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

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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

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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

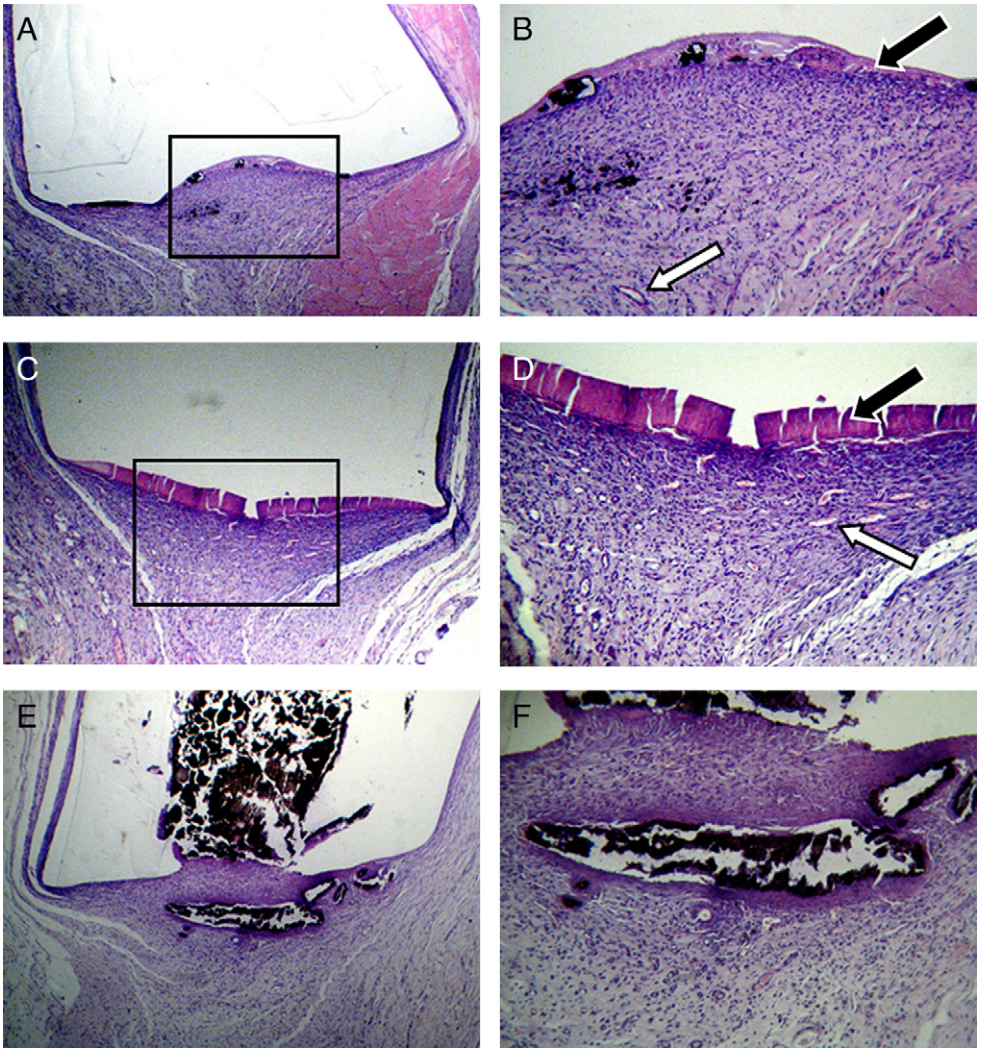

Figure 1. (*A-F*) Representative specimens of ER/ACC, RS, and PCS at the 10-day observation period. (*A*) ER/ACC: a low-power magnification of tissue/material contact (hematoxylin and eosin (H&E), original magnification ×40). (*A*) A higher magnification of the outlined area in *A*. A thin band of necrotic 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/.)