

Histopathologic evaluation of subcutaneous tissue response to three endodontic sealers in rats

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Abstract: The aim of this study was to evaluate the subcutaneous biocompatibility of three root canal sealers in rats. Thirty Wistar rats were divided into three groups according to three time periods (15, 30 and 60 days). Sterilized polyethylene tubes filled with root canal sealers (AH Plus, Epiphany & Grossman), and one empty tube (control) were implanted into four separate dorsal regions in each rat. At the end of each study period, 10 animals were sacrificed, and histologic sections of connective tissue at the open ends of the tubes were prepared. Severity of tissue inflammatory response was assessed. Grossman endodontic sealer had the most severe inflammatory response followed by the AH Plus, Epiphany and control groups. The tissue inflammatory response of the Epiphany and AH Plus sealers was not significantly different. Thus, Epiphany sealer showed acceptable biocompatibility when tested on rat subcutaneous tissue. (*J Oral Sci* 53, 15-21, 2011)

Keywords: endodontic sealers; tissue reaction; subcutaneous implantation.

Introduction

The success of root canal therapy depends on complete debridement and obturation of the root canal system with biocompatible materials in order to prevent periradicular tissue irritation (1). Gutta-percha with zinc oxide eugenol or calcium hydroxide-based sealers have been used for root canal obturation for many years (2). Shrinkage and dissolution of these sealers may occur over time, and the apical seal may be affected (3). None of these materials bond with root dentin and consequently complete apical seal may not be achieved (4). Although endodontic sealers are used for intracanal obturation, they frequently pass through apical constriction into the periradicular tissue. Thus, the importance of biocompatibility in endodontic sealers is an accepted principal for successful endodontic treatment (5).

Recently, a polyester-based thermoplastic material (polycaprolacton urethane dimethacrylate) known as Resilon was introduced as a root canal obturation material that has more bonding properties with its sealer. In the Epiphany obturation system, Resilon cones able to bond with root canal dentin via a dual cure resin sealer are used (6). Recent studies have shown that root canals obturated with the Resilon-Epiphany system have greater resistance to fracture and better sealing ability in comparison to root canals filled with Gutta-percha and traditional sealers (7).

With the introduction of new obturating systems, it is necessary to compare them to traditional materials to reach a more ideal selection (8). It is clear that all root canal

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filling materials (solid cores and sealers) cause tissue irritation when extruded into the periradicular area. Thus, the question is not about the presence or absence of tissue irritation but about severity and time span of tissue irritation of different materials (9). Sealers play an important role in root canal obturation as they fill all voids and spaces that core material cannot because of physical limitations (10). As endodontic sealers in association with core materials have a principal role in sealing of root canal systems, and considering the possibility of their extrusion into periradicular tissues, assessment of their biocompatibility is essential (11). Although Epiphany sealer has been approved by the United States Food and Drug Administration (FDA), there is little published data on its biocompatibility and cytotoxicity (12). Therefore, the aim of this study was a histopathological comparison of the severity of tissue inflammatory responses to Epiphany, AH Plus & Grossman sealers in rat connective tissue.

Materials and Methods

This study was approved by the ethics committee of Isfahan University of Medical Sciences and Torabinejad Dental Research Center. Thirty male Wistar rats weighing 150-200 g were used. After inhalation anesthesia with chloroform, general anesthesia was induced by intraperitoneal injection of 0.2 ml of ketamine and promazine in equal proportions. Four separate regions on the dorsum of each animal were disinfected with 10% betadine, followed by shaving. Four 10-mm cephalocaudal incisions were made with a scalpel, and the skin was undermined with blunt cotton pliers. Each sealer was mixed with a sterilized spatula on a sterilized glass slab according to the manufacturer's instruction. Sterilized polyethylene tubes (1.2 mm in diameter and 10 mm in length) filled with AH Plus (Dentsply De Trey, GmbH, Jonstanz, Germany), Epiphany (Pentron clinical technologies LLC, Wallingford, CT, USA), Grossman (Sultan Chemists, Englewood, NJ, US (sealers and an empty tube (control) were inserted in right front, right rear, left front and left rear incisions, respectively. One end of each tube was closed by heating before sealer placement. After each period of time in this study (15, 30 and 60 days) 10 rats were sacrificed by anesthetic drug overdose. The areas where the tubes were inserted were shaved, and 1.5 × 2.5 cm tissue sections containing each tube were removed and placed in 10% buffered formalin solution. Histologic sections of the connective tissue around the open end of each tube at a thickness of 5 μm were taken from specimens, placed in paraffin blocks and stained with hematoxylin and eosin. Quantitative assessment of inflammatory cells (lymphocytes, plasma cells, neutrophils, macrophages

and Gaint cells) was performed in 10 separate fields of each specimen at ×400 magnification of light microscope (Carl Zeiss, Oberkachen, Germany). The mean count of inflammatory cells for the 10 fields was determined, and severity of tissue inflammatory response was classified as follows (13,14):

1. Grade 0: absence of inflammatory cells or presence of fewer than 5 cells
2. Grade 1 (mild reaction): presence of 5 to 25 cells
3. Grade 2 (moderate reaction): presence of 25 to 125 cells
4. Grade 3 (severe reaction): presence of more than 125 cells

Severity of tissue inflammatory response in experimental (AH Plus, Epiphany, Grossman) and control groups was compared using Friedman statistical test for each period of time (15, 30 and 60 days). Wilcoxon complementary test was used to compare each individual group with other groups when a significant difference was detected ($P < 0.05$). Severity of tissue inflammatory response for each experimental group was then compared at three time periods (15, 30 and 60 days) using Kruskal-Wallis test. Mann-Whitney complementary statistical test was used to compare each single period with two other periods when significant differences between the three time periods were observed ($P < 0.05$).

Results

In all three time periods studies (15, 30 and 60 days) the severity of tissue inflammatory response of Grossman sealer was significantly different from other groups. The most severe response was due to Grossman sealer followed by the AH Plus, Epiphany and control groups. The tissue inflammatory responses in the AH Plus and Epiphany groups were not significantly different. Cellular distribution scores for the experimental and control groups and related reactions on 15th, 30th and 60th days are given in Table 1.

Overall, the tissue inflammatory response in the histologic sections ranged from acute for the 15-day period to chronic for the 30- and 60-day periods. Inflammatory cell infiltration decreased from day 15 to day 60 in all groups. The predominant cell types in the 15-day sections were PNL, lymphocytes, plasmocytes and macrophages; lymphocytes were predominant in the 30- and 60-day sections. Fibrous capsule formation was observed only in the Epiphany and control groups on day 60. Necrosis was seen in some sections of the AH Plus (day 15) and Grossman (day 15 and 30) groups. Calcified areas and foreign materials (sealer) were not detected in any of the sections.

Table 1 Cellular distribution scores of groups: 0, 0 or very few cells; 1, 5-25 cells; 2, 25-125 cells; 3, 125 or more cells.

Cell type	Day 15				Day 30				Day 60			
	Gr.1	Gr.2	Gr.3	Gr.4	Gr.1	Gr.2	Gr.3	Gr.4	Gr.1	Gr.2	Gr.3	Gr.4
Lymphocyte	2	2	2	2	1	1	2	1	1	1	1	1
Plasmocyte	2	2	2	2	1	1	2	0	1	0	1	0
PNL	3	2	2	1	1	1	1	0	0	0	1	0
Macrophages	2	2	1	2	1	0	1	1	0	0	1	0
Giant cells	1	2	1	1	1	0	1	0	0	0	1	0
Necrosis	+	-	+	-	-	-	+	-	-	-	-	-
Calcification	-	-	-	-	-	-	-	-	-	-	-	-

Gr = Group

Gr.1: AH Plus; Gr.2: Epiphany; Gr.3: Grossman; Gr.4: Control.

Necrosis and calcification rows were identified as yes (+) or no (-).

Table 2 P values between experimental study groups at different time periods

Groups	AH Plus		Grossman	Control	Epiphany		Grossman	Control
	Epiphany	Grossman			Grossman	Control		
15 days period	0.317	0.014	0.001	0.007	0.011	0.001		
30 days period	0.748	0.175	0.038	0.131	0.063	0.025		
60 days period	0.414	0.275	0.034	0.069	0.102	0.014		

15-day period:

The results for the 15-day period are shown in Fig. 1. The Friedman test indicated a significant difference between the groups ($P < 0.05$), with Grossman sealer showing the most severe response. The Wilcoxon complementary test confirmed that the difference in results was not statistically significant between the AH Plus and Epiphany groups (Table 2).

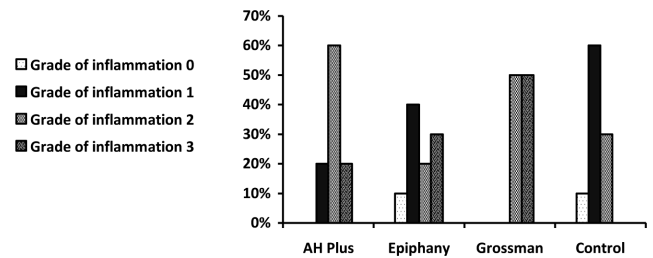


Fig. 1 Tissue inflammatory response grade for 15-day period.

30-day period:

The results for the 30-day period are shown in Fig. 2. The Friedman test indicated a significant difference between the groups ($P < 0.05$), but the Wilcoxon complementary test confirmed that there was no significant difference between the AH Plus-Control and Grossman-Control groups. The sealer groups did not show any significant differences from one another (Table 2).

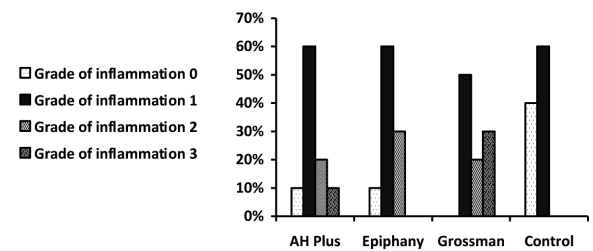


Fig. 2 Tissue inflammatory response grade for 30-day period.

60-day period:

The results for the 60-day period are shown in Fig. 3. The Friedman test indicated a significant difference between the groups ($P < 0.05$), but the Wilcoxon complementary test showed a significant difference between the AH Plus-Control and Grossman-Control groups. The sealer groups did not show any significant differences from one another (Table 2).

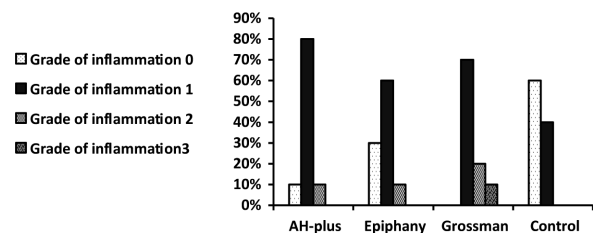


Fig. 3 Tissue inflammatory response grade for 60-day period.

Overall, the severity of tissue inflammatory response induced by all three sealers (AH Plus, Epiphany, Grossman) decreased with time (from day 15 to day 60).

Discussion

Three-dimensional obturation after cleaning, preparation and disinfection of the root canal system is one of the most important steps for successful root canal therapy (2). Root canal obturation materials are generally composed of core materials such as Gutta-percha and sealer. The biocompatibility of such materials is important because of the possible long-term contact with periradicular tissues (15). Toxic components of these materials can cause irritation and degeneration of periradicular tissues.

As the tissue inflammatory responses of all connective tissues are similar, animal subcutaneous implantation studies are one of the most reliable methods to assess the biocompatibility of dental materials (16). Rats were used in the present study because they are less susceptible to postoperative infection, economically accessible and an accepted model for determining tissue biocompatibility (17). To ensure standardization and similarity to clinical conditions, polyethylene tubes were selected for this experiment. Polyethylene tubes are inert in nature and are effective for testing materials in contact with the surrounding tissue (18,19).

Tissue inflammatory response in this study was assessed on the basis of the number of inflammatory cells. According to Olsson et al. (16) quantitative assessment of inflammatory response is possible only when there is a significant difference between the study groups. On the other hand, qualitative assessment of tissue inflammatory response cannot portray an accurate comparison between different materials or the same material at different periods of time (16). Therefore, lack of significant statistical differences between some groups in our study may be due to the quantitative assessment method used.

In this study, AH Plus sealer had moderate tissue inflammatory response at 15 days and mild inflammatory response at 30 and 60 days (mean grade, 2, 1.3 and 1, respectively). The severity of inflammatory response decreased over time in this group. There was a lack of significant differences between inflammatory response at 15-30 days and that at 30-60 days, but the difference between 15 and 60 days was significant. Scarparo et al. (20) compared the subcutaneous inflammatory responses of AH Plus, a zinc oxide eugenol, and a methacrylate resin sealer at 7, 30 and 60 days; their results were consistent with the findings of our study. All three groups of sealers showed a more severe inflammatory response than the control group, but the severity of inflammation decreased over time. The similarity in results may have been due to similar experimental conditions in the two studies. Sousa et al. (21) compared the intraosseous inflammatory response of AH Plus, Epiphany and EndoRez sealers. The severity

of the inflammatory response of AH Plus sealer was severe at 4 weeks and moderate at 12 weeks. The inflammatory response decreased over time, which was similar to the present observations. Bouillaguet et al. (22) placed Epiphany, AH Plus and Guttaflow sealers in contact with cultured fibroblast cells and assessed their cytotoxicity at 24 and 72 h using MTT [(methyl tetrazolium bromide)]-based colorimetric assay, and they found that all of these sealers had severe cytotoxicity. Similarly, Lodiene et al. (23) placed AH Plus, EndoRez, Epiphany and Roeko Seal sealers in contact with mouse fibroblast cells and found that fresh mixed AH Plus sealer is severely toxic.

Therefore, *in vivo* studies indicate moderate to severe tissue inflammatory responses to AH Plus, and its severity decreases over time. As *in vitro* studies assess cytotoxicity over short periods of time after mixing of sealer, the initial cytotoxic response is severe and decrease in cytotoxicity cannot be seen over time. The primary cytotoxicity of AH Plus sealer may be due to the release of traces of formaldehyde.

In this study, Epiphany sealer showed a moderate tissue inflammatory response at 15 days and a mild inflammatory response at 30 and 60 days (mean grade, 1.7, 1.2 and 0.8, respectively). The severity of the inflammatory response decreased over time. Statistical differences in inflammatory response were not significant between the 15-, 30- and 60-day periods. De compos-pinto et al. (24) assessed the subcutaneous biocompatibility of Epiphany sealer in four separate regions of the dorsum of 15 rats. They inserted polyethylene tubes filled with Epiphany sealer, light-activated Epiphany sealer, Epiphany sealer with self etch primer, and Epiphany sealer with self etch primer activated with light. The inflammatory responses of the four groups were compared at 7, 21 and 42 days. In all periods of the study, Epiphany sealer caused mild inflammatory responses. The design of that study was different from the present study in that they inserted the four experimental groups of material into the dorsum of each animal, and the one other animal served as a control. Onay et al. (25) inserted a tube filled with Epiphany sealer, an empty tube as a control, and 10 mm of Gutta percha or resilon into four separate dorsal regions in 36 rats. There were no significant statistical differences in tissue response at 1, 4 and 8 weeks between the groups. The inflammatory response was moderate to severe at 1 week and its severity decreased gradually at 4 and 8 weeks. The degree of the inflammatory response of the Epiphany sealer and the gradual decrease in severity over time was similar to those in the present study, probably as a result of the similar methods and conditions of the two studies. Sousa et al. (21) assessed intraosseous inflammatory response to Epiphany, AH Plus and EndoRez

sealers. They found that Epiphany sealer caused little or no inflammatory response at 4 and 12 weeks. The results of our study are similar, in that Epiphany and AH Plus sealers caused moderate tissue inflammatory response initially and severity decreased over time.

Conversely, Bouillaguet et al. (22) and Lodien et al. (23) demonstrated severe cytotoxicity for Epiphany sealer. This severe reaction may be due to released monomers prior to complete setting. The shortcoming of *in vitro* studies is that they assess cytotoxicity within a short period of time after mixing of the sealer and do not show the true cytotoxic behavior of the sealer over time.

In this study, Grossman sealer had moderate to severe tissue inflammatory response at 15 days, moderate

inflammatory response at 30 days, and at 60 days, the response was mild to moderate (mean grade, 2.5, 1.8 and 1.4, respectively). The severity of inflammatory response decreased over time. Significant statistical differences in inflammatory responses were present between the 15- and 60-day periods. Key et al. (8) placed Grossman, Thermaseal and Sealapex sealers in contact with human gingival fibroblasts. At 1 and 24 h, the cytotoxic effects of Grossman sealer were greater when compared with other sealers in the experiment. Erausquin and Muruzabal (26) also showed severe inflammatory responses to ZOE-based sealer. As eugenol is used in the preparation of Grossman sealer, a more severe tissue inflammatory response is anticipated than with Epiphany and AH Plus

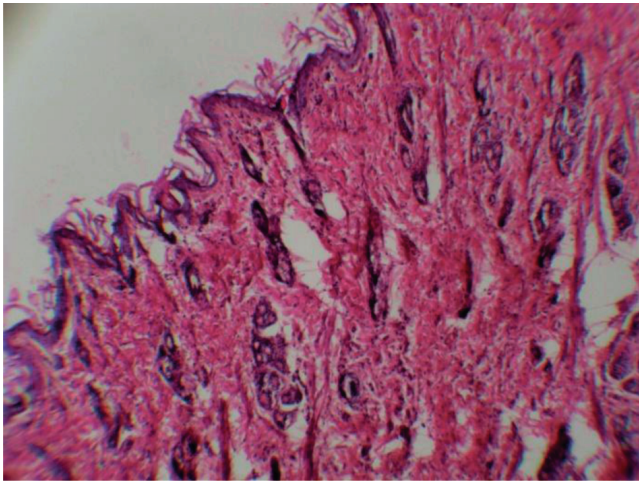


Fig. 4 Control group: 60 days. Connective tissue with few inflammatory cells (H&E; $\times 100$).

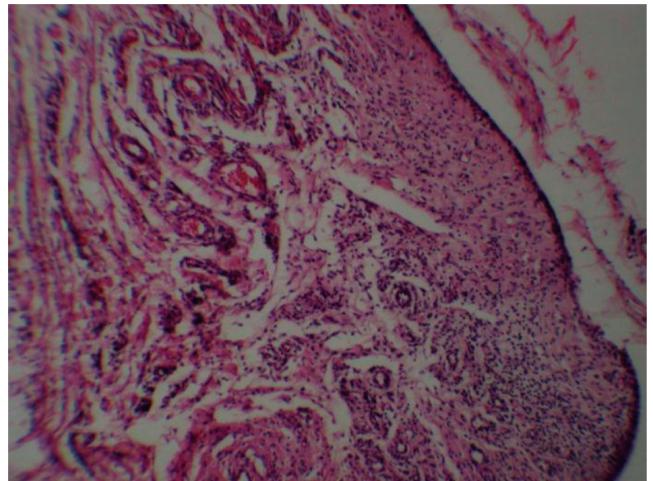


Fig. 6 AH Plus: 30 days. Moderate inflammatory reaction of connective tissue with medium inflammatory cell infiltration (H&E; $\times 100$).

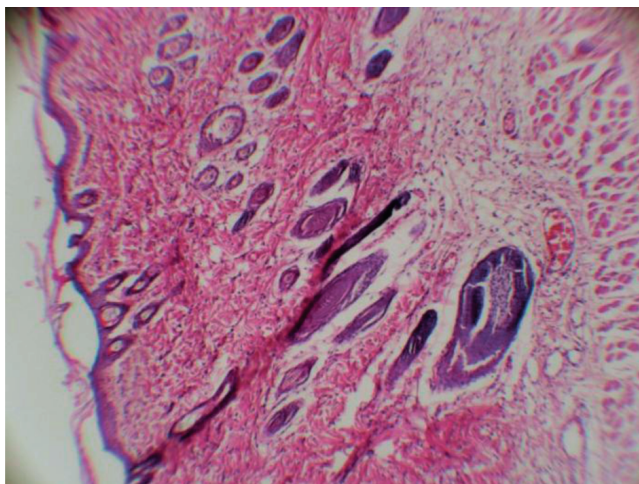


Fig. 5 Epiphany: 60 days. Mild inflammatory reaction in connective tissue with few inflammatory cells and thin fibrous capsule formation (H&E; $\times 100$).

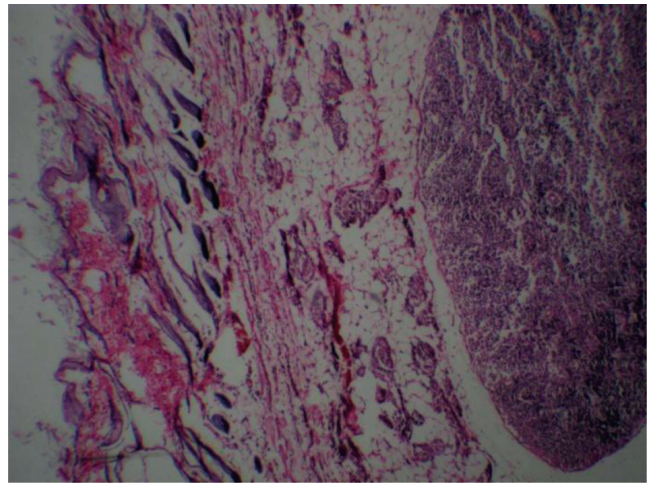


Fig. 7 Grossman: 15 days. Severe inflammatory reaction of connective tissue with severe inflammatory cell infiltration (H&E; $\times 100$).

sealers. Various studies have attributed the irritative potential of ZOE-based sealers to eugenol causing development of periapical inflammation (27-29). Confirming the above statement, in the present study, Grossman sealer caused a more severe tissue inflammatory response than AH Plus and Epiphany sealers.

The control group had mild tissue inflammatory response at 15 days, and mild to no inflammatory response at 30 and 60 days (mean grade, 1.2, 0.6 and 0.4, respectively). At 15 days, the tissue inflammatory response of the control group may be due to initial surgical trauma. In later periods of the study (30 and 60 days), the inflammatory response may be due to mechanical irritation of tube edges (Fig. 4).

Grossman sealer had the most severe tissue inflammatory response when compared to Epiphany and AH Plus sealers. AH Plus and Epiphany sealers had similar tissue inflammatory response (Figs. 5-7). The severity of inflammatory response in all groups of sealers decreased over time. Epiphany sealer had a mild tissue inflammatory response, and considering its reported advantages, it can be recommended for clinical use.

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