Analysis of Tissue Reactions to Methacrylate Resin-based, Epoxy Resin-based, and Zinc Oxide–Eugenol Endodontic Sealers

Roberta Kochenborger Scarparo, MSc, Fabiana Soares Grecca, PhD, and Elaine Vianna Freitas Fachin, PhD

Abstract

The purpose of this study was to investigate the reaction of the subcutaneous connective tissue of rats to methacrylate resin-based sealer (EndoREZ), epoxy resin-based sealer (AH Plus), and zinc oxide–eugenol sealer (EndoFill). Polyethylene tubes containing the test materials were implanted in 18 rats. After 7, 30, and 60 days, tissues were collected for biopsy and fixed and processed for histologic evaluation. Observations were made of the cellular inflammatory component, the fibrous condensation, and the abscess formation. Comparisons between groups and times were made with the Friedman and Kruskall-Wallis tests. EndoREZ and EndoFill sealers showed a more intense and longer-lasting inflammation. With AH Plus, the inflammatory reaction showed a tendency to diminish over time. The only group to show a statistically significant reduction in inflammation during the 60-day period was the control group. None of the materials tested proved to have ideal characteristics for biocompatibility. *(J Endod 2009;35:229–232)*

Key Words

Biocompatible materials, endodontics, root canal filling materials

From the School of Dentistry, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil.

Address requests for reprints to Roberta Kochenborger Scarparo, Avenida General Barreto Viana, 1175/1108, CEP 91330-630, Porto Alegre, RS, Brazil. E-mail address: robks@terra.com.br.

0099-2399/$0 - see front matter

doi:10.1016/j.joen.2008.10.025

To obtain an ideal seal, a good number of studies have been carried out in attempts to perfect sealers' adhesive characteristics, thus giving rise to the endodontic sealers based on epoxy resin, such AH Plus, and to those based on methacrylate resin, such EndoREZ (1, 2). However, treatment success can only be achieved if the obturation material is biocompatible and creates a biologic seal on the periapical region.

There are divergent reports on the biocompatibility of AH Plus. Some authors (3, 4) have stated that the cytotoxic effects of this material are reduced, but other authors (5) disagree. Nonetheless, in implant tests (6) and in tests of dental application (7, 8) performed on animals, AH Plus has proved to have biologic potential.

There is a lack of consensus as well regarding the biologic behavior of the EndoREZ sealer. Some authors (9, 10) have reported good tolerance in subcutaneous connective tissue and bone, whereas other authors (6, 11) have observed an intense and long-lasting inflammatory reaction.

These contradictions apparently arise from the many different methodologies available to analyze the inflammatory reactions, from qualitative analyses (6, 11–14) aiming at describing the inflammatory events to quantitative analyses of the extension of the tissue response (10, 15) and of the number of inflamed cells (10, 16), with the purpose of comparing materials and experiment duration in a more objective way.

Given that a detailed description of the reactions caused by the endodontic sealers available is of paramount importance, this study aimed at carrying out an evaluation of the reactions of rat subcutaneous connective tissue to EndoREZ, AH Plus, and a zinc oxide–eugenol sealer (EndoFill).

Material and Methods

This study was sanctioned by the Institutional Review Board and by the Research Ethics Committee of the School of Dentistry of the Federal University of Rio Grande do Sul, Brazil. Eighteen animals (*Rattus norvegicus albinus Wistar*) were grouped according to 3 experimental periods (7, 30, and 60 days). Inflammatory reactions were evaluated for 4 groups: group I, methacrylate resin-based sealer (EndoREZ; Ultradent, Inc, South Jordan, UT); group II, epoxy resin-based sealer (AH Plus; Dentsply D Trey, GmbH Konstanz, Germany); group III, zinc oxide–eugenol sealer (EndoFill; Dentsply Hero Indústria e Comércio Ltda, Petrópolis, RJ, Brazil); and group IV, the control group (empty tube).

Animals were anesthetized with 0.008 mL/100 g of ketamine and 0.004 mL/100 g of xylazine hydrochloride 2% (Virbac do Brasil Indústria e Comércio Ltda, São Paulo, SP, Brazil). Dorsal trichotomy was performed manually, and the area was disinfected with alcohol-iodine solution. Four 0.5-cm-long incisions were made on each animal's back, 2 cm from the spine and at least 2 cm apart. Lateral tearing of the subcutaneous tissue allowed for 4 surgical cavities, arranged in quadrants, equidistant from the center of the animal's back.

Polyethylene tubes approximately 10 mm long and 1.5 mm in diameter (Abott Lab do Brasil, São Paulo, SP, Brazil) were autoclaved. The sealers were prepared in accordance with the manufacturers' instructions and were carefully introduced into the tubes by means of sterile insulin syringes (Injex Indústria Cirúrgica Ltda, Ourinhos, SP, Brazil) in such a way that the material being used would fill up the tube entirely and

would not overflow. The tubes were inserted into the surgical cavities, parallel to the incisions, with the open end of each tube pointing toward the animal's head. The position in which each sealer was implanted was standardized. Incisions were sutured with a 3-0 silk thread (Johnson & Johnson Produtos Profissionais Ltda, São José dos Campos, SP, Brazil).

At the end of each experimental period, 6 animals were anesthetized and killed by means of cervical dislocation. Biopsy of the implant area was carried out with a 1-cm safety margin, and the resulting specimens were fixed in formalin at 10% for 24 hours. The tubes were removed from the specimens, which were then set in paraffin blocks and coded. Sections with thickness of 3–4 μm were taken along the axis of the tube, mounted on slides, and stained with hematoxylin-eosin. Three sections were evaluated per sample. The slides were examined under a light microscope by a blinded examiner, according to the criteria described by Figueiredo et al. (17). The examiner was calibrated before the data were analyzed.

The cellular inflammatory component was determined by the presence of neutrophils, lymphocytes/plasmacytes, eosinophils, macrophages, and giant cells; it was then classified according to the following scale: (1) absent (cells absent or within vessels); (2) mild (cells present, but sparse or in reduced clusters); (3) moderate (cells present, yet not dominating the microscopic field); and (4) intense (cells present in the form of infiltrate).

Fibrous tissue was classified according to the following scale: (1) absence of collagen fibers; (2) presence of a thin layer of collagen fibers; and (3) presence of a thick layer of collagen fibers.

Abscess formation was classified as follows: (1) absence of abscess; (2) presence of abscess in contact with the surgical cavity where the material had been; and (3) presence of abscess areas distant from the surgical cavity where the material had been.

The Friedman test was used to compare the variables between study groups, given that this study examined dependent samples. Whenever statistically significant differences were observed, the Friedman test was supplemented with the Wilcoxon test.

When the variables were evaluated across the different experimental periods, the comparison between results was carried out for independent samples, and the Kruskall-Wallis test was used followed by the Mann-Whitney *U* test for those cases in which statistically significant differences were detected. The significance level was set at .05.

Results

During the experimental periods, the histopathologic reaction to EndoREZ sealer (group I) was intense, with the presence of lymphoplasmacytic infiltrate and macrophages. Fibrous condensation scored lower after 7 days than after 60 days ($P = .05$). There were few neutrophils, eosinophils, or giant cells, and abscesses were observed in some samples. After 7 and 30 days, hemosiderin pigmentation was also observed.

In group II in which the AH Plus sealer was applied, the number of inflammatory cells tended to decrease during the 3 experimental periods. However, statistically significant differences were detected just in the increase in fibers after 60 days, when compared with the 7-day period ($P = .003$).

The histopathologic reaction to the EndoFill sealer (group III) was intense after all 3 experimental periods, with an abundant lymphoplasmacytic infiltrate and large quantities of macrophages. In some samples, eosinophils, neutrophils, giant cells, and abscess formation could be observed. Fibrous condensation was irrelevant or nonexistent after 7 days and became more evident, although not

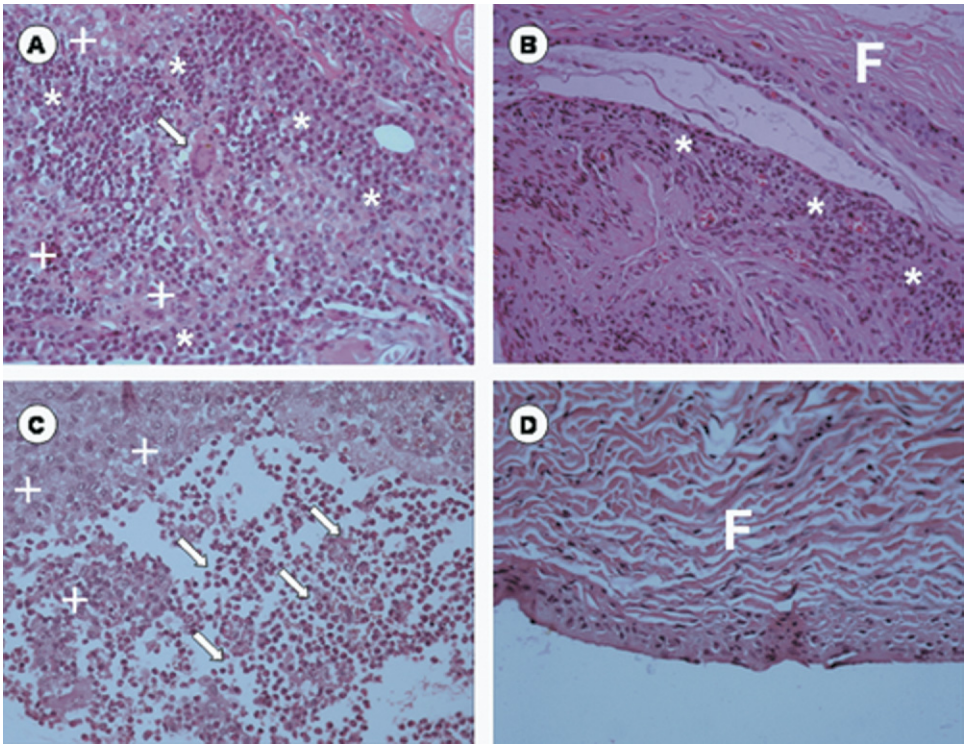

Figure 1. (*A*) Giant cell (arrow), macrophages (+), and intense lymphoplasmacytic infiltrate (*) in response to EndoREZ after 30 days. (*B*) Reaction to the presence of AH Plus after 30 days, presence of a moderate lymphoplasmacytic infiltrate (*) and of a thick fibrous condensation in response to AH Plus after 30 days. (*C*) Abscess (arrows) and macrophages (+) present in regions treated with EndoFill after the 60-day postoperative period. (*D*) Scar tissue and thick fibrous condensation (*F*), control group after 60 days.

very thick or else disorganized, after 30 and 60 days. Only the scores for the presence of fibers were statistically lower after 7 days than after 30 days ($P = .025$).

In group IV, there was a reduction in inflammatory cells and an increase in fibers as the experiment developed. Fibrous condensation scored statistically higher after 30 and 60 days ($P = .029$) Lymphocytes and plasmacytes showed statistically higher scores after 7 and 30 days than after 60 days ($P = .003$). Macrophages, eosinophils, neutrophils, giant cells, and abscess formation exhibited lower values throughout the experiment.

Fig. 1 shows some of the different parameters used in assessing the results during the 3 experimental periods.

Table 1 lists the results of the comparisons between the experimental groups in relation to the 3 experimental periods.

Discussion

The criteria for evaluation as presented by Figueiredo et al. (17) make it possible to supplement the descriptive analysis with an objective analysis of the results. According to Olsson et al. (18), the quantification of tissue response is only possible in materials with very distinct degrees of irritability. A quantitative analysis will not allow for the observation of each and every event that characterizes the inflammatory process, and these events are essential for the evaluation of materials. On the other hand, a descriptive analysis on its own does not allow for an accurate comparison between cements or between the different experimental periods for the same material.

The present study indicates a tendency; methacrylate resin-based and zinc oxide–eugenol sealers will apparently have greater potential for tissue irritation. EndoREZ seems to cause a more intense reaction than those observed either in the AH Plus group or in the control group, especially during longer periods. The same observation has been previously reported (6, 11). On the other hand, in a study carried out with bone tissue, Zmener et al. (10), by means of quantitative analysis, did not observe prolongation of an inflammatory reaction in the presence of EndoREZ.

Methacrylate resin-based and zinc oxide–eugenol sealers brought about greater quantities of macrophages. De Oliveira Mendes et al. (19) assessed the effects of zinc oxide–eugenol sealers on macrophages and

TABLE 1. Mean Scores and Standard Deviation (SD) Attributed to the EndoREZ, AH Plus, EndoFill, and Control (empty) Groups after Experimental Periods of 7, 30, and 60 Days for the 7 Events Assessed

Event	Time (days)		Group				P Value
			EndoREZ	AH Plus	EndoFill	Empty	
Lymphocytes/plasmacytes	7	Mean	3.50^{AB}	2.67^{AB}	3.83^{A}	2.33^{B}	.031
		SD	0.84	0.82	0.41	0.52	
	30	Mean	3.50	3.50	4.00	3.17	.340
		SD	0.84	0.84	0.00	0.98	
	60	Mean	3.00^{A}	2.33^{AB}	3.83^{A}	1.17^{B}	.007
		SD	0.63	1.37	0.41	0.41	
Macrophages	7	Mean	2.67^{A}	1.50^{B}	2.50^{A}	1.17^{B}	.007
		SD	0.52	0.55	0.55	0.41	
	30	Mean	2.67^{A}	1.67^{B}	2.83^{A}	1.67^{B}	.012
		SD	0.52	0.52	0.41	0.82	
	60	Mean	2.67^{A}	1.50^{AB}	2.50^{A}	1.00^{B}	.008
		SD	0.52	0.84	0.55	0.00	
Neutrophils	7	Mean	1.50	1.00	1.50	1.00	.052
		SD	0.55	0.00	0.55	0.00	
	30	Mean	1.17	1.00	1.33	1.33	.801
		SD	0.41	0.00	0.82	0.82	
	60	Mean	1.17	1.00	1.17	1.00	.572
		SD	0.41	0.00	0.41	0.00	
Eosinophils	7	Mean	1.17	1.00	1.33	1.00	.194
		SD	0.41	0.00	0.00	0.52	
	30	Mean	1.17	1.00	1.33	1.33	.532
		SD	0.41	0.00	0.52	0.52	
	60	Mean	1.00	1.00	1.00	1.00	—
		SD	0.00	0.00	0.00	0.00	
Giant cells	7	Mean	1.33	1.33	1.67	1.00	.149
		SD	0.52	0.52	0.52	0.00	
	30	Mean	1.50	1.67	1.50	1.33	.634
		SD	0.55	0.52	0.55	0.52	
	60	Mean	1.00	1.00	1.33	1.00	.112
		SD	0.00	0.00	0.52	0.00	
Fibers	7	Mean	1.50^{AB}	2.00^{B}	1.33^{A}	2.17^{B}	.020
		SD	0.55	0.00	0.52	0.41	
	30	Mean	2.17	2.50	2.50	2.83	.225
		SD	0.41	0.55	0.41	0.55	
	60	Mean	2.83	3.00	2.17	2.83	.080
		SD	0.41	0.00	0.75	0.41	
Abscess	7	Mean	1.67	1.00	1.50	1.00	.056
		SD	0.82	0.00	0.55	0.00	
	30	Mean	1.17	1.00	1.17	1.17	.801
		SD	0.41	0.00	0.41	0.41	
	60	Mean	1.17	1.00	1.33	1.00	.572
		SD	0.41	0.00	0.82	0.00	

Mean values followed by different superscript letters are significantly different.

concluded that they did not interfere with the macrophages' viability, but adherence and potential for phagocytosis were affected. This explains the increased mobilization of macrophages—to cope with the potential for tissue irritation. It could be inferred that the same reaction takes place with EndoREZ.

The fact that hemosiderin pigmentation was still present in tissues after 7 and 30 days also supports the hypothesis that macrophage efficacy is reduced with EndoREZ sealer. Hemosiderin is a pigment that will mark the excess of iron in tissues resulting from the degradation of erythrocytes. Under normal circumstances, this pigment is quickly degraded by macrophages, an event that did not take place in the presence of EndoREZ.

Giant cells participate in the organism's reactions to foreign bodies and are associated with the presence of material that the body finds hard to break down. In the present study they were observed in small numbers.

Few neutrophils were observed, perhaps because there was no experimental period shorter than 7 days. There were no significant differences between the study groups, although after 7 days the EndoREZ group and the EndoFill group tended to show a greater number of neutrophils. This indicates that the characteristics of acute inflammation lasted for prolonged periods; therefore, given the findings that characteristics remained of lymphoplasmacytic infiltrate plus the pronounced number of macrophages in those 2 groups, one can conclude that the inflammatory reaction is longer-lasting and more intense with those 2 materials. In accordance with this conclusion, Kolokouris et al. (13) observed neutrophils to be present in tissue reactions to zinc oxide–eugenol sealers.

The AH Plus sealer exhibited similar inflammatory infiltration when compared with the control group after longer periods. Other studies (6, 11) have already reported this finding. A tendency of the inflammatory process to be reduced during the experimental periods was observed. However, it did not exhibit a statistically significant reduction in lymphocytes and plasmacytes during the different periods.

Eosinophils, which are related to hypersensitivity reactions, were present in reduced numbers. Other authors also have failed to observe an abundance of this cell type in reactions to sealers (16, 20). Nonetheless, Görduysus et al. (14) did observe an abundance of eosinophils in reaction to the EndoFill sealer after 2 days. In the present study, no statistically significant differences were detected, but EndoFill was associated with an increased occurrence of eosinophils, marking a tendency toward increased potential for tissue irritation.

There was an increase in the thickness of fibers during the experimental periods in all of the study groups. However, the EndoFill group showed less intense fibrous condensation after the 7-day period. This is not a desirable characteristic, because the presence of an organized fibrous capsule limiting the area of inflammation prevents the inflammatory reaction from extending to regions distant from the area in contact with the experimental material.

No significant differences were observed between the groups in terms of abscess formation, which was proved to be greatly reduced and just occasionally detected, mostly with EndoFill and EndoREZ sealers, in accordance with data reported by Sousa et al. (6).

The control group alone showed significant reduction in inflammatory reaction characteristics. This observation makes it evident that none of the materials tested here offer the ideal characteristics of biocompatibility desirable in an endodontic sealer, which include the capacity to bring about no more than an acceptable level of inflammatory response, restricted to short periods of time (16).

References

1. Mannocci F, Ferrari M. Apical seal of roots obturation with latterally condensed gutta-percha, epoxy resin cement, and dentin bonding agent. J Endod 1998; 24:41–4.
2. Ahlberg KM, Tay WM. A methacrilate-based cement used as a root canal sealer. Int Endod J 1998;3:15–21.
3. Koulaouzidou EA, Papazisis KT, Beltes P, Geromichalos GD, Kortsaris AH. Cytotoxicity of three resin-based root canal sealers: an in vitro evaluation. Endod Dent Traumatol 1998;14:182–5.
4. Tai KW, Huang FM, Chang YC. Cytotoxic evaluation of root canal filling materials on primary human oral fibroblast cultures and a permanent hamster cell line. J Endod 2001;27:571–3.
5. Huang TH, Yang JJ, Li H, Kao CT. The biocompatibility evaluation of epoxy resin-based root canal sealers in vitro. Biomaterials 2002;23:77–83.
6. Sousa CJ, Montes CR, Pascon EA, Loyola AM, Versiani MA. Comparison of the intraosseous biocompatibility of AH Plus, EndoREZ and Epiphany root canal sealers. J Endod 2006;32:656–62.
7. Tanomaru Filho M, Leonardo MR, Silva LA, Utrilla LS. Effect of different root canal sealers on periapical repair of teeth with chronic periradicular periodontitis. Int Endod J 1998;31:85–9.
8. Leonardo MR, Salgado AA, da Silva LA, Tanomaru Filho M. Apical and periapical repair of dogs' teeth with periapical lesions after endodontic treatment with different root canal sealers. Pesqui Odontol Bras 2003;17:69–74.
9. Zmener O. Tissue response to a new methacrylate-based root canal sealer: preliminary observations in the subcutaneous connective tissue of rats. J Endod 2004;30:348–51.
10. Zmener O, Banegas G, Pameijer CH. Bone tissue response to a methacrylate-based endodontic sealer: a histological and histometric study. J Endod 2005;31:457–9.
11. Zafalon EJ, Versiani MA, de Souza CJ, Moura CC, Dechichi P. In vivo comparison of the biocompatibility of two root canal sealers implanted into the subcutaneous connective tissue of rats. Oral Surg Oral Med Oral Pathol Radiol Endod 2007;103:88–94.
12. Batista RF, Hidalgo MM, Hernandes L, Consolaro A, Velloso TR, Cuman RK. Microscopic analysis of subcutaneous reactions to endodontic sealer implants in rats. J Biomed Mater Res A 2007;81:171–7.
13. Kolokouris I, Economides N, Beltes P, Vlemmas I. In vivo comparasion of the biocompatibility of two root canal sealers implanted into the subcutaneous connective tissue of rats. J Endod 1998;24:82–5.
14. Görduysus MO, Etikan I, Gököz A. Histopathological evaluation of the tissue reactions to Endo-Fill root canal sealant and filling material in rats. J Endod 1998;24:194–6.
15. Kaplan AE, Ormaechea MF, Picca M, Canzobre MC, Ubios AM. Rheological proprieties and biocompatibility of endodontic sealers. Int Endod J 2003;36:527–32.
16. Yesilsoy C, Koren LZ, Morse DR, Kobayashi C. A comparative tissue toxicity evaluation of established and newer root canal sealers. Oral Surg Oral Med Oral Pathol 1988;65:459–67.
17. Figueiredo JA, Pesce HF, Gioso MA, Figueiredo MA. The histological effects of four endodontic sealers implanted in the oral mucosa: submucous injection versus implant in polyethylene tubes. Int Endod J 2001;34:377–85.
18. Olsson B, Sliwkowiski A, Langeland K. Subcutaneous implantation for the biological evaluation of endodontic materials. J Endod 1981;7:355–67.
19. de Oliveira Mendes ST, Ribeiro Sobrinho AP, de Carvalho AT, de Souza Côrtes MI, Vieira LQ. In vitro evaluation of the cytotoxicity of two root canal sealers on macrophage activity. J Endod 2003;29:95–9.
20. Leonardo MR, da Silva LA, Almeida WA, Utrilla LS. Tissue response to an epoxy resin-based root canal sealer. Endod Dent Traumatol 1999;15:28–32.