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## Susceptibility of methacrylate-based root canal filling to degradation by bacteria found in endodontic infections

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**Objectives:** To present a case of endodontic failure obturated with a methacrylate-based root filling material, Resilon/RealSeal (RS). To determine if RS is susceptible to biodegradation by endodontically relevant microbes by a method known to show RS degradation. **Method and Materials:** Emulsions of RS were dispersed in agar with minimal bacterial nutrients in culture plates. Lipase PS served as a positive control. *Pseudomonas aeruginosa*, *Fusobacterium nucleatum*, *Prevotella intermedia*, *Porphyromonas gingivalis*, *Porphyromonas asaccharolytica*, *Enterococcus faecalis*, *Streptococcus sanguis*, *Streptococcus mutans*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*

were tested for their ability to biodegrade RS. The bacteria were inoculated in the plates and examined daily for RS degradation for 7 days. **Results:** Degradation of the emulsified RS manifested in the formation of clear zones around *P aeruginosa*, *P intermedia*, *P asaccharolytica*, *S aureus*, and *S epidermidis*. No degradation was seen with the other tested bacteria or in plates that did not contain RS emulsion. **Conclusion:** Endodontic pathogenic bacteria can degrade RS. These findings complement other work and suggest that the seal and integrity of root canal fillings obturated with RS may be impaired by a microbial insult. (doi: 10.3290/j.qi.a32235)

**Key words:** clear zone, degradation, endodontic bacteria, Resilon, root canal treatment failure

Resilon/RealSeal (RS) is a methacrylate-based resin root canal filling material. It is used with a methacrylate-based sealer and a self-etching primer in an attempt to

create a bond between the sealer, the core material, and the canal dentin walls, resulting in a “monoblock”.<sup>1</sup> The monoblock has been claimed to improve the leakage resistance of the obturated root.<sup>2</sup> However, the clinical outcome of teeth obturated with RS was statistically indistinguishable from teeth obturated with conventional materials.<sup>3</sup> A review of the literature<sup>4</sup> on RS did not find it to be an evidence-based alternative to the traditional gutta-percha (GP)-based filling materials.

Retreatments of teeth obturated with RS are now being performed, and clinicians have become aware of blackening, softening, and degradation of the removed RS obturating material (Fig 1; unpublished results). The

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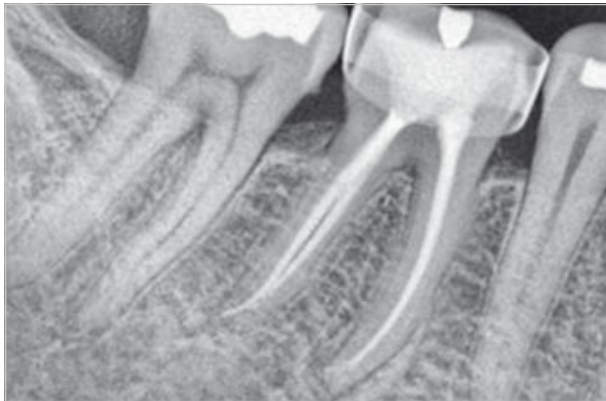
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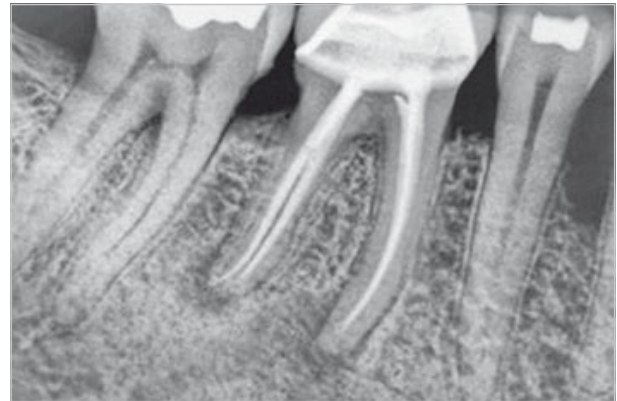
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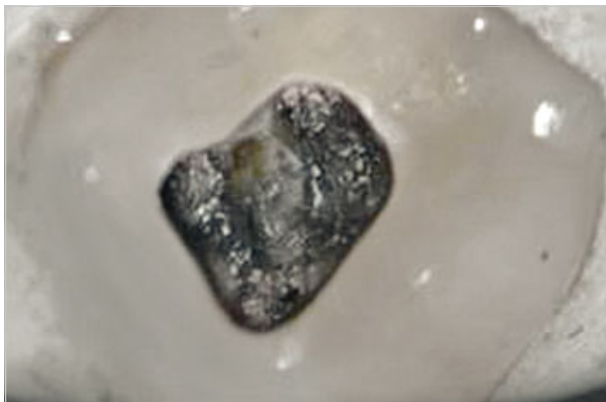
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**Fig 1a** Initial diagnosis of the right mandibular first molar was irreversible pulpitis. An orthodontic band was placed on the tooth after the root canal treatment until a porcelain-fused-to-metal crown was made.



**Fig 1b** The same tooth 3 years after initial treatment. The crown was intact and firmly in place. A diagnosis of apical periodontitis (AP) was made.



**Fig 1c** Blackened, soft, degraded RS was removed from the tooth chamber and from the canals during retreatment.



**Fig 1d** Resolution of AP in the right mandibular first molar 2 years after retreatment.

manufacturer, SybronEndo, has independently issued a communication addressing the blackening of RS. It states that the black substance results from microleakage. Proteins in the seeping fluid react with bismuth oxychloride, a radiopacifying agent. The bismuth sulfide formed is the black substance seen.<sup>5</sup>

RS contains polycaprolactone (PCL), which serves as a thermoplastic organic matrix for RS inorganic fillers. Hydrolytic bacterial enzymes can degrade RS in a similar fashion to PCL degradation by indicating that the PCL component in RS is degradable,<sup>6,7</sup> and although RS may contain other polymer/fillers within its PCL matrix, these do not prevent RS biotic degradation.<sup>6-8</sup> Nonspecific microbes from a dental sludge can blacken and degrade RS<sup>9</sup> and it was suggested that endodontically relevant bacteria may also have that ability.<sup>7</sup> Propo-

nents of RS have questioned the relevance of RS degradation studies to endodontics.<sup>10</sup>

The purpose of this work was to present a clinical case of a root canal treated tooth obturated with RS that failed. The softening and blackening of RS described in this case was frequently encountered during retreatment of teeth that were obturated with RS. Although the microbial degradation of RS has been documented,<sup>9</sup> we wished to examine if endodontic microbes known to be in the infected root canal system can degrade RS. The clinical relevance was that if endodontic microbes can degrade RS then it could be that the softening and blackening of RS seen in retreatments is of microbial origin and that root canals obturated with RS are more susceptible to degradation by residual bacteria or by secondary bacterial colonization.



## CASE REPORT

A 45-year-old patient was diagnosed with irreversible pulpitis in the mandibular right first molar (tooth 30 according to FDI notation) (Fig 1a). The tooth had a dental history of a large occlusal amalgam with clinical evidence of a fracture of the distal marginal ridge. After rubber dam isolation, four canals were located. Cleaning and shaping was done using NiTi EndoSequence rotary files sizes 15 to 40, .04 taper (Brasseler). Irrigation was performed with 5.25% NaOCl and 15% ethylenediaminetetraacetic acid (EDTA), which was also used for the final rinse. After drying all four canals, obturation was completed using RS and Epiphany SE (SybronEndo) via warm vertical condensation according to the manufacturer's instructions (Fig 1a). After completion of root canal therapy, an orthodontic band was placed to prevent further crack propagation until the tooth was finally restored. The tooth was restored with a porcelain-fused-to-metal crown (PFM) within 3 months of completion of root canal treatment (RCT).

Three years after the initial RCT was completed, the patient presented for retreatment of the mandibular right first molar, which was diagnosed with symptomatic apical periodontitis (AP) (Fig 1b). Retreatment was done through the existing PFM crown. RS was removed with Hedstrom hand files and Brasseler EndoSequence NiTi rotary files size 30 to 45, .04 taper. The infected RS was discolored (blackened) (Fig 1c) and soft. It was easily removed and no special solvents were used. Copious irrigation with 5.25% NaOCl and 15% EDTA was used throughout the treatment. Calcium hydroxide (UltraCal, Ultradent Products) was placed for 14 days as an inter-appointment medicament. Canals were obturated with GP and Roth Sealer (Roth International) via warm vertical condensation. Access closure was completed by placing a 4-mm layer of amalgam on the pulpal floor. The porcelain part of the PFM crown was then etched with hydrofluoric acid 9% (UltraDent Products). Silane (UltraDent Products) was applied to the etched porcelain, followed by the application of Clearfil SE (Kuraray) to the access walls. EndoSequence Core BuildUp Material (Brasseler) was used to finish the access closure.

The 2-year follow-up radiograph showed resolution of the periapical lesion (Fig 1d). The tooth was asymptomatic, and the restoration was intact and firmly in place.

The softening and blackening of RS seen in this case was frequently encountered during retreatment of teeth that were obturated with RS, and was rarely seen in retreatment of teeth in which GP served as the core material. These findings led us to investigate if bacteria found in the infected root canal treated tooth can degrade RS.

## METHOD AND MATERIALS

### Bacterial strains and media

*Prevotella intermedia* (ATCC 700610), *Pseudomonas aeruginosa* PA14 (a strain known to over-express lipase),<sup>11</sup> *Porphyromonas asaccharolytica* (ATCC 25260), *Staphylococcus aureus* (ATCC 6538), *Staphylococcus epidermidis* (ATCC 14990), *Enterococcus faecalis* (ATCC 29212), *Fusobacterium nucleatum* (ATCC 10953), *Streptococcus mutans* (ATCC 700610, UA159), *Streptococcus sanguis* (ATCC 10556), and *Porphyromonas gingivalis* (ATCC 33277) were used in the present study. The strains were stored at  $-80^{\circ}\text{C}$  in tryptic soy broth (TSB; Acumedia), with 20% glycerol, before use. *S mutans* and *S sanguis* were grown on mitis-salivarius sucrose bacitracin (MSSB; Anaerobe Systems) agar plates initially for culture of these bacteria in 5%  $\text{CO}_2$  at  $37^{\circ}\text{C}$ , while *P intermedia*, *P gingivalis*, *P asaccharolytica*, and *F nucleatum* were typically cultured for 48 hours on blood agar plates (Fisher Scientific) at  $37^{\circ}\text{C}$  in an anaerobic GasPak jar to late logarithmic or early stationary phase. *P aeruginosa* PA14, *S aureus*, *S epidermidis*, and *E faecalis* were aerobically grown on blood agar plates for 48 hours.

### Preparation of RS/GP stock emulsions

RS stock emulsions were prepared as described previously.<sup>8</sup> Briefly, 0.5 g of RS pellet (SybronEndo) was dissolved in 12.5 ml of chloroform for 2 hours at room temperature, and shaken occasionally. The mixture was centrifuged for 6 minutes at 3,000 rpm. The supernatant containing the dissolved PCL was decanted and



added to a 0.1% solution of water, and a phosphoric ester type anionic surfactant (0.05 g of Plysurf A-210G; Dai-ichi Kogyo Seiyaku) was added to make a total of 50 ml of emulsion. Sonication for 5 minutes with a Branson Sonifier 450 resulted in the formation of nano-droplets of hydrophobic polymers that were eventually stabilized in water by the surfactant molecules. This was left overnight on a stirring plate at 40°C to evaporate the chloroform. This resulted in a RS stock emulsion with an initial polymer concentration of 1 g/L. A GP emulsion was prepared in the same manner, whereby 0.5 g of GP pellet (Obtura Spartan) was used instead of the RS pellet.

### Agar plate preparation

TSB (2 g) and 1.5 g of Agar, Bacteriological (Acumedia) were dissolved in 90 ml of deionized distilled water and mixed with 10 mL of the 1% stock emulsion of RS to result in a 0.1% RS-Agar solution. The final mixture was autoclaved and 10 mL of the sterilized RS-Agar emulsion was poured into 90-mm diameter sterile Petri dishes to a depth of 5 mm and allowed to solidify. This resulted in homogeneously turbid RS-Agar plates. Negative control plates and GP plates were prepared in the same manner without adding the RS emulsion and using 0.5 g of GP pellets instead of the RS pellets, respectively.

### Agar well diffusion (AWD) assay

Lipase PS (*Burkholderia cepacia*, Amano Enzyme), a true lipase (EC 3.1.1.3), was previously shown<sup>6,8</sup> to degrade RS emulsions and was used as described. Lipase PS (0.06 g; 1,800 U) was dissolved in 2 mL of 0.1 mol/L phosphate-buffered saline (pH 7.0). Lipase PS concentrations of 30 U/ml and 300 U/ml were tested in the agar plates. Wells were punched in the agar with a Fischer cork borer and filled with 20 µl of enzyme solutions. The plates were left at room temperature for 24 hours to allow hydrolysis of the dispersed RS within the agar. The negative control consisted of 20 µl sterile saline used in lieu of lipase PS in the wells. Subsequent enzymatic hydrolysis of the emulsified RS was manifested by the formation of clear zones circumferentially

around the cylindrical wells. For assessing bacterial hydrolysis approximately equal volumes of the tested bacterial colonies growing in stationary phase were placed on the prepared agar plates. A 3-mm microbiologic loop was used to collect and place the bacteria on the agar plates. The plates were then grown at 37°C anaerobically/aerobically as required. The bacteria on the plates were followed for 1 week and checked daily for the presence of clear zones. Experiments were repeated 8 times for a suitable confidence interval.

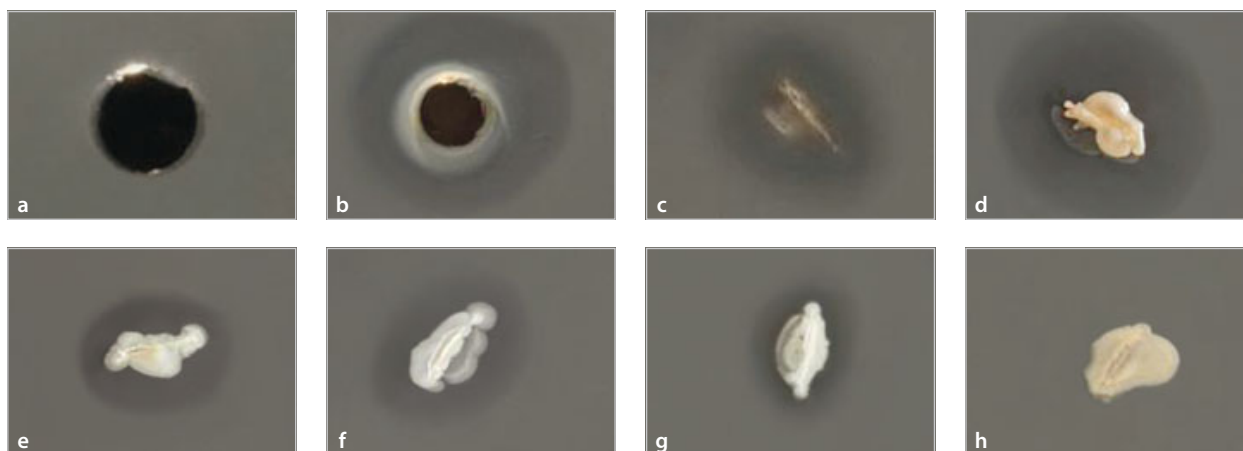
## RESULTS

Hydrolytic clear zones were observed on the RS-containing TSB plates when they were exposed to lipase PS, and no clear zones were seen when using TSB agar plates that did not contain RS. All bacteria grew on their respective RS and GP agar plates. Clear zones on the RS-agar plates were demonstrated with *P intermedia*, *P aeruginosa*, *P asaccharolytica*, *S epidermidis*, and *S aureus* 100% of the time at each of the eight inoculation sites (95% confidence interval [CI], 63% to 100%) (Fig 2). Clear zones were not seen (0%) with *E faecalis*, *F nucleatum*, *P gingivalis*, *S mutans*, or *S sanguis* at any inoculation site (95% CI, 0% to 37%). No clear zones were observed with any the bacteria grown on TSB agar plates that did not contain RS emulsion. No clear zones were demonstrated when GP replaced the RS and was exposed either to lipase (positive control) or to *P aeruginosa* PA14GA. The appearance of the clear zones and the width of the clear zone created varied greatly (1 to 5 mm), both between the species and within the same species.

## DISCUSSION

*P intermedia*, *P aeruginosa*, *P asaccharolytica*, *S epidermidis*, and *S aureus* were able to hydrolyze RS emulsion, as was evident by the clear zones observed around the bacterial colonies (Fig 2). No such clearing was demonstrated around the bacterial colonies in identical plates not containing the RS emulsion.





**Figs 2a to 2h** Biodegradation of RS by endodontic bacteria: hydrolysis and the formation of clear zones in TSB agar plates infused with RS emulsion. Growth was observed for up to 1 week and clear zones were usually seen within 48 to 71 hours. When halos were seen they occurred 100% of the time at each of the eight inoculation sites for each bacteria (95% CI, 63% to 100%). Likewise when not seen, they did not occur at any of the sites (95% CI, 0% to 37%). (a) Negative control. (b) Positive control: enzymatic degradation-hydrolysis with clear zone formation after exposure to 30 µg/mL lipase PS. (c) Positive control: biodegradation by *P aeruginosa*, known to secrete lipase. (d to g) Biodegradation by equal volumes of *P intermedia*, *P asachharolytica*, *S aureus*, and *S epidermis*, respectively. (h) No clear zone was seen with *E faecalis*. Clear zones were also not seen with *P gingivalis*, *F nucleatum*, *S sanguis*, and *S mutans*.

Bacterial enzymes can hydrolyze both untreated RS<sup>7</sup> and RS emulsions.<sup>6,8</sup> Thus hydrolysis of RS emulsion would also indicate susceptibility of untreated RS. The AWD assay<sup>8</sup> was modified to check for direct bacterial degradation by specific endodontic bacteria found in the root canal. This modification did not affect its ability to detect hydrolysis of the incorporated RS emulsion by lipase PS or by *P aeruginosa* (positive controls).

The present qualitative findings indicate that RS is biodegradable by specific bacteria seen in endodontic infections. It could be that had there been a more sensitive test than the AWD assay to detect RS enzymatic hydrolysis more bacteria would have shown that ability.

In a pilot experiment, RS pellets were incubated in vials containing *P intermedia*, *E faecalis*, or *P aeruginosa*. Blackening of the RS pellet was noted in all the vials, similar to what was seen clinically in retreatments of RS obturated teeth with AP.

The retreatment shown (Fig 1) was typical of many of the RS retreatments that were done. The periapical lesion (Fig 1) that developed indicates that microleakage had occurred, and bacteria had gained access to the treated root canal system. Degradation/softening and blackening of the removed RS were seen. It could be that these are the result of endodontic microbial

activity and not just seeping of proteins as had been suggested.<sup>5</sup> The resolution of the periapical lesion was the result of the disinfection that removed or at least reduced the microbial insult. GP was chosen as the obturating material due to the problems the treating clinician encountered when obturating with RS.

The manufacturer recommends that during obturation the RS core material be coated with sealer. The biodegradation of the sealer was not evaluated. RS sealer was previously shown to have no antibacterial properties and even encouraged bacterial growth.<sup>12</sup> The core material is a dominant component in the obturated canal, and it is likely that during obturation there will be areas of RS not coated with sealer in contact with the canal walls that may contain residual bacteria, thus emphasizing the importance of the RS biodegradation.

Bacteria may persist even after instrumentation and irrigation with sodium hypochlorite.<sup>13,14</sup> Obturation with RS or GP does not entomb bacteria or prevent regrowth,<sup>15</sup> and residual bacteria in root canal treated teeth have been shown to contribute to the formation of AP.<sup>16</sup> Resin-dentin bonds are not as durable as was previously thought,<sup>17</sup> and degradation of resin-based materials such as RS and RS sealer could also impair the entombment of bacteria.



The AWD assay was not suitable for checking for bacterial degradation of GP. No clear zone was seen when GP was substituted for RS or when GP was subjected to lipase PS or *P aeruginosa* (positive controls). GP rarely undergoes bacterial degradation.<sup>18</sup> However, it does chemically decompose over time and can support biofilm growth.<sup>19-21</sup>

The present observations support other studies<sup>6-9,22</sup> that question the suitability of RS as a root canal filling material as the environment it creates may be biodegradable and as such may support residual bacterial growth or colonizing bacteria.

Materials are introduced into the dental market with an emphasis on their positive qualities and the promise they hold to improve the quality of our treatment. RCT failures with RS were not seen immediately and not in all cases. Nonetheless, it would not be wise to disregard the failures seen and the in-vitro work performed, or to wait until studies with a high level of evidence, investigating the nature of RCT failures of teeth obturated with RS, become available.

## CONCLUSION

RS, a methacrylate-based root canal filling, is susceptible to degradation by bacteria found in the infected root canal system. This may impair the seal and integrity of root canal fillings sealed with RS over time.

## ACKNOWLEDGMENT

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