>17,18</sup> and the following scores were attributed: 1 - no/mild inflammation (thickness of reaction zone similar or only slightly larger than along side tube; no or few inflammatory cells), 2 - moderate inflammation (increased reaction zone; presence of macrophages and/or plasma cells), 3 - Severe inflammation (increased reaction zone; presence of macrophages and plasma cells; occasional foci of neutrophil granulocytes and/or lymphocytes), 4 - Extreme (focal areas of necrosis; tissue densely infiltrated by inflammatory cells).

Statistical analysis was performed by one-way ANOVA and Tukey's post-hoc test to determine significant differences among the groups. Significance level was set at 5%.

## RESULTS

The GMA-embedded tissue showed the original orientation of the tube filled with the endodontic sealer and an inflammatory cell infiltrate at the end of tube. The quality of cell definition and staining allowed analyzing the cell infiltrate at the end of the tube and distinguishing chronic from acute inflammatory cells.

### Materials

#### *Endométhasone*

A severe reaction was observed on the 3rd day. The tissue was disorganized and infiltrated with neutrophils, but there were no giant cells or areas of necrosis (Figures 1A and 1B). On the 7th day, the tissue was more organized and was characterized by the presence of chronic cells and absence of fibrous capsule formation and areas of necrosis (Figures 1C and 1D). The intensity of the reaction was milder on the 30th day and an organizing fibrous capsule was observed. The tissue was infiltrated with macrophages, giant cells and fibroblasts (Figures 1E and 1F).

#### *AH-Plus*

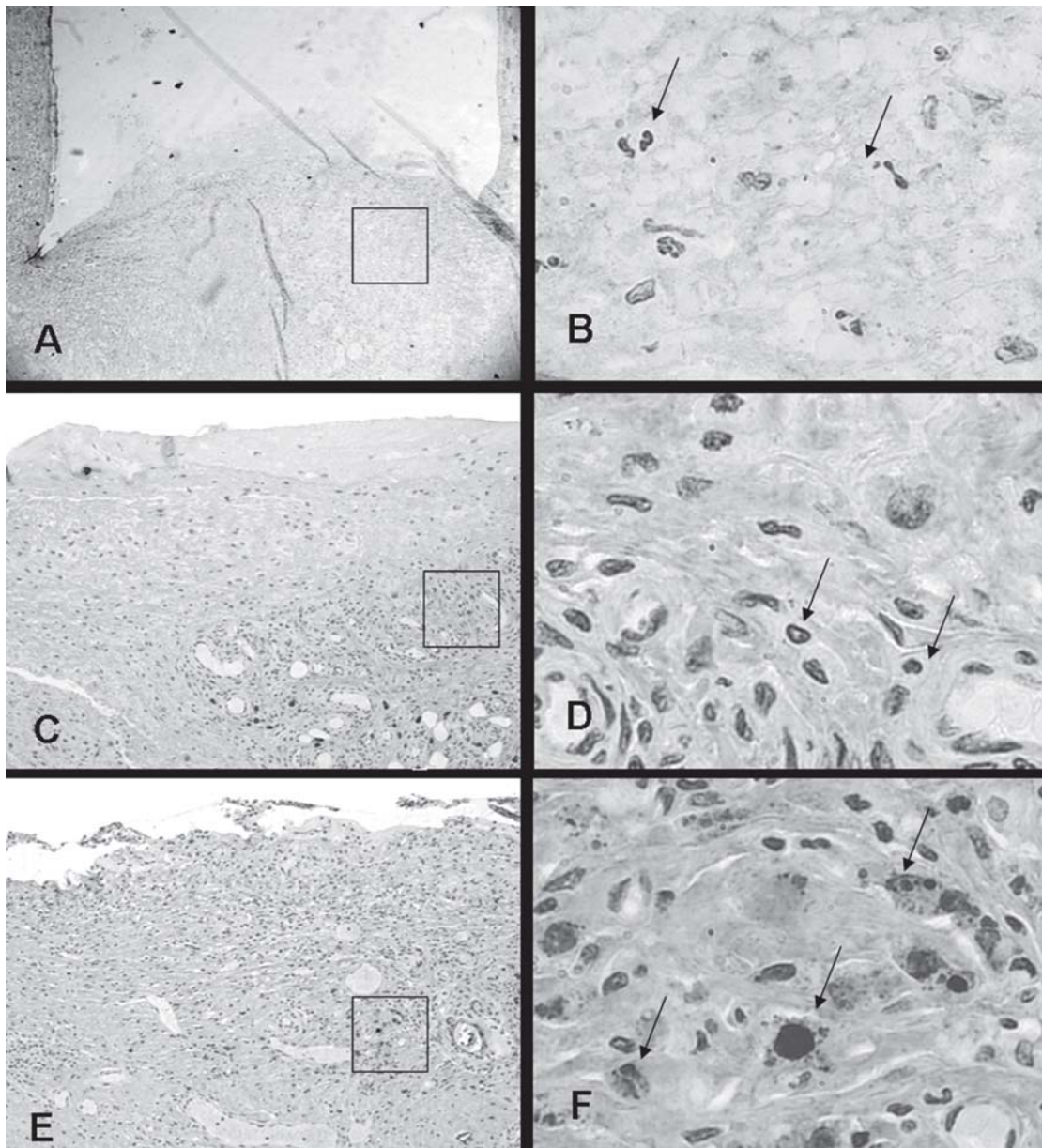
A severe inflammatory reaction was observed on the 3rd day. The tissue was infiltrated with neutrophils and few macrophages. There were no giant cells or areas of necrosis

(Figures 2A and 2B). The intensity of the inflammatory reaction was milder on the 7th day and the tissue was more organized exhibiting the formation of connective fibers. The tissue was infiltrated with macrophages, plasma cells and lymphocytes. There were no giant cells or area of necrosis and organization of a fibrous capsule was observed in this period of time (Figures 2C and 2D). On the 30th day, the capsule was still not completely formed. There inflammatory reaction was more severe than in the 7th day and was characterized by presence of chronic cells including giant cells (Figures 2E and 2F).

**Pulp Canal Sealer EWT**

Moderate inflammation without areas of necrosis was

observed on the 3rd day. The inflammatory reaction was characterized by presence of neutrophils and macrophages, although fibroblasts and few connective fibers were seen (Figures 3A and 3B). The intensity of the reaction was attenuated at the 7th day and chronic inflammatory cells were predominantly observed. The tissue was in an initial state of organization with presence of few fibroblasts and connective fibers (Figures 3C and 3D). On the 30th day, connective tissue with fibers and few fibroblasts was observed and a fibrous capsule tissue was present between the tube opening and the tissue. Macrophages and giant cells with material in their cytoplasm were also observed (Figures 3E and 3F).



**FIGURE 1-** Rat subcutaneous tissue reaction to Endomethasone. The tissue on the tube end is disorganized and infiltrated with neutrophils on the 3rd day - HE 50x (A). Greater magnification showing neutrophils (arrows) and absence of collagen fibers between the cells - HE 400x (B). On the 7th, day the tissue presented more organized with the presence of chronic cells - HE 50x (C). Greater magnification showing chronic cells (Arrows) - HE 400x (D). Milder reaction on the 30th day, with the presence of an organizing fibrous capsule - HE 50x (E). Greater magnification showing chronic cells (arrows) and collagen fibers between the cells - HE 400x (F)

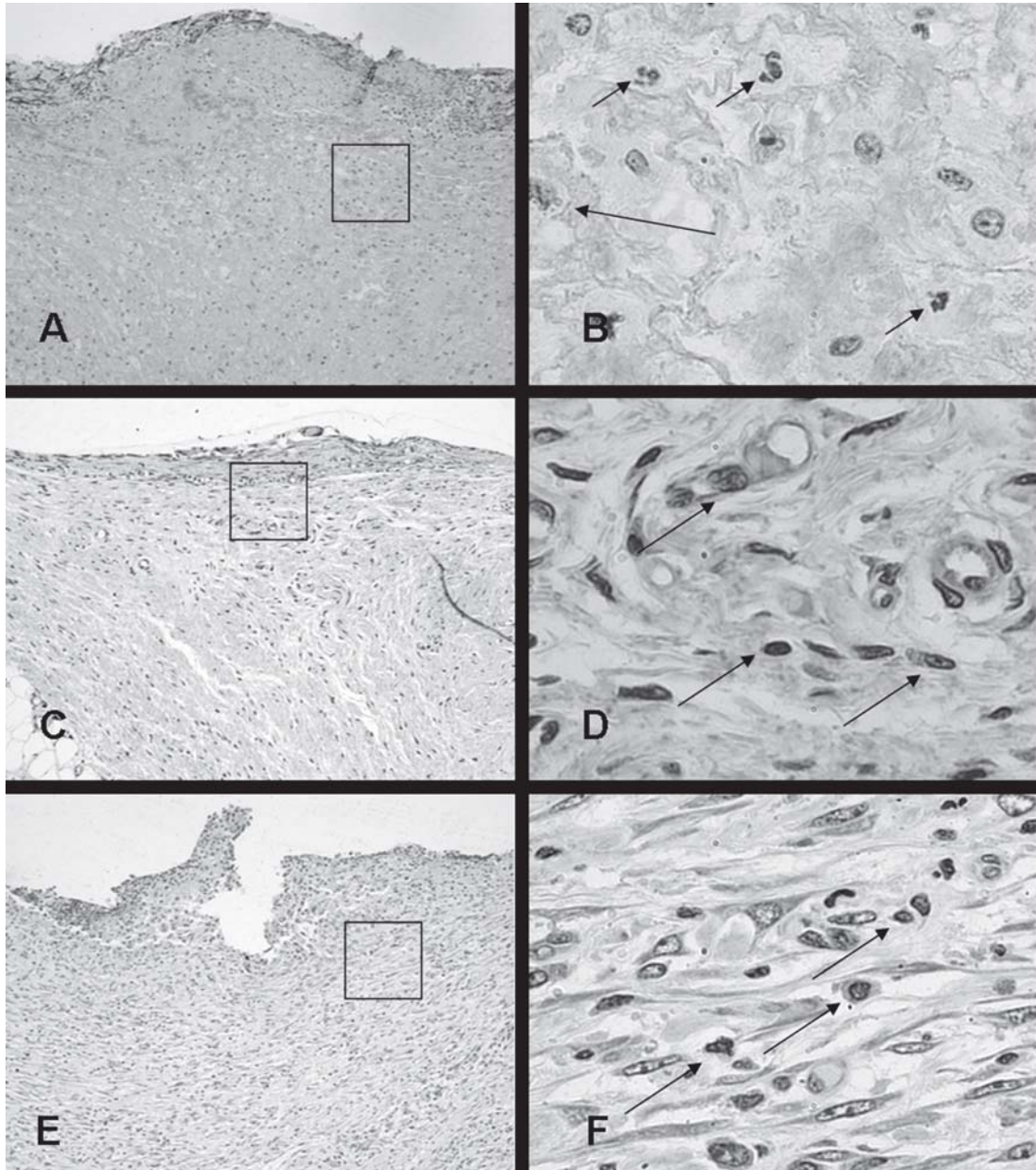
**Control (no sealer)**

A moderate reaction was observed on the 3rd day. The tissue was infiltrated with chronic cells and few neutrophils. No areas of necrosis or formation of fibrous capsule were observed (Figures 4A and 4B). On the 7th day, the intensity of the reaction was milder and the tissue was characterized by the presence of chronic cells, fibroblasts and blood vessels as well as absence of giant cells and fibrous capsule (Figures 4C and 4D). On the 30th day, it was possible to observe a more organized tissue with predominance of connective fibers, fibroblasts and absence of necrotic areas

and giant cells. A fibrous capsule was present though not completely formed (Figures 4E and 4F).

**Evaluation Periods****Day 3**

The distribution of the scores attributed to the materials is given on Table 1. There was statistically significant difference ( $p < 0.01$ ) among the scores of inflammatory response (Table 2). Except for Pulp Canal Sealer EWT ( $p > 0.05$ ), all sealers differed significantly from the control group (no sealer) ( $p < 0.01$ ). Pulp Canal Sealer EWT was significantly different ( $p < 0.01$ ) from the other two root canal



**FIGURE 2-** Rat subcutaneous tissue reaction to AH-Plus. A severe inflammatory reaction was observed on the 3rd day - HE 50x (A). Greater magnification showing that the tissue was infiltrated with neutrophils (small arrows) and macrophages (large arrows) - HE 400x (B). The intensity of reaction was milder on the 7th day and the tissue was more organized with formation of connective fibers - HE 50x (C). Greater magnification showing chronic cells, such as macrophages, plasma cells and lymphocytes (arrows) - HE 400x (D). On the 30th day, the capsule was still not completely formed - HE 50x (E). Greater magnification showing a more severe reaction than that observed on the 7th day with the presence of chronic cells (arrows) - HE 400x (F)

sealers. AH-Plus and Endométhasone were not significantly different from each other ( $p>0.05$ ).

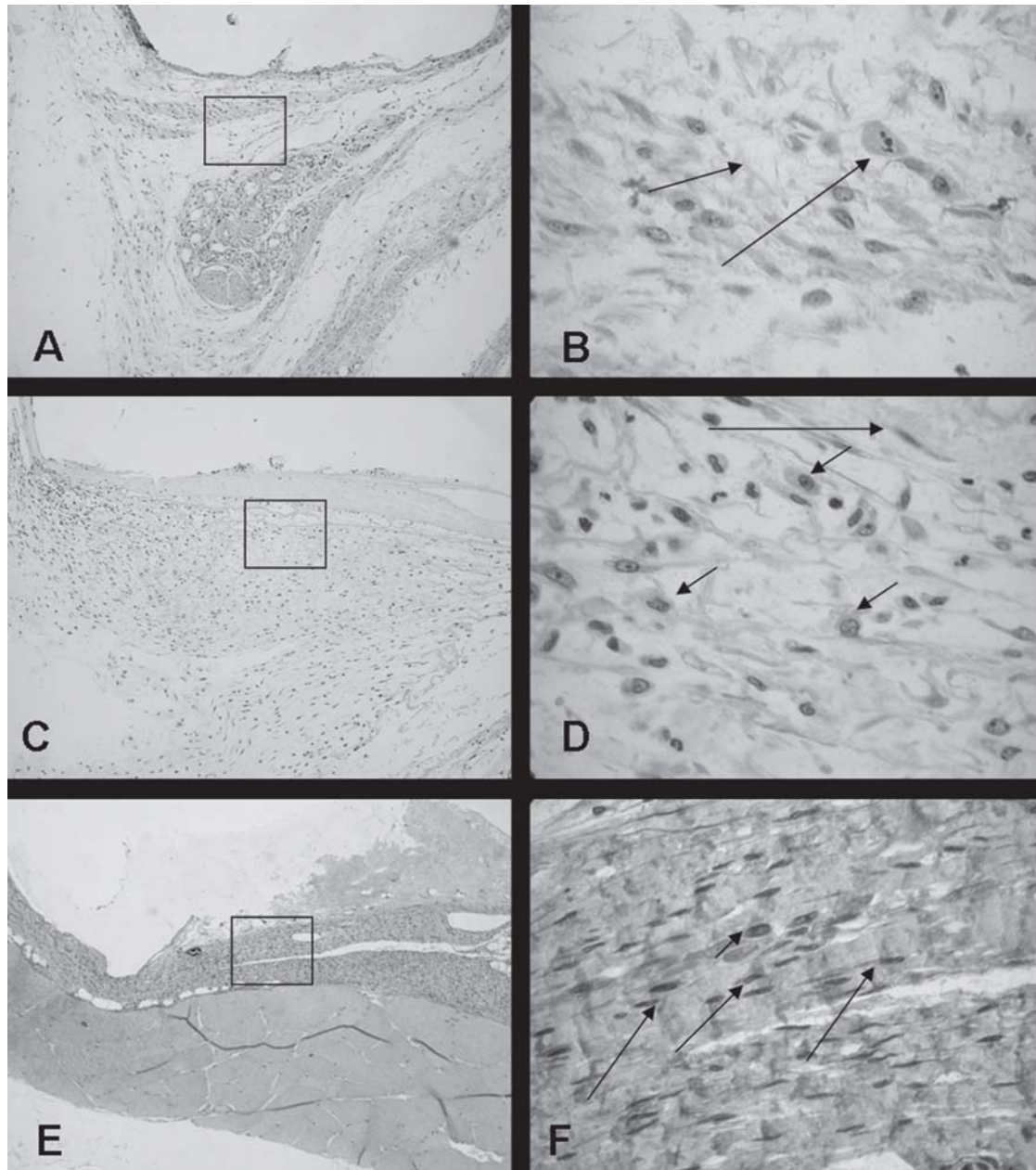
#### Day 7

The distribution of the scores attributed to the materials is given on Table 3. There was statistically significant difference ( $p<0.01$ ) among the scores of inflammatory response (Table 4). Pulp Canal Sealer EWT and AH-Plus were not significantly different from the control group (no sealer) ( $p>0.05$ ). However, Endométhasone was significantly different ( $p<0.01$ ) from the control. Pulp Canal Sealer EWT was significantly different ( $p<0.01$ ) from Endométhasone

but did not differ significantly from AH-Plus ( $p>0.05$ ). AH-Plus and Endométhasone were significantly different from each other ( $p<0.01$ ).

#### Day 30

The distribution of the scores attributed to the materials is given on Table 5. There was statistically significant difference ( $p<0.01$ ) among the scores of inflammatory response (Table 6). Pulp Canal Sealer EWT and Endométhasone were not significantly different from the control group (no sealer) ( $p>0.05$ ), whereas AH-Plus was significantly different from the control. Pulp Canal Sealer



**FIGURE 3-** Rat subcutaneous tissue reaction to Pulp Canal Sealer EWT. Moderate inflammation was observed on the 3rd day - HE 50x (A). Greater magnification showing the presence of neutrophils (large arrow), macrophages, fibroblasts and few connective fibers (small arrow) - HE 400x (B). The intensity of the inflammatory reaction was attenuated on the 7th day and chronic inflammatory cells were predominant - HE 50x (C). In a greater magnification, it was possible to identify fibroblasts (large arrow), inflammatory cells (small arrows) and connective fibers between the cells - HE 400x (D). On the 30th day, a fibrous capsule was present between the opening tube and the tissue - HE 50x (E). Greater magnification showing remaining chronic cells (small arrow) and fibroblasts (large arrows)- HE 400x (F)

EWT was significantly different from AH-Plus ( $p < 0.01$ ) but did not differ significantly from Endométhasone ( $p > 0.05$ ). AH-Plus and Endométhasone were significantly different from each other ( $p < 0.01$ ).

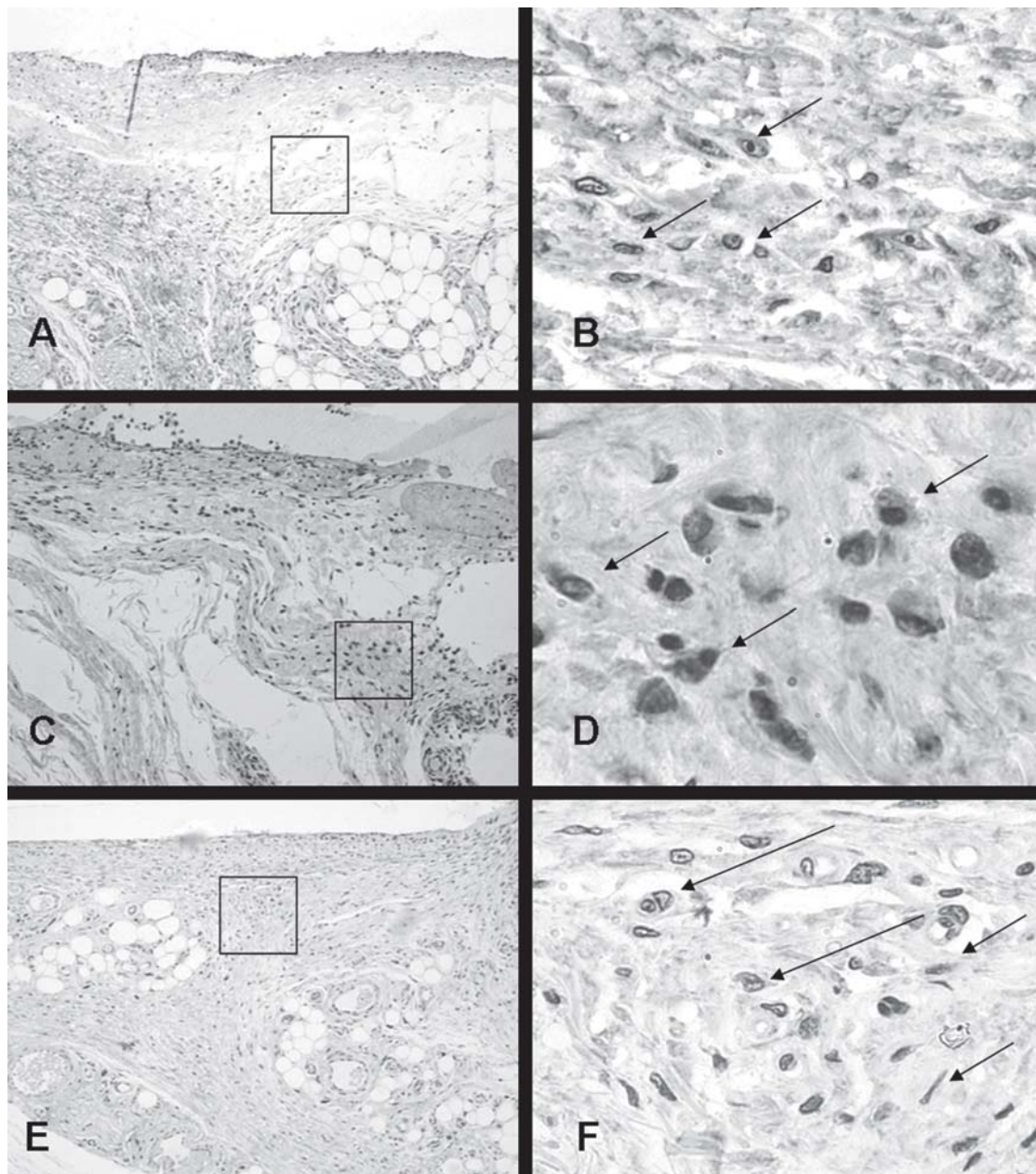
## DISCUSSION

The GMA embedding technique offers several advantages over paraffin embedding, such as producing less distortion, providing thin sections that offer good cellular definition, allowing the preparation of sections

without removal of the tubes and yielding good-quality staining and only few technical artifacts<sup>7,9</sup>.

Several studies have evaluated sealer cytotoxicity using *in vitro* cell culture assays<sup>16</sup>, implantation into muscle<sup>17</sup> and periradicular response<sup>26</sup>. *In vivo* tests are based on clinical and histological evaluation of tissue responses<sup>17</sup>.

In the present study, polyethylene tubes were used because of their suitability for maintaining the test materials in contact with the tissue in a controlled manner<sup>31</sup>. All sealers used in this study were aggressive to the subcutaneous tissue in the beginning of the experiment. The inflammatory reaction, however, become milder on the 30th day. A stronger



**FIGURE 4-** Rat subcutaneous tissue reaction to the empty tube. A moderate reaction without the organization of fibrous capsule was observed on the 3rd day - HE 50x (A). Greater magnification showing that the tissue was mainly infiltrated with chronic cells (arrows) - HE 400x (B). The intensity of the reaction was milder and the fibrous capsule was still not formed on the 7th day - HE 50x (C). Greater magnification showing chronic cells (arrows) - HE 400x (D). On the 30th day, a more organized tissue was observed but the fibrous capsule was not completely organized (E). Greater magnification showing a predominance of connective fibers, fibroblasts (small arrows) and chronic cells (large arrows) - HE 400x (F)

action of the sealers in the beginning and attenuation of the inflammatory response over time have been reported elsewhere<sup>5,8,15,16,23,24,26,29</sup>.

On the 3rd day, the reaction observed to all sealers was more likely due to the surgical trauma rather than caused by the materials' toxicity. However, it allowed evaluating the behavior of the materials along the experimental time and

during the natural skin healing process as the initial period. At this time, the tissue was disorganized and infiltrated with neutrophils, which is consistent with the findings of other studies<sup>29</sup>. On the 7th day, the tissue was more organized in all sealer groups and was infiltrated with chronic cells, such as macrophages, lymphocytes and plasma cells. Fibrous capsule formation was observed only with AH-Plus and

**TABLE 1-** Distribution of the scores attributed to the materials (Day 3)

Material	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6	Rat 7	Rat 8
Control (no sealer)	2	2	3	2	2	2	2	2
Endométhasone	3	3	3	2	3	3	3	3
Pulp Canal Sealer EWT	2	2	2	2	3	2	2	2
AH-Plus	3	3	3	3	3	3	2	3

**TABLE 2-** Results of Tukey's multiple comparison test for the scores attributed to the materials (Day 3)

Tukey's multiple comparison test	Mean Difference	q	p value
Control vs Endométhasone	-0.75	6	p<0.01
Control vs Pulp Canal Sealer EWT	0	0	p>0.05
Control Vs AH-Plus	-0.75	6	p<0.01
Endométhasone vs Pulp Canal Sealer EWT	0.75	6	p<0.01
Endométhasone vs AH-Plus	0	0	p>0.05
Pulp Canal Sealer EWT vs AH-Plus	-0.75	6	p<0.01

**TABLE 3-** Distribution of the scores attributed to the materials (Day 7)

Material	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6	Rat 7	Rat 8
Control (no sealer)	1	1	2	1	1	2	1	1
Endométhasone	2	2	2	2	2	3	2	2
Pulp Canal Sealer EWT	1	1	1	2	1	2	1	1
AH-Plus	1	1	2	1	2	1	1	1

**TABLE 4-** Results of Tukey's multiple comparison test for the scores attributed to the materials (Day 7)

Tukey's multiple comparison test	Mean Difference	q	p value
Control vs Endométhasone	-0.875	5.649	p<0.01
Control vs Pulp Canal Sealer EWT	0	0	p>0.05
Control Vs AH-Plus	0	0	p>0.05
Endométhasone vs Pulp Canal Sealer EWT	0.875	5.649	p<0.01
Endométhasone vs AH-Plus	0.875	5.649	p<0.01
Pulp Canal Sealer EWT vs AH-Plus	0	0	p>0.05

Pulp Canal Sealer EWT implants. On the 30th day, although the tissue inflammatory reaction to all sealers was milder than that observed on the 3rd day after implantation, it was still present. Surrounding Pulp Canal Sealer EWT implants, a persistent inflammatory response was observed, which has already been reported<sup>15</sup>. This could be attributed to eugenol release from this material whose eugenol content is high right after mixing, but decreases with time. Pulp Canal Sealer EWT and Endométhasone are zinc-oxide-and-eugenol-based sealers and both have residual eugenol after mixing. As previously stated<sup>22</sup>, this residual eugenol (~5%) is sufficient to cause an inflammatory reaction. Tissue response to Endométhasone implants was stronger than that developed to Pulp Canal Sealer EWT. It may be explained by the presence of hydrocortisone in Endométhasone composition. Radostina, et al.<sup>25</sup> (1989) observed a strong positive correlation between the inhibition of the synthetic apparatus of fibroblast development under hydrocortisone effect. It is thus possible to associate the toxicity of Endométhasone with the presence of hydrocortisone. On the other hand, Orstavick and Mijör<sup>24</sup> (1988) found a low toxicity to Endométhasone after 90 days of implantation, which may be attributed to presence of corticosteroids. The persistent response to the AH-Plus implants may be due to the release of formaldehyde as a product of the hexamethylenetetramine decomposition as occurs with AH-26<sup>5</sup>. Leonardo, et al.<sup>18</sup> (1999), reported minimal but existent formaldehyde release from AH-Plus, which also may contribute to the observed tissue response.

In view of the methodological differences among *in vivo* investigations, it is difficult to compare directly our results to those of previous studies. Further research should be conducted to contribute to the development of a root canal

sealer that fulfills all properties of an ideal material.

## COCLUSIONS

Under the tested conditions, it may be concluded that the sealers had a similar pattern of irritation, which was more severe in the beginning and milder with time, in such a way that all sealers showed a persistent mild reaction. Pulp Canal Sealer EWT yielded better tissue organization than Endométhasone and AH-Plus, which, in turn, showed similar results to each other.

## REFERENCES

- 1- Bennett HS, Wyrick AD, Lee SW, McNeil JH. Science and art in preparing tissues embedded in plastic for light microscopy, with special reference to glycol methacrylate, glass knives and simple stains. *Stain Technol.* 1976;51:71-97.
- 2- Berbert FL, Leonardo MR, Silva LA, Tanomaru M Filho, Bramante CM. Influence of root canal dressings and sealers on repair of apical periodontitis after endodontic treatment. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2002;93(2):184-9.
- 3- Berbert CCV. Reação dos tecidos periapicais a sobreobturações com diferentes cimentos endodônticos em duas consistências: histopatologia em dentes de cães [tese]. Piracicaba: Universidade de Campinas, Faculdade de Odontologia de Piracicaba; 1996.
- 4- Brodin P, Roed A, Aars H, Orstavik D. Neurotoxic effects of root filling materials on rat phrenic nerve in vitro. *J Dent Res.* 1982;61:1020-3.

**TABLE 5-** Distribution of the scores attributed to the materials (Day 30)

Material	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6	Rat 7	Rat 8
Control (no sealer)	1	1	1	1	1	1	1	1
Endométhasone	1	1	1	2	1	1	1	1
Pulp Canal Sealer EWT	1	1	1	1	1	1	1	1
AH-Plus	1	1	1	2	2	2	2	2

**TABLE 6-** Results of Tukey's multiple comparison test for the scores attributed to the materials (Day 30)

Tukey's multiple comparison test	Mean Difference	q	p value
Control vs Endométhasone	-0.125	1.128	p>0.05
Control vs Pulp Canal Sealer EWT	0	0	p>0.05
Control vs AH-Plus	-0.625	5.641	p<0.01
Endométhasone vs Pulp Canal Sealer EWT	0.125	1.128	p>0.05
Endométhasone vs AH-Plus	-0.5	4.513	p<0.05.
Pulp Canal Sealer EWT vs AH-Plus	-0,625	5,641	p<0.01



- 5- Economides N, Kotsaki-Kovatsi VP, Pouloupoulos A, Kolokuris I, Rozos G, Shore R. Experimental study of the biocompatibility of four root canal sealers and their influence on the zinc and calcium content of several tissues. *J Endod.* 1995;21:122-7.
- 6- Erisen R, Yucel T, Kucukay S. Endomethasone root canal filling material in the mandibular canal: a case report. *Oral Surg Oral Med Oral Pathol.* 1989;63:343-5.
- 7- Feder N, O'Brien TP. Plant microtechnique: some principles and new methods. *Am J Bot.* 1968;55:123-42.
- 8- Gomes BP, Pedrosa JA, Jacinto RC, Vianna ME, Ferraz CC, Zaia AA, et al. In vitro evaluation of the antimicrobial activity of five root canal sealers. *Braz Dent J.* 2004;15:30-5.
- 9- Gomes-Filho JE, Gomes BP, Zaia AA, Novaes PD, Souza-Filho FJ. Glycol methacrylate: an alternative method for embedding subcutaneous implants. *J Endod.* 2001;27:266-8.
- 10- Grecca FS, Leonardo MR, Silva LA, Tanomaru M Filho, Borges MA. Radiographic evaluation of periradicular repair after endodontic treatment of dog's teeth with induced periradicular periodontitis. *J Endod.* 2001;27(10):610-2.
- 11- Grossman LI. Physical properties of root canal cements. *J Endod.* 1976;2:166-75.
- 12- Gumru OZ, Yalcin S. Surgical treatment of paresthesia following over-extension of root canal filling material: a case report. *J Nihon Univ Sch Dent.* 1991;33:49-53.
- 13- Kaufman AY, Rosenberg L. Paresthesia caused by Endomethasone. *J Endod.* 1980;6:529-31.
- 14- Koch MJ. Formaldehyde release from root-canal sealers: influence of method. *Int Endod J.* 1999;32:10-6.
- 15- Kolokuris I, Beltes P, Economides N, Vlemmas I. Experimental study of the biocompatibility of a new glass-ionomer root canal sealer (Ketac-Endo). *J Endod.* 1996;22:395-8.
- 16- Langeland K, Olsson B, Pascon EA. Biological evaluation of hydron. *J Endod.* 1981;7:196:204.
- 17- Langeland K. Correlation of screening tests and usage tests. *J Endod.* 1978;4:300-2.
- 18- Leonardo MR, Bezerra da Silva LA, Tanomaru M Filho, Santana da Silva R. Release of formaldehyde by 4 endodontic sealers. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 1999;88(2):221-5.
- 19- Leonardo MR, Silva LA, Almeida WA, Utrilla LS. Tissue response to an epoxy resin-based root canal sealer. *Endod Dent Traumatol.* 1999;15(1):28-32.
- 20- Leonardo MR, Hernandez ME, Silva LA, Tanomaru M Filho. Effect of a calcium hydroxide-based root canal dressing on periapical repair in dogs: a histological study. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2006;102(5):680-5.
- 21- Leonardo MR, Salgado AA, Silva LA, Tanomaru M Filho. Apical and periapical repair of dogs' teeth with periapical lesions after endodontic treatment with different root canal sealers. *Pesqui Odontol Bras.* 2003;17(1):69-74.
- 22- Molnar EJ. Residual eugenol from oxide-eugenol compounds. *J Dent Res.* 1967;46:645-9.
- 23- Olsson B, Sliwowski A, Kaare L. Subcutaneous implantation for the biological evaluation of endodontic materials. *J Endod.* 1981;7:355-69.
- 24- Orstavic KD, Mijör IA. Histopatoloy and X-ray microanalysis of the subcutaneous tissue response to endodontic sealers. *J Endod.* 1988;14:13-23.
- 25- Radostina AL, Zumangi N, Malomu UUF. Changes in the ultrastructure and fibrogenic activity of rat dermal fibroblasts as affected by various doses of hidocortisone. *Arkh Anat Gistol Embriol.* 1989;96:52-8.
- 26- Rowe AHR. Effect of root filling materials on the periapical tissues. *Br Dent J.* 1967;7:98-102.
- 27- Schroder A. Mitteilungen uber die abschcludichtigkeit von wurzelfullmaterialien und erster Hinweis auf ein neuartiges Wurzelfullmaterial. *Schweiz Monatsschr Zahnheilkund.* 1954;64:921-31.
- 28- Schwarze T, Fiedler I, Leyhausen G, Geurtsen W. The cellular compatibility of five endodontic sealers during the setting period. *J Endod.* 2002;28:784-6.
- 29- Spangberg L, Langeland K. Biological effects of dental materials. *Oral Surg Oral Med Oral Pathol.* 1973;35:402-14.
- 30- Spangberg LSW, Barbosa SV, Lavigne GD. AH26 release formaldehyde. *J Endod.* 1993;19:596-8.
- 31- Torneck CD. Reaction of rat connective tissue to polyethylene tube implants. I. *Oral Surg Oral Med Oral Pathol.* 1966;21(3):379-87.
- 32- Tronstad L, Yang ZP, Trope M, Barnett F, Hammond B. Controlled release of medicaments in endodontic therapy. *Endod Dent Traumatol.* 1985;1(4):130-4.