Antibacterial temporary restorative materials incorporating polyethyleneimine nanoparticles

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Objectives: Temporary restorative materials (TRMs) often rapidly lose their dimensional stability and antibacterial properties after exposure to humidity and bacterial infection. Quaternary ammonium polyethyleneimine (QPEI) nanoparticles (NP) are long-lasting, stable, biocompatible, and nonvolatile antibacterial polymers. In the present study, we incorporated QPEI NP into standard TRMs and examined their influence on dimensional stability and their ability to reduce bacterial leakage. **Method and Materials:** A modified split-chamber model was used in vitro to test calcium sulfate-based and zinc oxide–eugenol-based TRMs (Coltosol and IRM, respectively). Both materials were tested with and without 2% wt/wt incorporated QPEI NP for fluid and bacterial leakage. **Results:** The calcium sulfate-based TRM displayed the lowest microleakage and highest antibacterial resistance. Two-way ANOVA analysis of the fluid transport test results showed that incorporation of 2% wt/wt QPEI NP significantly increased the sealing ability of both TRMs ($P < .01$). Analysis of survival curves by the Kaplan-Meier method showed that the calcium sulfate-based TRM with 2% wt/wt QPEI NP survived the bacterial load significantly more effectively than did the zinc oxide–eugenol-based TRM ($P < .0001$). **Conclusion:** Incorporation of 2% w/w QPEI NP may prominently improve the sealability and the antibacterial properties of TRMs. TRMs incorporating antibacterial nanoparticles may be clinically advantageous for sealing the endodontic access cavity to avoid reinfection of the root canal system during endodontic treatment. *(Quintessence Int 2013;44:209–216; doi: 10.3290/j.qi.a29056)*

Key words: nanoparticles, polyethyleneimine, temporary restorative materials

The coronal seal has a crucial impact on the prevention of reinfection during endodontic sessions and following endodontic treatment.[1] Temporary restorative materials (TRMs) are used to prevent coronal leakage during the short intervals between and following endodontic sessions.[2] However, the average service time of TRMs is often to a great extent longer than recommended.[3] As a result, recently filled root canals are prone to rapid recontamination that occurs due to structural changes in the materials, such as shrinkage, cracking dissolution, and disintegration.[4,5] In addition, TRMs have a weak short-range antibacterial effect that fades within several days.[6]

The most frequently used TRMs are calcium sulfate-based and zinc oxide–eugenol-based materials.[3] For example, Coltosol (Coltene Whaledent), a hydrophilic calcium sulfate-based material, tightly adheres to the cavity walls; however, bacteria may penetrate through the calcium sulfate body, thereby resulting in recontamination.[4] Another TRM is IRM, a hydrophobic material that contracts after thermal changes,[5] resulting in microgaps between the material and dentin walls that may cause leakage. No antibacterial properties have been reported for Coltosol. However, IRM showed short-term antibacterial activity,[6] which was probably due to the eugenol content. This effect diminishes with time, depending on

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the release of the eugenol. Since temporary restorations are expected to seal the endodontic access cavity to avoid reinfection of the root canal system during the endodontic treatment, TRMs should prevent contamination of the root canal by fluids, organic materials, and oral bacteria from the oral cavity. Moreover, Ray and Trope[7] showed that the quality of coronal restoration is equally important to that of the endodontic treatment. However, Madison and Wilcox[8] demonstrated in vivo that temporary fillings failed to seal coronally the root canal system of endodontically treated teeth within 10 days of service. Regrettably, the short-term antibacterial effect of TRMs fades within days.[6] It seems from the above, that within a short period of time TRMs fail to adequately seal the endodontic access cavity, making the recently filled root canals susceptible to recontamination. Thus, TRMs that possess good sealing ability and possess antibacterial properties may be advantageous in preventing recontamination.

Quaternary ammonium polyethyleneimine (QPEI) nanoparticles (NP) are cross-linked cationic macromolecules that are nanometric, insoluble, nonvolatile, chemically stable, positively charged, and hydrophobic.[9–16] QPEI NP kill bacteria upon contact without releasing antibacterial agents from the material bulk.[17] Recent studies have shown that QPEI NP impart antibacterial activity to restorative composite resins,[18–21] ie QPEI NP resulted in total growth inhibition of *Streptococcus mutans* when incorporated in restorative composite resins. Moreover, the antibacterial effect was retained for at least 3 months.[22] Furthermore, NP incorporated at low concentrations in provisional cement exhibited an antibacterial effect against *S mutans* and *Enterococcus faecalis* for a period of 14 days in another group of dental materials.[23]

In the present study, we investigated in vitro the dimensional changes and antibacterial properties of two TRMs with and without QPEI NP. The study tested the null hypothesis that incorporation of QPEI NP into TRMs enhances their antibacterial properties without compromising their sealability.

METHOD AND MATERIALS

Preparation of test samples

QPEI NP were synthesized as previously described.[19] Two commercially available TRMs were homogeneously mixed with 2% wt/wt synthesized polymers: Group A was IRM (Dentsply) (N = 10) and Group B was

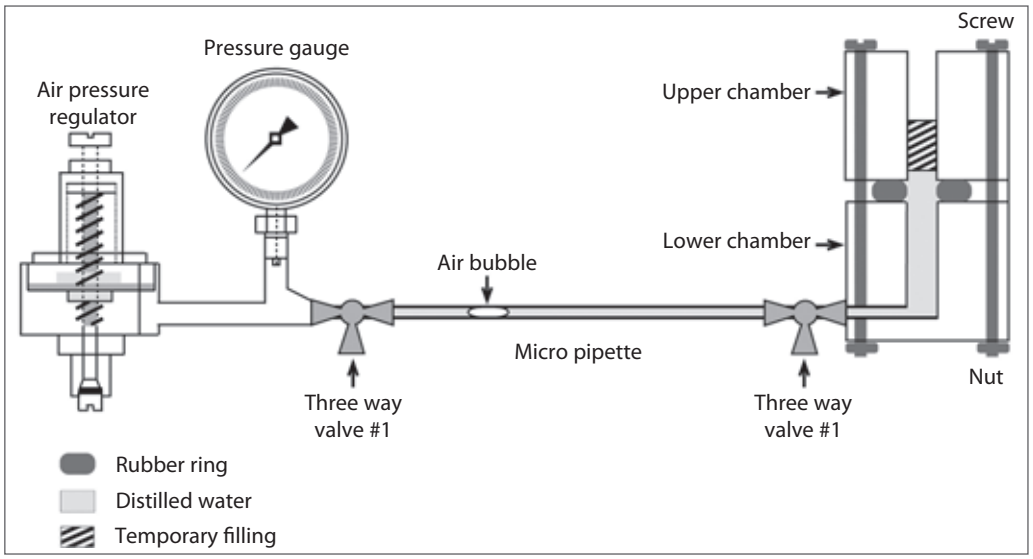

Fig 1 The fluid filtration configuration of the split-chamber device.

Coltosol (Coltene Whaledent) (N = 10). Base materials without QPEI NP were used as the control groups: Group C was IRM (N = 10) and Group D was Coltosol (N = 10).

Experimental model

A split-chamber device was used in this study to measure the fluid volume filtrated by the TRMs (Fig 1) and thereby allow bacterial passage (Fig 2). A total of 40 cylindrical cavities (4 mm diameter × 20 mm height) were prepared in eight 20-mm thick plexiglass plates. Each cavity served as an upper chamber (UC) for filling packed to a 4 mm height against a sterile aluminum foil coated surface. Lower cylindrical chambers (LC) that were 5 mm shorter than the UC were prepared in similar size plexiglass plates and in accordance with the UC position. Each LC ended in a perpendicular 2-mm hole at the base. This hole connected the LC to the measuring or sampling device, which will be further described. The LC and UC were separated from each other by a 1-mm rubber O-ring and tightened against each other with four screws and nuts. Each UC/LC unit was connected to a fluid filtration device, tested for fluid transport, disconnected, sterilized, and subjected to a bacterial leakage test. The TRMs were allowed to set at 37°C and 100% humidity for 24 h and were subjected to 500 temperature cycles (5 ± 2 to 55 ± 2 °C, dwell time 30 seconds) (PRC thermocycler, ADAHF [American Dental Association Health Foundation]).

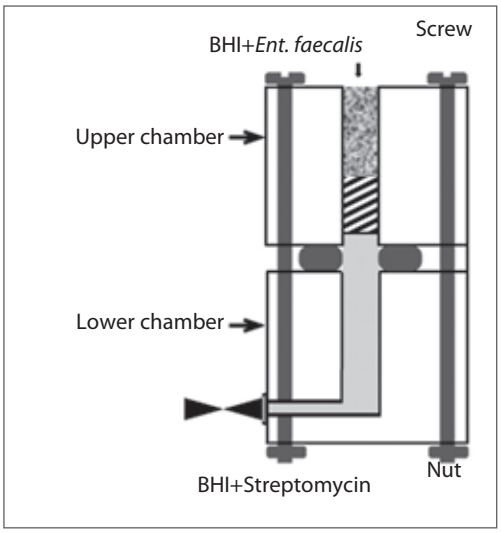

Fig 2 The bacterial leakage configuration of the split-chamber device.

Fluid transport test

A fluid transport device was used as previously described (Fig 1).[24] In brief, a 0.2-µl gradient micropipette (Fotuna Opticolor) was connected in each free edge to a three-way valve (Biometrics, Jerusalem). The first valve was connected to a pressure gauge that was connected to a pressurized air source, and the second valve was connected to the 2-mm hole in the LC of the split device. Prior to system activation, the tightness of the device was tested under positive pressure (2 bars) in a water bath to ascertain sealing ability. Next, the first valve was closed, the entrapped air was vacuumed and the micropipettes and LCs were filled with distilled water. A needle attached to a 1-ml syringe was inserted into each valve. The first needle was used to insert an air bubble inside the micropipette, while the second was used to expel extra water from the micropipette. Additional distilled water was added into the micropipette through the first valve to form an intercalated air bubble. Following bubble formation, all syringes were removed. For assembly activation, positive air pressure (0.2 bars) was applied to push the water toward the temporary filling. The water movement was recorded by registering air bubble movement in the micropipette. Fluid transport was measured using the micropipette scale at 10 min, 30 min, 1 h and 6 h.

System re-checks. Following the leakage test, each assembly was tested again under positive air pressure (2 bars) in a water bath to ascertain that no breach in system sealability had occurred during the experiment.

Bacterial leakage test

A bacterial suspension of clinically isolated *E faecalis* was used in this study. The bacteria were cultured overnight in 5 ml of brain-heart infusion (BHI) broth (Difco) at 37°C. Each bacterial suspension was adjusted to an optical density of 0.2 at

650 nm (2.5×10^9/ml). To minimize contamination, the BHI broth was supplemented with 0.5 mg/ml of streptomycin (Sigma-Aldrich).

Set-up design (Fig 2). Following the fluid transport test, the upper plates were separated from the lower ones and were gas sterilized (ethylene oxide) overnight. A sterile rubber O-ring was fixed to the LC upper surface with cyanoacrylate glue (Parson Adhesives). A sterile rubber valve (Biometrics) was fitted at each sampling port exit.

The LCs were filled with sterile BHI broth supplemented with streptomycin (0.5 mg/ml) to the surface level of the O ring. A syringe needle was inserted into the valve to allow venting of excess fluid, and the plates were tightened as previously described. The empty space in the UC was filled with the previously prepared *E faecalis* suspension and sealed with a polyvinyl chloride band. For homogenization, the plates were agitated on a shaker before sampling. BHI broth (a volume of 10 µl) was collected from the LC every 24 hours for 80 days. In addition to the LC sampling, broth from the UC was sampled to ascertain that the bacteria were vital throughout the entire test period. All sampling was performed under sterile conditions. A new band was placed after each sampling to prevent cross-contamination. Fresh broth was added just below the surface level, ie the original level of each UC and LC. During the experimental period, the assemblies were maintained in an incubator at 37°C and 100% humidity. The samples collected from LC at each time period were plated on BHI/AGAR (Acumedia) agar plates supplemented with 0.5 mg/ml streptomycin. Bacterial growth on the agar plates was defined as bacterial leakage, and the absence of bacterial growth on the plate was defined as no bacterial leakage.

Statistical analysis

Quantitative data from the fluid test was analyzed using two-way ANOVA, and the level of significance was set at $P < .05$. The descriptive data of the bacterial test was plotted on a Kaplan-Meir survival graph.

RESULTS

The fluid transport test

The results showed an increase in fluid transport over time for all tested materials, which was less evident in the modified TRMs with incorporated NP. The greatest leakage was observed in the IRM group (0.065 µl ± 0.061) followed by the IRM group with 2% QPEI NP (0.03 µl ± 0.03) and the Coltosol group (0.01 µl ± 0.002). Coltosol with 2% wt/wt QPEI NP exhibited the least leakage (0.005 µl ± 0.0007) (Fig 3). The differences between all tested groups were significant ($P < .01$).

Bacterial leakage test

Bacterial growth was detected within 4 days for the nonmodified IRM group. The Coltosol group samples showed bacterial growth after 11.5 (± 1.5) days, and the IRM group samples with incorporated QPEI NP showed bacterial growth after 18 (± 1) days. For samples collected from the Coltosol group with incorporated QPEI NP, 50% of samples showed bacterial growth after 24 days, and the other 50% showed no bacterial growth for at least 80 days (Fig 4).

DISCUSSION

Improving the clinical performance of dental temporary restorations by prolonging their ability to withstand the harsh conditions in the oral environment is a desired aim. Specifically, dimensional stability and antibacterial properties may be advantageous. Coronal microleakage appears to have equal or greater clinical relevance as a factor in endodontic failure than apical leakage due to risk of recontamination. Moreover, in cases with adequate root canal seal that was achieved by a well-filled root filling, a faulty coronal restoration seal may lead to bacterial penetration and recontamination. In the present study, we modified two clinically available TRMs by incorporating polyethyleneimine antibacterial NP. These results showed that the two tested temporary TRMs with incorporated QPEI NP scored much higher in the fluid transport and bacterial leakage tests compared to the base materials.

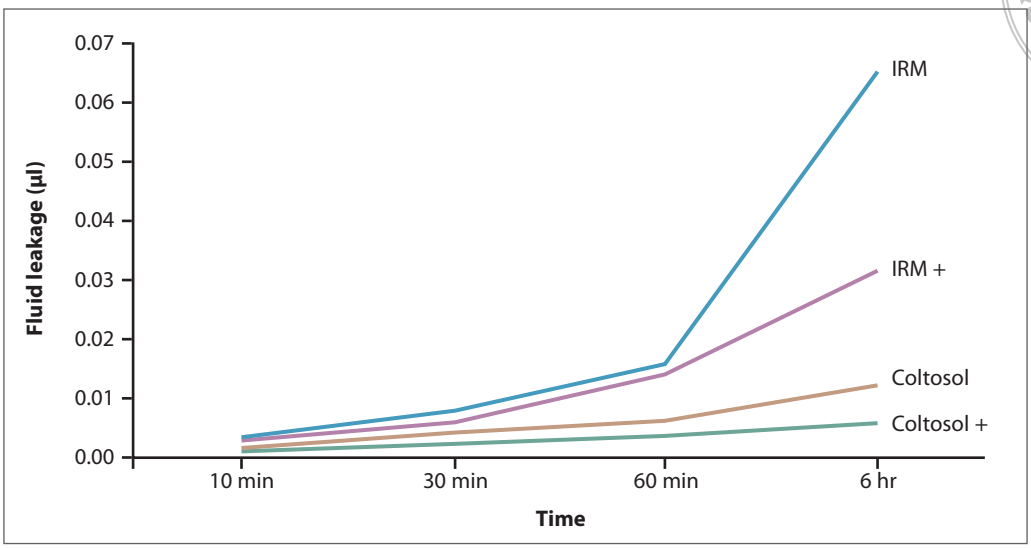

Fig 3 Fluid transport test. Fluid leakage was detected in all the tested groups. The IRM group showed leakage (mean 0.065 µl ± 0.061) > IRM + 2% QPEI NP group (0.03 µl ± 0.03) > Coltosol group (0.01 µl ± 0.002) > Coltosol + 2% QPEI NP group (0.005 µl ± 0.0007). One-way ANOVA revealed significant differences between all tested groups ($P < .01$).

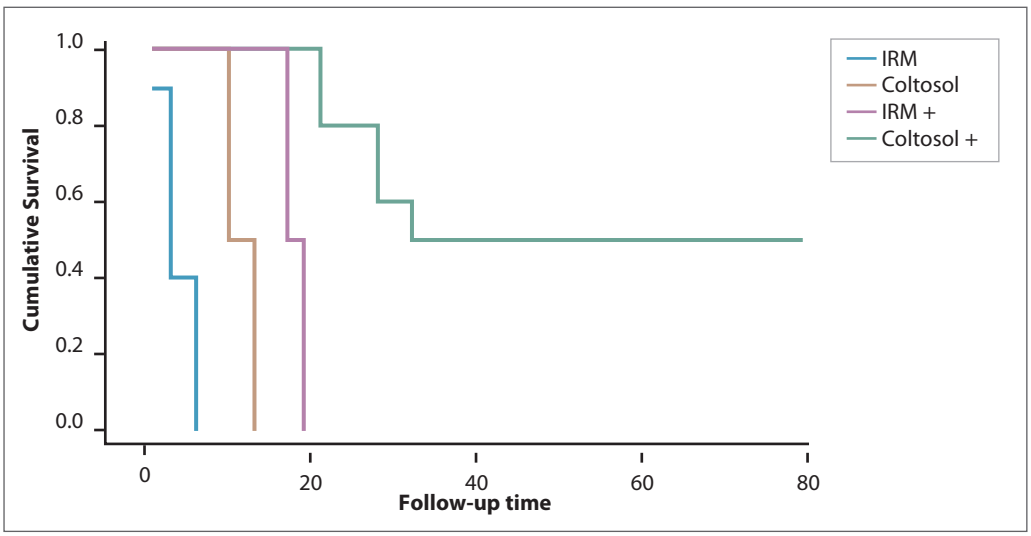

Fig 4 Bacterial leakage test. Bacterial growth on agar plates was defined as bacterial leakage, and no bacterial growth on plates was defined as no bacterial leakage. The results are plotted on a Kaplan-Meir survival graph.

The role of coronal leakage in endodontic failure is well established.[25] Coronal leakage may occur soon after temporization,[8] thereby resulting in endodontic failure due to bacterial recontamination.[2] A retrospective clinical study[7] showed that a fair endodontic treatment outcome may be achieved even in inadequately filled root canals when the quality of the coronal restoration is sufficient. These findings emphasize the significance of the coronal seal and may challenge the rationale of endodontics, with the quality of the coronal restoration being more noteworthy than the quality of root canal treatment in eliminating apical periodontitis. Furthermore, Madison and Wilcox[8] showed in vivo that temporary sealed cavities following endodontic treatment demonstrated an

equal leakage to non-sealed endodontically treated teeth, thus demonstrating the critical need in a long-term antibacterial temporary filling material.[8] Zinc oxide/calcium sulfate-based and zinc oxide–eugenol-based materials are the most commonly used materials for temporary restorations.[3] These materials are recommended for short-term temporization not exceeding 2 weeks.[2] However, 79% of temporary fillings are replaced by a permanent restoration only after 4.4 months.[26] Consequently, the clinical reality calls for radical improvements in the properties of these materials.

Zinc oxide–eugenol-based and calcium sulfate-based materials have a limited antibacterial capacity.[27] To overcome this disadvantage, volatile and soluble materials, such as eugenol and zinc ions, are added. These materials are released from the restorative material over time and thus have limited antibacterial activity.[28,29] A recently suggested approach advocates the use of antibacterial macromolecular disinfectants that can inactivate target bacteria on contact, disrupt cell-cell communication, and prevent bacterial aggregation and biofilm formation or kill bacteria directly (lysis of the cell membrane).[30] Given their stability, these macromolecules are not released from the bulk phase. Recently, QPEI were developed to enhance the antibacterial activity of restorative composite resins.[19] Since satisfactory coronal restoration clearly is among the factors that may certainly influence the outcome of endodontic treatment, we thought of incorporating QPEI to gain the advantage of antibacterial properties in TRMs.

The rationale behind the present study was that temporary restoration properties should include the ability to prevent the access of any further bacteria during and after endodontic treatment. Thus, a more predictable and favorable healing of the apical periodontium may be achieved. In the present study, we further investigated the effect of incorporated QPEI NP in TRMs. For this purpose, fluid transport and bacterial leakage tests were used. Although many previous leakage studies examined the penetrability of temporary filling material, the majority of these studies were semiquantitative and thus difficult to compare.[2] In addition, many of the studies did not use thermocycling, which has a dramatic effect on the IRM contraction.[31]

The fluid transport model allows repetitive, comparable, and nondestructive measurements of a given sample. Despite the associated advantages, the fluid filtration model merely indicates a potential path of infection. Bacterial leakage depends not only on potential fluid penetration through the marginal gaps of restoration, but also on the antibacterial properties of the material. The present in vitro study examined the impact of incorporated antibacterial NP on the sealing ability and level of bacterial microleakage of Coltosol (a calcium sulfate-based material) and IRM (a zinc oxide–eugenol-based material). Each material was tested for fluid leakage and bacterial leakage in a two-compartment model. All samples were tested simultaneously under similar conditions, which allowed for reliable comparison between the end results. Equipped with this model, fluid transport could be tested over time using the same specimens. In addition, the standardized preparation minimized the number of variables affecting the results. Coltosol showed less fluid transport compared to IRM, in concurrence with previous studies[5,32,33] that tested calcium sulfate-based materials (Cavit and Cavit G) and zinc oxide–eugenol-based materials (IRM). In a recent study,[34] no difference was observed between the fluid filtration rate of IRM and Coltosol. However, in contrast to the three studies mentioned above, there was no reference to the duration of the experiment and timing of sampling. In the present study, significant differences were detected only after 6 hours of fluid transport, thereby indicating that test time is an important variable in this assay. Interestingly, the incorporated QPEI NP decreased the fluid transport by > 50 % in both tested materials compared to the nonmodified materials. Therefore, the decreased fluid transport might substantially improve the sealability of TRMs and thus prolong their clinical performance.

Using the fluid transport test, it is impossible to differentiate between leakage along the dentin-material interface and through the material body. These patterns were

demonstrated to coexist in both calcium sulfate-based material and IRM.[4] The success of TRMs depends on both fluid transport through the bulk materials and bacterial leakage in the material-tooth interphase. The same specimen was tested for bacterial leakage to investigate further the leakage properties of the material. Incorporated QPEI NP significantly prevented bacterial leakage in both groups, although the effect was dramatically prolonged in the modified Coltosol (Fig 2). A possible explanation for this effect is that the body leakage of Coltosol is higher than IRM.[4] Coltosol and IRM have a similarly limited antibacterial effect that decreases after 14 days.[6] Therefore, the differences between Coltosol and IRM may be attributed to the presence of QPEI NP in the Coltosol body, which allowed a larger contact area with the invading bacteria, in comparison to IRM with bacterial contact mainly in the dentin interface.

In summary, a unique combined in vitro model tested both fluid transport and bacterial leakage of Coltosol and IRM with and without QPEI NP. The endpoint results of both tests indicated a significant correlation between the material group and leakage. QPEI NP improved the sealing ability of both IRM and Coltosol and dramatically upgraded the antibacterial property of Coltosol. Incorporating QPEI NP into TRMs seems advisable, provided these additives do not alter the physical properties of the material. The use of a bilayer composed of Coltosol with incorporated QPEI NP and modified IRM with incorporated QPEI NP may combine the advantages of both modified materials. Additionally, TRMs incorporating QPEI NP may serve as canal orifice plugs and retrofillings. Current studies are focusing on these potential applications.

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