

# Bone Tissue Response to a Methacrylate-Based Endodontic Sealer: A Histological and Histometric Study

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## Abstract

Biocompatibility of endodontic sealers is a prime requisite for successful endodontic therapy. The purpose of this study was to evaluate the reaction of bone to a methacrylate-based endodontic sealer. Silicone tubes, filled with a methacrylate-based sealer and silicone rods used as controls, were implanted in the tibiae of white male Wistar rats. Ten and 60 days after implantation the reaction of the bone was analyzed by means of histological and histometric methods. After 10 days the amount of reactionary bone in contact with the methacrylate-based sealer was significantly lower than that observed for the controls ( $p < 0.05$ ). Cell counts demonstrated that the number of inflammatory cells that were in contact with the sealer were significantly higher ( $p < 0.05$ ). After 60 days, no significant differences were observed in the amount of reactionary bone that was in contact with either material. The initial inflammatory reaction observed for the methacrylate-based sealer was resolved. Furthermore the cell counts demonstrated no statistically significant differences ( $p > 0.05$ ) between the methacrylate based sealer and control.

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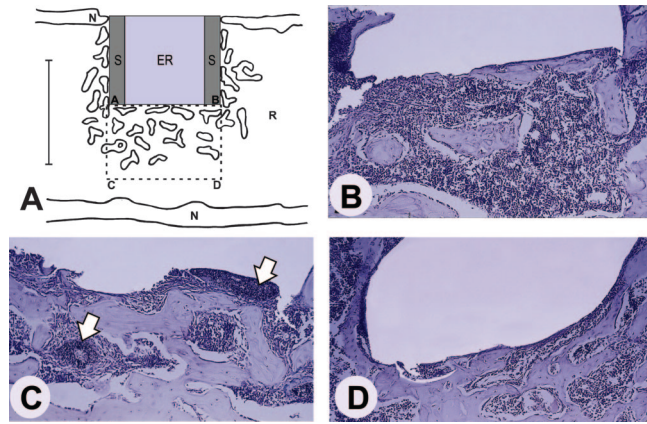
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Successful endodontic treatment depends on thorough debridement of the root canal(s) and total obturation of the root canal space with an inert filling material. The use of gutta-percha in conjunction with a sealer is one of the most reliable methods for root canal obturation (1). Of a variety of sealers that have been developed to accomplish this, methacrylate resin-based sealers have shown promising results (2–4). They exhibit excellent adhesion to root canal walls (2) and good sealing properties (3, 4). Recently, a new methacrylate-based endodontic sealer, EndoRez (ER) (Ultradent Products, Inc., South Jordan, UT) has been introduced for filling root canals. ER is an hydrophilic, two-component, chemical-set material containing zinc oxide, barium sulfate, resins, and pigments in a matrix of urethane dimethacrylate resin. Preliminary observations (5–7) revealed that ER exhibited excellent adaptation to root canal walls (5), that is well tolerated by subcutaneous connective tissues in rats (6) and periapical tissues in subhuman primates (7). To date no studies have been conducted to analyze histologically the effect of ER implantation in bone. Therefore, the purpose of this study was to evaluate histologically and histometrically the effect of ER implantation in the tibiae of rats.

## Materials and Methods

After autoclaving, silicone tubes closed at one end (Raholin SRL, V. Madero, Buenos Aires, Argentina) and measuring 1 mm long with an outer diameter of 1 mm and a lumen of 0.5 mm, were filled flush with freshly mixed ER. The ER was handled aseptically and mixed according to the manufacturer's instructions. Twenty (20) white male Wistar rats weighing approximately 100 g each had one sample of the experimental material implanted in the right tibia. A solid silicone rod of the same length and external diameter as the tubes was implanted in the left tibia and served as the negative control (8). The implantation technique was performed according to a technique described in a previous report (9). The animals were anesthetized by intraperitoneal administration of sodium pentobarbital (0.025 g/1000 g body weight). The tibiae were shaven and the skin disinfected with 70% alcohol. Under sterile conditions, a longitudinal incision of 1.5 cm was made at the lateral aspect of the anterior border of the tibia reaching the bone. After reflection of the periosteum, a 1 mm deep cylindrical opening was prepared in the area of the diaphyseal bone (DB), approximately 8 mm from the lateral external side. To prevent overheating of the bone, the openings were made manually by rotating an end-cutting bur measuring 1 mm in diameter. The tubes filled with ER and the silicone rods were then introduced into the bone in such a way, that they projected into the marrow space while the closed ends were level with the outer surface of the cortical bone. Care was taken to prevent smearing of ER on the outside of the tubes. The wounds were sutured and the animals maintained on a regular diet and water ad libitum. The animals were killed by means of ether suffocation. Ten experimental and 10 control tibiae were harvested after subsequently 10 and 60 days and fixed in a 10% neutral buffered formalin solution. The specimens were decalcified in 10% formic acid and processed for paraffin embedding. Serial sections parallel to the long axis of the implants of approximately 7- $\mu$ m thick were obtained from the center of the specimens and stained with hematoxylin and eosin. Because of complications, three animals had to be excluded from the experiment. They were replaced by new animals that underwent the same surgical procedures.



**Figure 1.** (A) Drawing of silicone tube filled with ER, implanted in DB areas of a rat tibia. Points ABCD define the rectangular area used for measurement of BDF and cell quantitation. N = Cortical bone; R = Marrow space. Bar = 1.00 mm. (B) ER, 10 days. In the tissues adjacent to the sealer, scattered areas of new bone formation can be observed (H & E, original magnification  $\times 400$ ). (C) ER, 60 days. Note the presence of newly formed trabecular bone in direct contact with ER. In addition, remnants of the sealer (arrows) are present at the end of the tube and in the bone marrow. (H & E, original magnification  $\times 400$ ). (D) Silicone rod, 60 days. The implant (empty space) is surrounded by a thin fibrous connective capsule and newly formed trabecular bone (H & E, original magnification  $\times 150$ ).

**Evaluation Parameters**

Trabecular bone density formation (BDF) and the cell population at the area in direct contact with ER or the silicone implants were used as evaluation parameters to determine tissue reactions to the implanted materials. Histometric measurements of BDF were performed with a LECO-300 stereo microscope (LECO Corp., St. Joseph, MI) which was connected to a LECO 2001–2.02 image analyzer (LECO). The BDF, was calculated at a magnification of  $\times 110$  within a rectangular field measuring  $1100 \times 850 \mu\text{m}$  (Fig. 1A). The bone trabeculae located within the specified field of measurement were analyzed on three randomly selected sections that belonged to the center area of each specimen. These measurements were expressed as a percentage of new bone. Mean values of BDF were then calculated and expressed as the ratio between new trabecular bone and the total area of observation. For quantitative cell counts, the total number of lymphocytes, polymorphonuclear leukocytes, macrophages, multinucleated giant cells, plasmocytes, and fibroblasts were recorded in the areas surrounding the central end of the implants. For this purpose, using a specially devised ocular at a magnification of  $\times 400$ , three different fields of vision measuring  $7.5 \text{ mm}^2$  each were identified. They were spaced at regular intervals in the area of direct tissue contact with ER or the silicone rods. The sections that were used for BDF analysis were also used for determining the number of cells. Each section was counted twice and a mean value was calculated from the total number of ER and control specimens. All measurements were performed by a single examiner who was not aware of the identity of the sections. Cumulative data consisted of the measurements of BDF and the cell counts made for each material and for each observation period. The effect of time and material was subjected to a statistical analysis by means of an ANOVA repeated measures, while a nonparametric Wilcoxon signed rank test was used to compare the cell counts for ER and controls at each observation period. For both tests a significance level of  $p < 0.05$  was used.

**TABLE 1.** Trabecular bone density formation

Days	EndoRez	Control	p
10	0.17 (0.04)	0.31 (0.06)	$<0.05$
60	0.39 (0.04)	0.42 (0.04)	$>0.05$

The results of BDF are expressed as the ratio between the trabecular area and the total area analyzed. Numbers in parenthesis = SD.

**Results**

At the end of the 10 and 60-day observation periods wound healing was clinically satisfactory for all cases and all implants remained in situ. The mean values of the BDF measurements are listed in Table 1. The results of an ANOVA for BDF demonstrated a statistically significant difference between experimental and control material at the 10-day period of observation. The amount of reactionary bone formation in direct contact with ER (Fig. 1B) was significantly less ( $p < 0.05$ ) than what was observed for the controls. However, after 60 days (Fig. 1C, D) no difference was seen ( $p > 0.05$ ). The mean values of the cell counts are shown in Table 2. At the 10-day observation, the number of inflammatory cells that was observed in direct contact with ER was higher and differed significantly ( $p < 0.05$ ) from those seen at the control sites. This cell population was mainly composed of lymphocytes, polymorphonuclear leukocytes, macrophages and an occasional multinucleated giant cell. After 60 days, no significant differences ( $p > 0.05$ ) for the cell counts were observed between ER and the control specimens. Fibroblastic proliferation in the contact area of ER and control implants was observed in both evaluation periods.

**Discussion**

Based on previous findings (9) the intraosseous implantation of endodontic filling materials in rat tibias provides a suitable model to evaluate biocompatibility of materials (9, 10). The analysis of BDF constitutes a sensitive indicator of bone damage under different experimental conditions (9, 11). The implantation periods used in this study, a short and long-term time interval, are according to the recommended standard practices for biological evaluation of dental materials (12). The fact that all implants remained in situ throughout the experiment was somewhat unusual since intraosseous implants have a tendency to be displaced with some frequency (13). Success for long-term retention may be dependent on the exact fit of the implants in the bone. In this study, we used silicone tubes as carriers for ER and silicone rods as negative controls because of their proven suitability (8, 9). The test materials were implanted in the areas of DB in which the mechanisms of endochondral ossification are absent and only reactionary bone formation to a foreign material is to be expected (9). Implantation of test materials in the area of DB allows the evaluation of possible adverse effects of ER since it has been previously demonstrated that toxic materials exert an inhibitory effect on the normal development of reactionary bone trabeculae in jaws or the endochondral ossification of long bones (9, 11, 14). The results after 10 days of implantation indicated that reactionary bone formation was significantly reduced in the tissues that were in direct contact with ER. No significant differences were found between ER and the controls after 60 days, indicating that after initial irritation the sealer does not interfere with normal bone healing. The results of the cell counts revealed an initial inflammatory reaction in the area that was in direct contact with ER. However, the severity of this reaction decreased over time and it appeared to be resolved after 60 days. The differences between both materials were statistically significant only at the 10-day observation period. The mild concentration of inflammatory cells observed for the controls in the short-term period may have occurred as a result of the trauma from surgery. After 60 days

**TABLE 2.** Cell counts in diaphyseal bone

	10 days			60 days		
	EndoRez (n = 10)	Control (n = 10)	p	EndoRez (n = 10)	Control (n = 10)	p
Lymphocytes	3.31 (1.67)	1.36 (1.58)	<0.05	0.90 (0.74)	0.65 (0.81)	>0.05
Polymorphonuclear Leukocytes	14.71 (2.21)	1.16 (0.99)	<0.01	0.55 (0.83)	0.17 (0.20)	>0.05
Macrophages	4.90 (2.07)	0.96 (1.12)	<0.01	0.44 (0.41)	0.21 (0.20)	>0.05
Giant cells	1.20 (0.60)	0.00 (0.00)	<0.05	0.00 (0.00)	0.00 (0.00)	—
Plasmocytes	0.23 (0.32)	0.17 (0.44)	>0.05	0.00 (0.00)	0.00 (0.00)	—
Fibroblasts	21.35 (4.02)	19.02 (3.02)	>0.05	23.19 (3.42)	21.01 (4.96)	>0.05

Cell counts obtained from all specimens of each material and expressed in mean values.

Numbers in parenthesis = SD.

this appeared to have been resolved. It may be hypothesized that the initial reduction of bone formation in the area of contact with ER may be a result of a depressed differentiation of osteoblasts caused by the presence of certain components of the sealer. Irritation to ER in short observation periods have been reported previously (6, 7). It has been demonstrated (6) that after subcutaneous implantation of fresh ER, components such as zinc and barium were present in tissues in direct contact with the sealer. Maryon and Jakeman (15), Smith et al. (16), and Cathers et al. (17) have reported various degrees of toxicity from different concentrations of zinc and barium. In our study, the leaching of components from freshly placed ER may occur during the setting period. Consequently, the presence of inflammatory cells at the 10-day observation period may, therefore, be attributed to the presence of ER components in the surrounding tissues. This is a common finding after intraosseous implantation of endodontic materials (13, 18). Long-term biocompatibility of ER was supported by the absence of inflammatory cells, the increased fibrous tissue response and by the presence of healthy bone trabeculae that were observed at the 60-day observation period. These results suggest that the behavior of ER in contact with bone tissue of rat tibias is comparable to other commonly used endodontic sealers (18, 19). Friend and Browne (18) reported that bone repair occurred around polyethylene tubes filled with the epoxy resin AH 26 (Dentsply/deTrey, Konstanz, Germany) and a polyvinyl resin Diaket (3M/Espe GmbH, Seefeld, Germany) within a time interval of 90 to 180 days after implantation in the tibias of rabbits. Deemer and Tsaknis (19) using rat tibias, reported similar results in a study in which polyethylene tubes were filled flush or were overfilled with gutta-percha and a zinc oxide-eugenol endodontic sealer. It should be pointed out that histologic and histometric measurements cannot predict the outcome of endodontic therapy; they merely are indicators of the biocompatibility of a material. Clinical data evaluating the clinical behavior of ER used in conjunction with laterally condensed gutta-percha (20) over a period of 18 to 24 months has demonstrated comparable results to other conventional filling materials and techniques (21–24). Longer term data will ultimately determine the clinical success of ER as a root canal sealer.

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