Subcutaneous Connective Tissue Reaction to Methacrylate Resin–based and Zinc Oxide and Eugenol Sealers

Osvaldo Zmener, DDS, Cornelis H. Pameijer, DMS, MScD, DSc, PhD,[†] Gabriel A. Kokubu, DDS,* and Daniel R. Grana, DVM**

Abstract

Introduction: An evaluation was made of the connective tissue reaction in rats after subcutaneous implantation of methacrylate resin-based sealers (EndoREZ [Ultradent Products, Inc, South Jordan, UT] with a polymerization accelerator and RealSeal [Sybron Dental Specialties, Orange, CA]) and Pulp Canal Sealer (Sybron Dental Specialties), a zinc oxide and eugenol-based sealer used as the control. **Methods:** Silicone tubes containing the test materials were implanted in 24 Wistar rats. Solid silicone rods of the same size served as the negative controls. After 10, 30, and 90 days, the animals (n = 8 per period) were euthanized and the implants with surrounding tissues dissected and processed for routine histological evaluation. A four-category evaluation system was used to measure and record the microscopic observations according to the thickness of a fibrous capsule, the vascular changes, and the various types of inflammatory cells. **Results:** Initially, a severe inflammatory reaction was observed of the soft tissues in direct contact with both EndoREZ/Accelerator and Real Seal. The severity decreased over time and was resolved at the end of the experiment. Pulp Canal Sealer showed a severe tissue reaction for all observation periods. The negative controls showed an initial mild to moderate inflammatory reaction. After 30 days, healthy fibrous connective tissue was observed, which increased over time. After 10 days, no statistically significant differences between the experimental groups were observed. After 90 days, EndoREZ and RealSeal were statistically significantly less toxic than Pulp Canal Sealer ($p > 0.05$). **Conclusions:** After 90 days, both methacrylate resin-based sealers were considered biologically acceptable when implanted in subcutaneous connective tissues of the rat. Pulp Canal Sealer remained toxic for the duration of the study. *(J Endod 2010;36:1574–1579)*

Key Words

Biocompatibility, endodontics, methacrylate-based sealers, tissue response

The current concept among clinicians is that after complete debridement, total obliteration of the root canal space with a biocompatible material constitutes the key factor for successful endodontic therapy (1). Different materials have been advocated for filling root canals; gutta-percha cones complemented with a sealer cement is the most widely used (2). During the last decade, methacrylate resin-based sealers (MRBSs) have gained popularity for root canal obturation (3). Preliminary reports have shown that two well-established MBRSs (ie, EndoRez [ER; Ultradent Products, Inc, South Jordan, UT] and RealSeal (RS; Sybron Dental Specialties, Orange, CA), formerly Epiphany, are both well tolerated by living tissues (4–7) and have shown promise for *in vivo* human clinical trials (8–10). More recently, an ER Accelerator (ACC, Ultradent Products Inc.) has been introduced. The ACC is composed of triethylene glycol dimethacrylate, tertiary amines, and a proprietary ingredient. The technique the manufacturer recommends is the following. When the master gutta-percha cone has been placed to length, two or three #20 to #25/.02 taper accessory cones dipped in ACC are harpooned in the sealer and pushed into the canal space as far as possible. The combination of ER and ACC accelerates the polymerization of the sealer, thus allowing for an immediate continuation of the coronal restoration. It also prevents dislodgement of the obturating material when a post space is prepared immediately after obturation, potentially causing early bacterial leakage (11).

Previous reports (12, 13) have shown that certain components from methacrylate resin-based materials may remain unpolymerized even after setting and can subsequently be released from the resin matrix. When the sealer is accidentally extruded through the apex or through a lateral canal, which is not an uncommon experience in endodontics (14), the unpolymerized components may be toxic to the periapical tissues. Although the biocompatibility of ER and RS has been investigated (4–7), the effect of ER/ACC in contact with living tissues and compared with RS has not been reported yet. Therefore, the purpose of this study was to evaluate the biocompatibility of ER/ACC and RS and to compare them with Pulp Canal Sealer (PCS, Sybron Dental Specialties), a zinc oxide and eugenol-based sealer, when implanted subcutaneously in connective tissue of rats.

Materials and Methods

The protocol of this study was approved by the Research Ethics Committee of the Argentine Dental Association. Autoclaved silicone tubes closed at one end (Raholin SRL, V. Madero, BA, Argentina) and 10-mm long with an internal diameter of 1 mm were filled flush with freshly prepared ER/ACC, RS, or PCS (positive control). Solid silicone rods (SIRODs) of the same size as the tubes were used as negative controls. ER alone was not tested because this had been done previously under similar conditions (5). The methacrylate resin–based sealer samples were prepared in such a way that the

From the *School of Dentistry, University of El Salvador, Buenos Aires, Argentina; and [†]University of Connecticut School of Dental Medicine, Farmington, CT, USA.
Supported in part by Ultradent Dental Products Inc.
Address requests for reprints to Dr Cornelis H. Pameijer, 10 Highwood, Simsbury, CT 06070. E-mail address: cornelis@pameijer.com.
0099-2399/$0 - see front matter
Copyright © 2010 American Association of Endodontists.
doi:10.1016/j.joen.2010.06.019

TABLE 1. Severity of Tissue Reaction to the Test Materials

		ER/ACC				RS				PCS				SIROD			
Days	n	NO	MI	MO	SE	NO	MI	MO	SE	NO	MI	MO	SE	NO	MI	MO	SE
10	8	0	0	0	8	0	0	0	8	0	0	0	8	0	6	2	0
30	8	0	0	8	0	0	0	8	0	0	0	0	8	8	0	0	0
90	8	7	1	0	0	6	2	0	0	0	0	0	8	8	0	0	0

NO, no reaction; MI, mild reaction; MO, moderate reaction; SE, severe reaction.

formation of an oxygen-inhibited layer was prevented. The sealers were prepared under aseptic conditions according to the following method.

In group ER/ACC (n = 8), the experimental design necessitated a slight modification of the manufacturer's recommendations. ER was injected through an automixing tip in a glass syringe measuring 25-mm long with a 5-mm internal diameter (De Luca SA, Buenos Aires, Argentina). Two size #40 gutta-percha cones dipped in ACC were subsequently inserted in the sealer and left for 3 seconds each after which they were removed. The sealer was then immediately injected into the silicone tubes through a 30-G needle. The procedure was repeated using a new syringe for each animal.

In group RS (n = 8), the sealer was extruded through an automixing tip attached to the two-barrel delivery syringe directly into a plastic

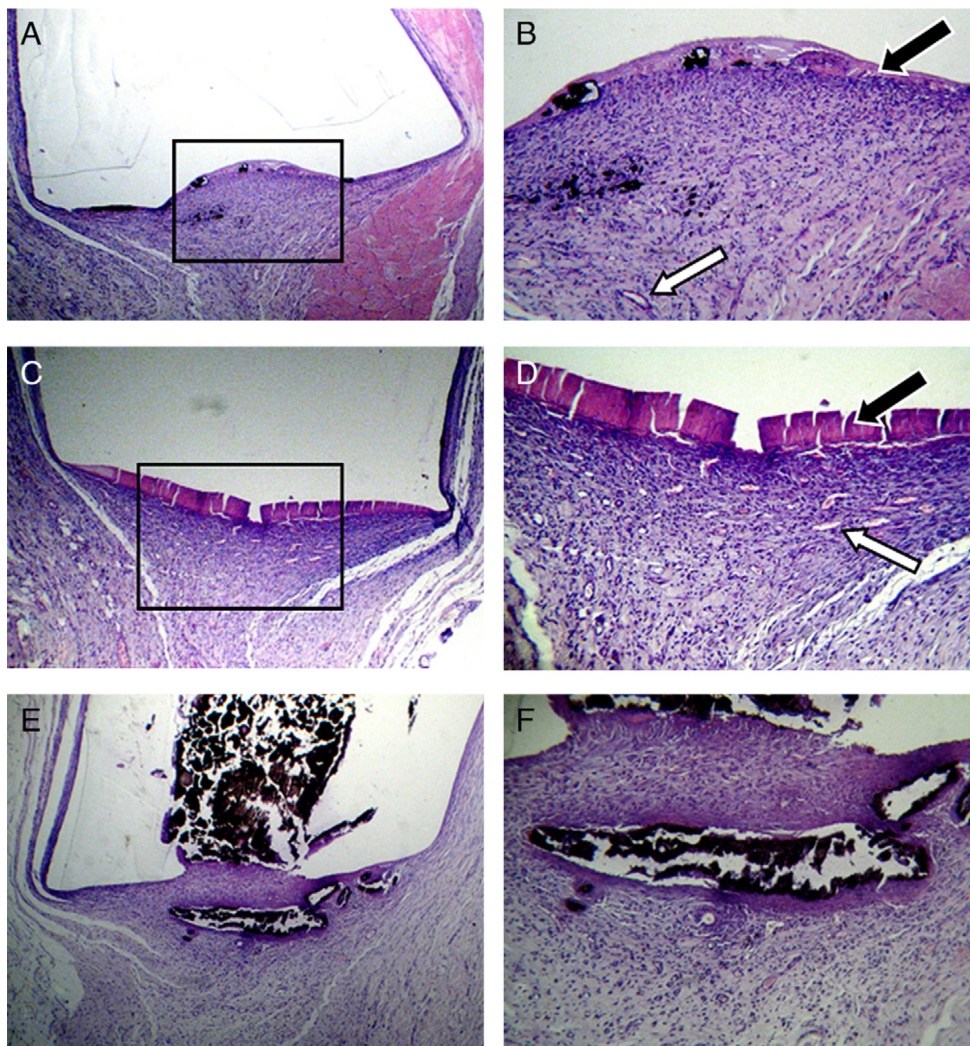

Figure 1. (*A-F*) Representative specimens of ER/ACC, RS, and PCS at the 10-day observation period. (*A*) ER/ACC: a low-power magnification of tissue/material contact (hematoxylin and eosin (H&E), original magnification ×40). (*A*) A higher magnification of the outlined area in *A*. A thin band of necrotic tissue in direct contact with the sealer (black arrow) and a severe granulomatous tissue reaction with dark material particles and newly formed capillaries (white arrow) can be seen (H&E, original magnification ×100). (*C*) RS: a low magnification of tissue/material contact (H&E, original magnification ×40). (*D*) A higher magnification of the outlined area in *C*. A thick layer of necrotic tissue in direct contact with the sealer (black arrow) is present. Below it, a severe granulomatous tissue reaction containing many newly formed capillaries (white arrow) can be seen (H&E, original magnification ×100). (*E*) PCS: low magnification of the area of tissue/material contact (H&E, original magnification ×40). (*F*) A higher magnification of the area of tissue/material contact in *E* showing extruded dark particles surrounded by a severe granulomatous tissue reaction (H&E, original magnification ×150). (This figure is available in color online at www.aae.org/joe/.)

syringe, which in turn delivered the mixture through a 30-G needle into the silicone tube.

In group PCS (n = 8), the sealer was prepared on a glass slab according to the manufacturer's recommendations. The mixture was loaded in a plastic syringe and injected into the silicone tube though a 30-G needle.

Excess material was removed from the open end of the tubes with a sterile plastic instrument. After preparation, the samples were immediately implanted in the subcutaneous connective tissues of white male Wistar rats weighing approximately 200 g each. The husbandry and management of the animals met the requirements of the ISO 10993–1 (1992) and ISO 10993-2 (1992) standards (15, 16) as well as the International Regulatory Requirements for the Care and Use of Laboratory Animals (17). All surgical procedures were performed under strict aseptic conditions. After anesthesia through the intraperitoneal administration of ketamine chloride (14 mg/kg body weight) and acepromazine (10mg/kg body weight), the dorsal skin was shaved and disinfected with 10% iodine-povidone solution (Phoenix SAIC, BA, Argentina). Four approximately 18-mm-long incisions were made through the dermis with a scalpel and further prepared by blunt dissection. Subsequently, one sample of each of the four groups was carefully placed into the pocket. A distance of at least 20 mm between the samples was present to avoid interference of tissue response between two materials. Finally, the wounds were closed with silk sutures, and the animals were maintained on a regular diet and water ad libitum. Eight animals were euthanized after 10, 30, and 90 days with an anesthetic overdose resulting in eight samples per group per time period.

The implants with surrounding tissues were dissected and fixed in 10% neutral buffered formalin (pH 7.4). After 48 hours of fixation, the samples were processed for routine histological evaluation. Paraffin blocks were oriented parallel to the long axis of the tubes and longitudinal serial sections of approximately 7-μm thick were cut from the middle of the implants and stained with hematoxylin and eosin. To evaluate the tissue response, three sections of the center of each specimen

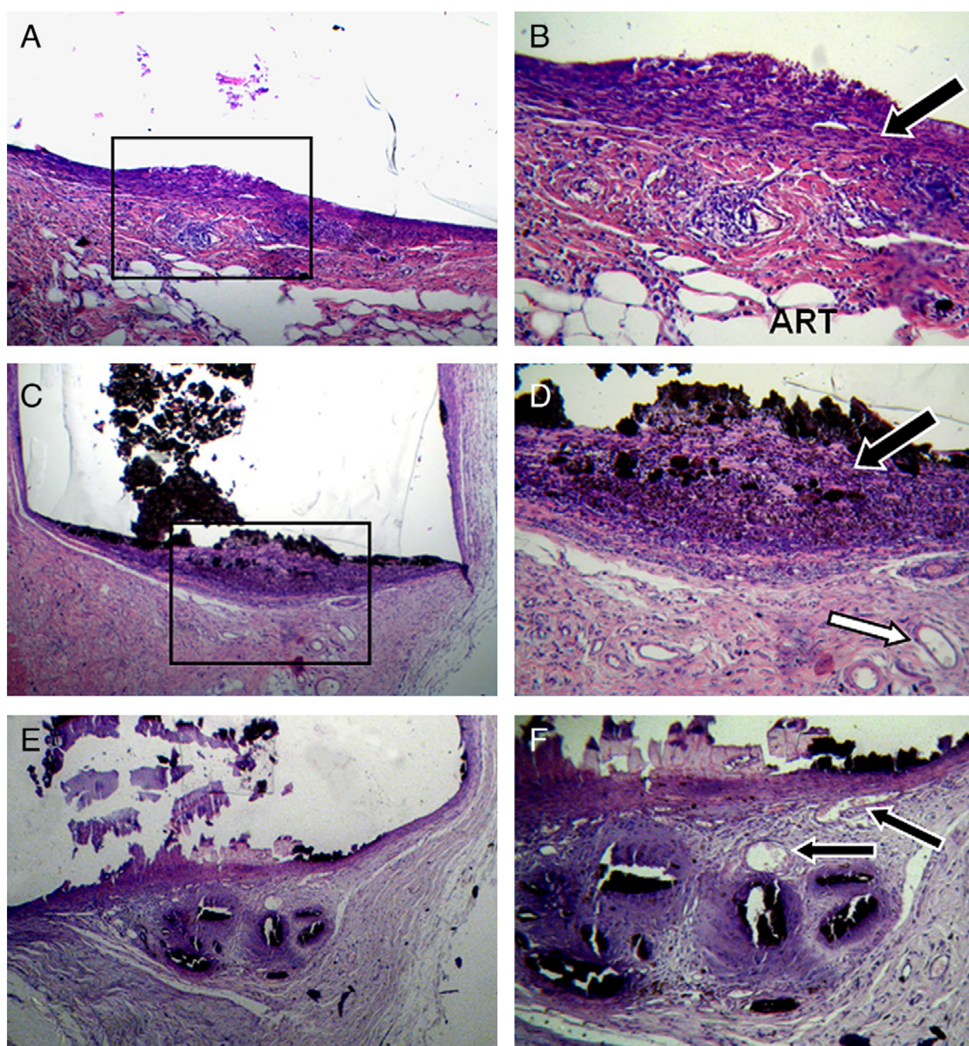

Figure 2. (*A-F*) A representative sample of ER/ACC, RS, and PCS of the 30-day observation period. (*A*) ER/ACC: a low magnification of tissue/material contact (H&E, original magnification ×80). (*B*) A higher magnification of the outlined area in *A*. In direct contact with the sealer, there is a dense fibrogranulomatous tissue concentration (black arrow). Below it is an artifact (ART) within an area of fat cells (H&E, original magnification ×150). (*C*) RS: a low magnification of tissue/material contact (H&E, original magnification ×40). (*D*) A higher magnification of the outlined area in *C*. A thick granulomatous zone containing many material particles can be seen in direct contact with the sealer (black arrow). Below it is a number of wide newly formed capillaries (white arrow) (H&E, original magnification ×150). (*E*) PCS: a low power of tissue/material contact (H&E, original magnification ×40). (*F*) A higher magnification of *E* showing material particles within a severe granulomatous tissue reaction. Note the presence of numerous wide newly formed capillaries (arrows) (H&E, original magnification ×150). (This figure is available in color online at www.aae.org/joe/.)

were analyzed and digitally photographed at different magnifications under a light microscope. All sections were analyzed independently, and two trained evaluators who were blind to the study scored the tissue reactions using the following criteria: NO: no reaction, fibrous-capsule formation, and absence of inflammatory cells; MI: a mild reaction and the presence of a fibrous-capsule formation with few inflammatory cells; MO: a moderate reaction and fibrous-capsule formation with the presence of polymorphonuclear leukocytes, lymphocytes, plasmocytes, and macrophages; and SE: a severe reaction and the presence of large accumulations of polymorphonuclear leukocytes, lymphocytes, plasmocytes, macrophages, foreign-body giant cells, and congested capillaries. Before the analysis, both evaluators were calibrated by having them analyze a set of 70 similar but unrelated slides displaying various types of inflammatory cells to endodontic sealers. In case of a disagreement between the evaluators, the sample under discussion was analyzed jointly until a consensus was reached. Data were analyzed by the Wilcoxon signed rank test to determine if there was a statistically significant difference between materials at each observation period. The total effect of time and material upon the tissue reaction was analyzed using the Kruskal-Wallis and the Dunn test. The significance level was set at $p < 0.05$. A sealer was considered to be biologically acceptable when tissue reactions were recorded as NO to MI.

Results

One animal from the 10-day time period had to be excluded from the study and was replaced with another undergoing the same implantation procedures. Macroscopic examination showed that wound healing

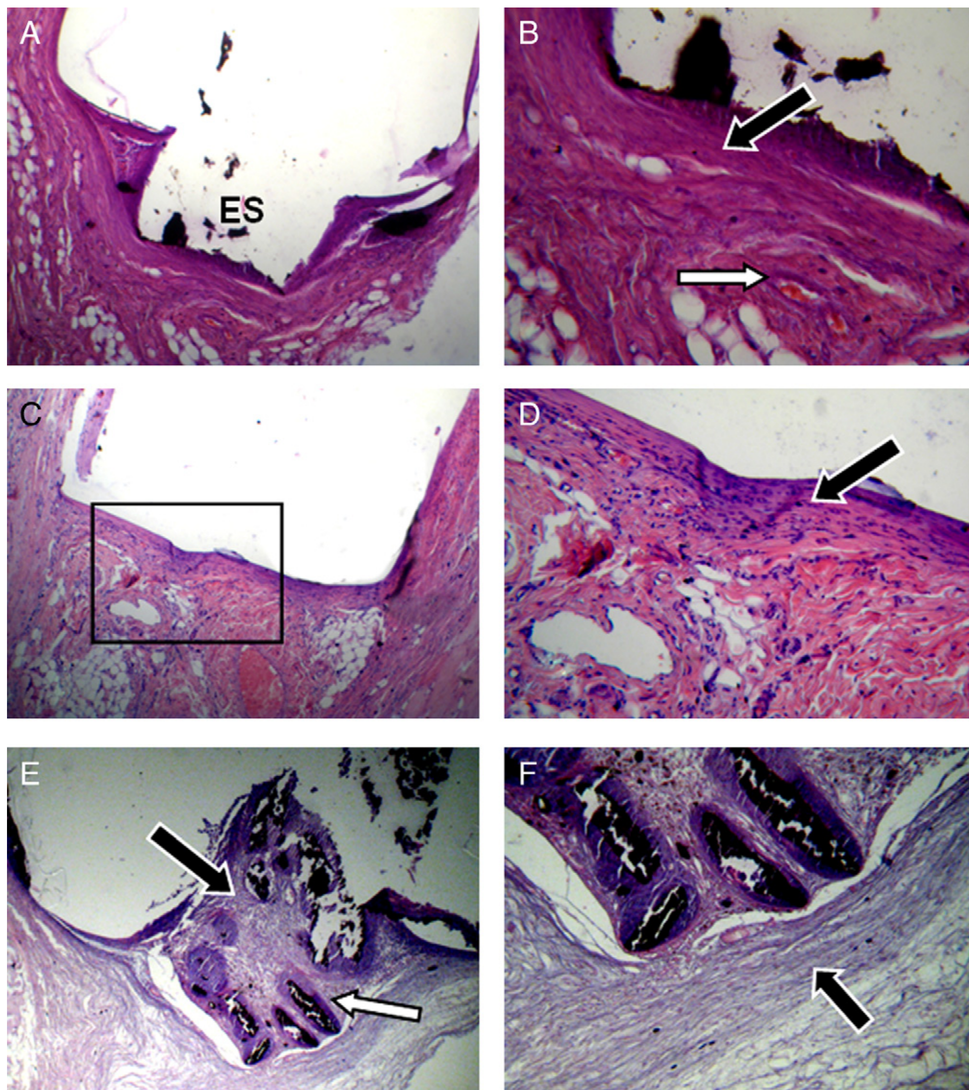

Figure 3. (*A-F*) A representative sample of ER/ACC, RS, and PCS at the 90-day observation period. (A) ER/ACC: an overview of the area of tissue/material contact showing extrusion (ES) of the sealer (H&E, original magnification ×40). (*B*) A higher magnification of *A*. In contact with the extruded material, a dense fibrous connective tissue encapsulation free of inflammatory cells is present (black arrow). The white arrow indicates a wide newly formed vessel (H&E, original magnification ×850). (*C*) RS: a low magnification of tissue/material contact (H&E, original magnification ×40). (*D*) A higher magnification of the outlined area in *C*. Note the presence of a thick fibrous connective tissue capsule (arrow). A few inflammatory cells can be seen within the capsule as well as in the surrounding tissues (H&E, original magnification ×850). (*E*) PCS: an overview of the area of tissue/material contact showing numerous material particles (white arrow) surrounded by granulomatous tissue slightly invaginated within the lumen of the tube (black arrow) (H&E, original magnification ×40). (*F*) A higher magnification of *E* showing a fibrous connective tissue (arrow) that appeared to isolate the inflammatory reaction from the surrounding tissues (H&E, original magnification ×850).(This figure is available in color online at www.aae.org/joe/.)

was satisfactory at all observation periods. Histological evaluation showed that the implants were surrounded by fibrous connective tissue of irregular thickness. It could be easily distinguished from the tissue reaction at the site where the tissues were in direct contact with the test material. The severity of tissue reaction is presented in Table 1.

After 10 days, the tissue reaction to ER/ACC, RS, and PCS was scored severe and was extensively dispersed around the end of the tubes (Figs. 1*A-F*). The majority of ER/ACC samples with direct tissue contact presented with an inflammatory reaction with slight invagination into the lumen of the tubes. Some ER/ACC and RS samples exhibited a thin necrotic zone in the direct contact area. Each group displayed many newly formed vessels and randomly dispersed dark particles, which appeared to have been released from the implanted materials. A mild to moderate reaction was observed in SIROD implants.

After 30 days, the intensity of the inflammatory reaction to ER/ACC and RS decreased slightly and was scored as moderate (Fig. 2*A-D*). In direct tissue contact, both materials displayed a fibrogranulomatous tissue, which appeared less dispersed and showed a tendency to be surrounded by a fibrous tissue containing many inflammatory cells. In contact with PCS, a persistent severe granulomatous tissue invagination containing many dark particles was observed. This granulomatous reaction was surrounded by an incipient layer of fibrous tissue, which was free of inflammatory cells (Fig. 2*E* and *F*). In contact with SIRODs, there was a thin fibrous connective tissue containing a few inflammatory cells.

After 90 days, some ER/ACC samples showed extrusion of the material into the surrounding tissues. In seven samples, a thick dense and mature fibrous tissue capsule (approximately 70- to 90-μm thick) free of inflammatory cells was present (Fig. 3*A* and *B*), whereas only one sample had a mild tissue reaction. The RS samples showed a dense fibrous capsule (50- to 80-μm thick) without inflammatory cells, whereas two cases were scored as mild with a few inflammatory cells still persisting within the capsule (Fig. 3*C* and *D*). PCS samples exhibited a severe persistent granulomatous tissue reaction containing necrotic areas as well as randomly distributed inflammatory cells. Particles of the material were observed at the end and within the lumen of the tubes in all samples. Higher magnifications showed the granulomatous tissues to be isolated by a thick fibrous tissue encapsulation. Many newly formed capillaries and a high concentration of acute and chronic inflammatory cells were also seen (Fig. 3*E* and *F*). A thick dense fibrous connective tissue, free of inflammatory cells, was observed in all SIROD samples (Fig. 4).

The Wilcoxon signed rank test showed no statistically significant differences ($p > 0.05$) between the reaction to ER/ACC, RS, and PCS at the 10-day observation period. After 30 days, no statistical differences were found between ER/ACC and RS ($p > 0.05$), whereas both ER/ACC and RS significantly differed from PCS ($p < 0.05$). For both periods, ER/ACC, RS, and PCS differed significantly from SIRODs. After 90 days, ER/ACC and RS differed significantly from PCS ($p < 0.05$) but not from SIRODs, whereas PCS remained significantly different from SIRODs. The effect of time for ER/ACC and RS showed that the 10 and 30 days differed significantly ($p < 0.05$) from the 90-day observation period. However, no significant differences ($p > 0.05$) were observed for PCS between all time intervals.

Discussion

The implantation of endodontic filling materials into subcutaneous connective tissue of rats is a valid screening method for testing biocompatibility (18, 19). PCS was used as the positive control because its toxicity has been previously determined by *in vitro* (20, 21) and *in vivo* experiments (22, 23). SIRODs were used as the negative control because they have been proven to be biocompatible (5, 6, 24, 25), a finding that was confirmed in this study. The initial inflammatory reaction of SIRODs may be a consequence of the surgical trauma. This subsided rapidly, and after 90 days a well-organized healthy dense fibrous tissue free of inflammatory cells was observed. The biocompatibility of ER has previously been tested (4–6); however, the incorporation of the ACC changes not only the original chemical composition of ER but also gives the sealer a shorter setting time; an evaluation of the toxicity is therefore essential to determine its safety.

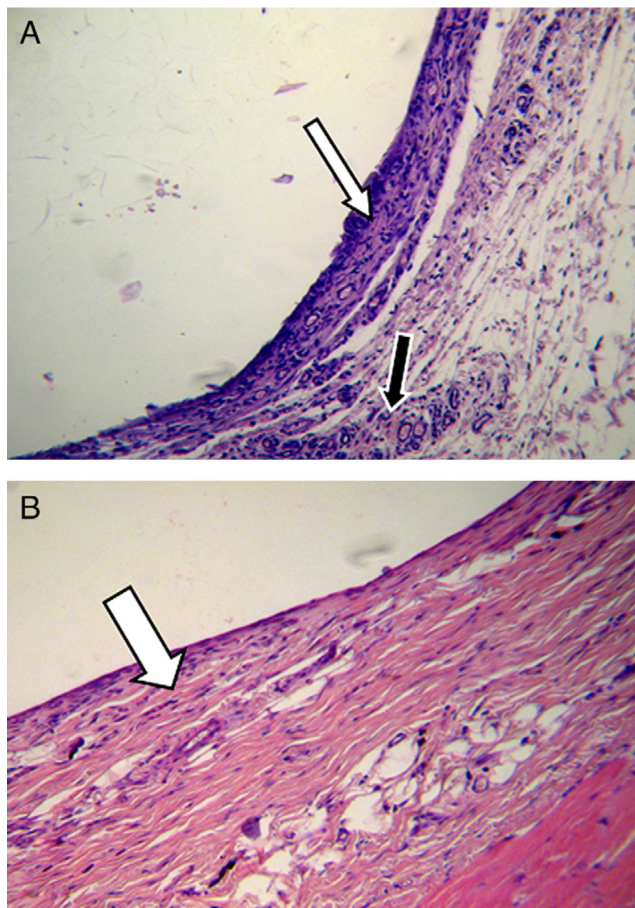

Figure 4. (*A*) A representative specimen of SIRODs after the 10-day observation period. In contact with the material (empty space), there is a moderate fibrogranulomatous tissue (black arrow) with inflammatory cells and newly formed capillaries (H&E, original magnification ×100). (*B*) A representative specimen of SIRODs after 90 days. In contact with the material (empty space), a thick dense fibrous connective encapsulation (arrow) without inflammatory cells can be seen (H&E, original magnification ×850).(This figure is available in color online at www.aae.org/joe/.)

The preparation of ER/ACC and RS samples was performed in such a way that no oxygen-inhibited layer was formed. Oxygen inhibits free-radical polymerization of resin-based materials yielding an uncured surface layer (26, 27), which is of particular concern for MBRS because it greatly affects the outcome in toxicity tests. Elution of uncured chemical components from the oxygen-inhibited layer is one of the main causes for tissue damage. However, severe reactions were observed for ER/ACC and RS at the short-term period, revealing that even after setting they still are an irritant. As has been shown (28), unreacted monomer persists in polymerized methacrylate resins. These unreacted monomers (28) undergo a rapid elution and the leaching of these unbound molecules (29), and other components caused the severe inflammatory reactions observed after 10 days. These findings are consistent with those of Costa et al (30, 31) and are supported by previous experiments of Ferracane and Condon (12)

who found that the loss of components from a methacrylate resin-based material in a water-based medium *in vitro* is initially rapid and, therefore, a similar behavior can be expected *in vivo*. It is significant to note that the initial severe reaction decreased over time and had been resolved at the end of the experiment although a few persistent inflammatory cells still remained adjacent to RS. After the rapid initial loss, the material depletes progressively slower over time (12), thus causing less irritation. Therefore, the setting time is significant with respect to irritation. ER/ACC sets in 7 minutes, RS in 25 minutes, and PCS in 260 minutes (Zmener and Pameijer, unpublished data, 2009). The faster setting of ER/ACC could be a contributory factor in reducing the release of components, in effect locking in potential irritants (12).

The tissue reaction to PCS was severe at all observation periods and is most likely caused by unreacted eugenol, which is highly toxic to tissues (32). Our findings for PCS are in agreement with others who reported that, like other zinc oxide and eugenol-based sealers (33–35), PCS reacted toxic in cell cultures (20, 21, 36) and in contact with living tissues (22, 37–39). However, the results for ER/ACC or RS disagree with those of Scarparo et al (39) who showed intense tissue reactions to ER. Their observations did not extend beyond 60 days, however. In the study reported here, a 90-day observation period was used. Longer time periods allow more time for elution of components causing a depletion of chemical components that are potential irritants. On the other hand, shortening of setting time may contribute to a lower output of uncured components from ER as shown here. Within the limitations of this study, it was concluded that ER/ACC and RS exhibited similarly and were both well tolerated by the subcutaneous connective tissue of rats after 90 days of implantation. PCS, however, remained toxic even after a 90-day observation period.

Acknowledgment

Dr. Pameijer is a consultant for Ultradent Products Inc.

References

1. Nguyen TN. Obturation of the root canal system. In: Cohen S, Burns RC. Pathways of the Pulp. 4th ed. St Louis: Mosby; 1987:183–94.
2. Taintor JF, Ross PN. Opinions and practices of American Endodontic Diplomates. Dent J 1978;44:321–5.
3. ADA Council of Scientific affairs. Statement on posterior resin-based composites. ADA Council on Dental Benefit Programs. J Am Dent Assoc 1998;129:1627–8.
4. Louw NP, Pameijer CH, Norval G. Histopathological evaluation of a root canal sealer in subhuman primates. J Dent Res 2001;80:654.
5. 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.
6. 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.
7. Sousa CJA, Montes CRM, Pascon EA, et al. Comparison of the intraosseous biocompatibility of AH Plus, EndoREZ and Epiphany root canal sealers. J Endod 2006;32:656–62.
8. Zmener O, Pameijer CH. Clinical and radiographic evaluation of a resin-based root canal sealer. Am J Dent 2004;17:19–22.
9. Zmener O, Pameijer CH. Clinical and radiographical evaluation of a resin-based root canal sealer: a 5-year follow-up. J Endod 2007;33:676–9.
10. Cotton TP, Schindler WG, Schwartz SA, et al. A retrospective study comparing clinical outcomes after obturation with Resilon/Epiphany or gutta-percha/Kerr sealer. J Endod 2008;34:789–97.
11. Zmener O, Pameijer CH, Alvarez Serrano S. Effect of immediate and delayed post space preparation on coronal bacterial microleakage in root canals obturated with a methacrylate-based sealer with an without accelerator. An ex vivo study. Am J Dent 2010;23:116–20.
12. Ferracane JL, Condon JR. Rate of elution of leachable components from composite. Dent Mat 1990;6:282–7.
13. Ferracane JL. Elution of leachable components from composites. J Oral Rehabil 1994;21:441–52.
14. Augsburger RA, Peters DD. Radiographic evaluation of extruded obturation materials. J Endod 1990;16:492–7.
15. ISO 10993-1:1992, Biological Evaluation of Medical Devices—Part 1: Guidance on Selection of Tests. Geneva: ISO; 1992.
16. ISO 10993-2:1992, Biological Evaluation of Medical Devices—Part 2: Animal Welfare Requirements. Geneva: ISO; 1992.
17. Bayne K. Developing guidelines of the care and use of animals. Ann N Y Acad Sci 1998;30:105–10.
18. Olsson B, Sliwkowsky A, Langeland K. Subcutaneous implantation for the biological evaluation of endodontic materials. J Endod 1981;7:355–69.
19. FDI. Féderation Dentaire Internationale. Recommended standard practices for the biological evaluation of dental materials. Int Dent J 1980;30:174–6.
20. Zmener O, Cabrini RL. Monocyte-lymphocyte adhesion to three zinc oxide and eugenol-based endodontic cements: an in vitro assay. Acta Odontol Latinoam 1986;3:27–32.
21. Pinna L, Brackett MG, Lockwood PE, et al. In vitro cytotoxicity evaluation of a self-adhesive methacrylate resin-based root canal sealer. J Endod 2008;34:1085–8.
22. Pascon EA, Leonardo MR, Safavi K, et al. Tissue reaction to endodontic materials: methods, criteria, assessment and observations. Oral Surg Oral Med Oral Pathol 1991;72:222–37.
23. Kolokouris I, Economides N, Beltes P, et al. In vivo comparison of the biocompatibility of two root canal sealers implanted into the subcutaneous connective tissue of rats. J Endod 1998;24:82–5.
24. Zmener O, Guglielmotti B, Cabrini RL. Biocompatibility of two calcium hydroxide-based endodontic sealers: a quantitative study in the subcutaneous connective tissue of the rat. J Endod 1988;14:229–35.
25. Zmener O, Guglielmotti B, Cabrini RL. Tissue response to an experimental calcium hydroxide-based endodontic sealer: a quantitative study in the subcutaneous connective tissue of the rat. Endod Dent Traumatol 1990;6:66–72.
26. Andrzejewska E, Linden LA, Rabek JF. The role of oxygen in camphorquinone-initial photopolymerization. Macromol Chem Phys 1998;199:441–9.
27. Versiani MA, Carvalho-Junior JR, Padilha MIAF, et al. A comparative study of physicochemical properties of AH Plus and Epiphany root canal sealants. Int Endod J 2006;39:464–71.
28. Rueggeberg FA, Margeson DH. The effect of oxygen inhibition on an unfilled/filled composite system. J Dent Res 1990;69:1652–8.
29. Rathbun MA, Craig RG, Hanks CT, et al. Cytotoxicity of a BIS-GMA dental composite before and after leaching in organic solvents. J Biomed Mater Res 1991;25:443–57.
30. Costa CAS, Hebling J, Teixeira MF. Preliminary study of the biological compatibility of the dentine adhesives All-bond 2 and Scotchbond MP. Histological evaluation of subcutaneous implants in rats. Rev Odontol USP 1997;11:11–8.
31. Costa CAS, Teixeira HM, Lopes Nascimento AB, et al. Biocompatibility of two current adhesive resins. J Endod 2000;26:512–6.
32. Molnar EJ. Residual eugenol from zinc-oxide-eugenol compounds. J Dent Res 1967;46:645–9.
33. Rodrigues H, Spängberg L, Langeland K. Biologic effects of dental materials. 9. Effect of zinc oxide-eugenol cements on He La cells in vitro. Estomat Cult 1975;9:191–4.
34. Zmener O, Cabrini RL. Effects of three calcium hydroxide-based materials on human blood monocytes and lymphocytes. Endod Dent Traumatol 1987;3:28–32.
35. Zmener O, Goldberg F, Cabrini RL. Effects of two gutta-percha formulations and one zinc oxide-eugenol and Canada balsam mixture on human blood monocytes and lymphocytes. Endod Dent Traumatol 1989;5:73–7.
36. Geurtsen W, Leyhausen G. Biological aspects of root canal filling materials—histocompatibility, cytotoxicity and mutagenicity. Clin Oral Invest 1997;1:5–11.
37. Tagger M, Tagger E. Subcutaneous reactions to implantation of tubes with AH26 and Grosman's sealers. Oral Surg Oral Med Oral Pathol 1986;62:434–40.
38. Yesilsoy C, Koren LZ, Morse DR, et al. A comparative tissue toxicity evaluation of established and newer root canal sealers. Oral Surg Oral Med Oral Pathol 1988;65:459–67.
39. Scarparo RK, Grecca FS, Fachin EVF. Analysis of tissue reactions to methacrylate resin-based, epoxy resin-based, and zinc oxide-eugenol endodontic sealers. J Endod 2009;35:229–32.