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Preliminary evaluation of a novel polymer nanocomposite as a root-end filling material

S. M. A. Chogle[1], C. F. Duhaime[1], A. K. Mickel[1], S. Shaikh[2], R. Reese[1], J. H. Bogle, D. K. Chan[1], S. Potluri[1] & S. Qutubuddin[2]

[1]Department of Endodontics, School of Dental Medicine; and [2]Department of Chemical Engineering, School of Engineering, Case Western Reserve University, Cleveland, OH, USA

Abstract

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Aim To evaluate two nanoparticle-enhanced polymer root-end filling materials (NERP1 and NERP2) on the initial apical seal as compared to a polymer-based commercial compomer.

Methodology One hundred and forty extracted roots with completely formed apices were resected 3 mm from the apex. Cavities were then prepared in the apical openings of the resected root ends using an ultrasonic handpiece to a depth of 3 mm. The roots were then randomly divided into five groups to receive one of five root-end filling materials. Roots in groups 1 and 2 received NERP1 and NERP2, whilst those in groups 3 and 4 received identical polymers as groups 1 and 2 but without nanoparticle addition (RP1 and RP2, respectively). The root-end cavities for the fifth group were filled with commercially available root-end filling compomer. Roots were mounted in a dual-chamber leakage apparatus and inoculated coronally with *Enterococcus faecalis*. Turbidity of the apical broth was assessed daily for 4 weeks as a sign of initial leakage. The results were statistically analysed using odds ratio and Fisher's chi-square analysis.

Results Nanoparticle-enhanced root-end filling polymer 1 displayed significantly fewer leaked samples compared to all other tested groups as early as 5 days, whilst NERP2 was not significantly different compared to other groups. Odds ratio analysis revealed leakage of the commercial compomer was 12 times more likely than NERP1.

Conclusion Nanoparticle-enhanced root-end filling polymer 1 can reduce apical microleakage significantly under laboratory conditions.

Keywords: microleakage, nanocomposites, nanoparticles, organoclays, retrofill.

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Introduction

The criteria for an ideal root-end filling material include factors such as stability, biocompatibility, lack of toxicity and periapical tissue regeneration (Gartner & Dorn 1992). Super-EBA®, Geristore® and Mineral Trioxide Aggregate (MTA) are three of the most widely accepted root-end filling materials today. However, none of them satisfy the requirements of an ideal root-end filling material (Gartner & Dorn 1992). MTA meets the criterion of biocompatibility; however, handling difficulty in a surgical crypt and its prolonged setting time may impede its routine use. The main drawbacks of Super EBA® are its solubility and short setting time (Biggs *et al.* 1995). Compomers such as Geristore® are known to shrink during setting, which may ultimately increase bacterial leakage.

Another drawback common to the current root-end filling materials previously discussed is that none of them have indisputable evidence of inherent antimicrobial properties after setting. One of the ideal

Correspondence: Dr. Sami Chogle, Boston University Institute for Dental Research and Education, Academic Medical Center, Dubai Healthcare City, P O Box 505097, Dubai, UAE (e-mail: sxc89@case.edu).

characteristics of materials used in close approximation to periradicular tissues is their antibacterial properties (Abdal & Retief 1982). Therefore, a material that addresses all of these issues could improve the outcome of periradicular surgery.

Nanocomposites are a newer class of composites that have shown great potential. A polymer nanocomposite is a generalized term for polymeric materials that are loaded with minimal amounts of nanoparticles such as clays, carbon nanotubes, etc. dispersed at a nanoscale (Krishnan *et al.* 2005). As opposed to conventional composites, the dispersed phase has a high surface-to-volume ratio. Polymer nanocomposites (PNCs) have therefore shown greatly improved mechanical and thermal properties even at very low filler content (typically between 0% and 5%). Previous studies have indicated substantial improvements in heat resistance (Tanaka *et al.* 2003, Krishnan *et al.* 2005), dimensional stability (Petkov *et al.* 2005), stiffness (Pandey *et al.* 2005, Gaboune *et al.* 2006), reduced electrical conductivity (Cho *et al.* 2004, Li *et al.* 2006) and, most uniquely, drug elution capabilities (Cypes *et al.* 2003, Shaikh *et al.* 2007). The authors have produced two such novel nanocomposites (NERP1 and NERP2). The present pilot study investigated these two nanoparticle-enhanced root-end filling polymers (NERPs) on the apical seal as compared to another polymer-based compomer commonly used as a root-end filling material.

Materials and methods

NERP and RP preparation

The C18 organoclay particles were synthesized by modifying hydrophilic montmorillonite (MMT) clay with octadecyl amine (C18-amine) via a previously described procedure (Fu & Qutubuddin 2005, Shaikh *et al.* 2007). The two NERP groups (NERP1 and NERP2) were prepared by dispersing the C18 organoclay particles in two separate combinations of monomers along with photo-initiators and activators by stirring overnight in a covered conical flask (Table 1). Two control root-end filling polymer groups (RP1 and RP2) were prepared similarly, but no C18 organoclay was added. Polymer (polymethyl methacrylate) was then added to all RP and NERP groups and thoroughly mixed with a hand spatula. Proportions were precisely controlled to yield a paste consistency for each of the materials to facilitate loading into an opaque syringe and expression through a wide-gauge needle into the root-end cavity.

Root preparation and root-end filling

One hundred and forty palatal roots of extracted human maxillary molars were selected. The inclusion criteria for the samples were based on length (16.0 mm), curvature (<10°) and radiographic evidence of canal space. Each tooth was decoronated and sectioned through the furcation to yield individual roots. The canals were instrumented to a size 40, 0.04 taper rotary file using 5.25% sodium hypochlorite irrigation. All canals were flushed with 17% EDTA to facilitate smear layer removal and then irrigated with sterile water before drying the canals with paper points. The apical 3 mm of each root was resected and a root-end cavity prepared to a depth of 3 mm using surgical ultrasonic tips. An appropriately cut single gutta-percha point without sealer was inserted from the coronal access cavity to fit just coronal to the root-end

Table 1 Monomer matrix components with designations

Groups	Nanoparticle	Polymer	Monomers	Chemical components
Retrofill Polymer1 (RP1)	None	PMMA	Bis-GMA, TEGDMA, HEMA	2,2-bis[p-(2-hydroxy-3-methacryloxy propoxy) phenyl]propane, Triethylene glycol dimethacrylate, Hydroxyethyl methacrylate Poly(methyl methacrylate)
Retrofill Polymer2 (RP2)	None	PMMA	HEMA, UDMA	Urethane dimethacrylate, Hydroxyethyl methacrylate, Poly(methyl methacrylate)
Nanoparticle-enhanced retrofill polymer 1 (NERP1)	C18	PMMA	Bis-GMA, TEGDMA, HEMA	2,2-bis[p-(2-hydroxy-3-methacryloxy propoxy) phenyl]propane, Triethylene glycol dimethacrylate, Hydroxyethyl methacrylate, Poly(methyl methacrylate), C18 organoclay
Nanoparticle-enhanced retrofill polymer 2 (NERP2)	C18	PMMA	HEMA, UDMA	Urethane dimethacrylate, Hydroxyethyl methacrylate, Poly(methyl methacrylate), C18 organoclay

preparation. The roots were then randomly divided into five groups of 24 roots each. The root-end cavities in groups 1 and 2 received NERP1 and NERP2, respectively, as root-end fillings. Groups 3 and 4 received control monomers, RP1 or RP2, respectively, whilst the fifth group (CCG) was root-end-filled with the commercially available compomer Geristore® (DEN-MAT, Santa Maria, CA, USA). Each material was placed into the prepared root-end cavity through a 25-gauge needle/syringe, gently adapted and condensed into the cavity and photo-polymerized for 30 s. Radiographs were taken to confirm proper adaptation to cavity walls and absence of voids within the filling. The gutta-percha point was then removed to ensure that the leakage was a function of the apical filling material alone. Ten roots were retained as negative controls, and ten additional roots were prepared as positive controls. Negative controls consisted of teeth that were prepared and then completely filled and covered with cyanoacrylate. For the positive controls, roots were resected and prepared similar to group 1 through 5, but root-end cavities were left unfilled.

Leakage apparatus, assessment and analysis

The dual-chamber leakage apparatus was assembled using a set-up described in earlier work (Chogle *et al.* 2005) and sterilized with gamma radiation (Rodrigues *et al.* 2004). The roots were positioned in the dual chamber such that the coronal portion of each root fitted in the upper chamber, whilst the apical portion was suspended in the lower chamber vial. A litre of sheep brain–heart infusion (BHI™ Laboratories, Detroit, MI, USA) was prepared and autoclaved for 30 min. Sterile BHI™ broth was pipetted into the vials to submerge the apical 2 mm of each root end. The entire apparatus was placed in an incubator at 37 °C for 24 h to confirm maintenance of a sterile environment.

Pure isolates of the *Enterococcus faecalis* (ATCC 29212) were inoculated in a 5.0-mL vial of sterile BHI™ broth and incubated for 1 day at 37 °C in a Fisher (Fisher Scientific, Pittsburgh, PA, USA) low-temperature incubator. The culture medium turned turbid after 24 h and was tested using Gram stain to test the purity of the inoculums after the culture medium turned turbid. The upper chambers were then inoculated with 50 µL of *E. faecalis* and maintained daily. Apical leakage was assessed each day with the naked eye by observing visible turbidity in the lower vials containing BHI broth. Turbidity in the apical broth indicated leakage of intracanal *E. faecalis* into the apical broth and compromise of the root-end filling material. The turbid broth was tested using Gram stain to confirm the presence of *E. faecalis*. Daily and cumulative turbidity (number of turbid samples) in each group was assessed over 4 weeks, and data were statistically analysed using Fisher chi-square test and odds ratio analysis.

Results

Prior to inoculation with *E. faecalis*, no contaminated samples were found during the 24-h pre-inoculation incubation period. All positive controls showed apical broth turbidity within 2 days, and the negative controls demonstrated clear apical broth throughout the experiment. The cumulative bacterial leakage revealed significant results at the end of 4 weeks (Fig. 1). The RP1 and NERP1 groups were the only two groups that did not display all turbid samples by the end of the experiment. As seen in Table 2, the NERP1 group had significantly fewer leaked samples as compared to other groups.

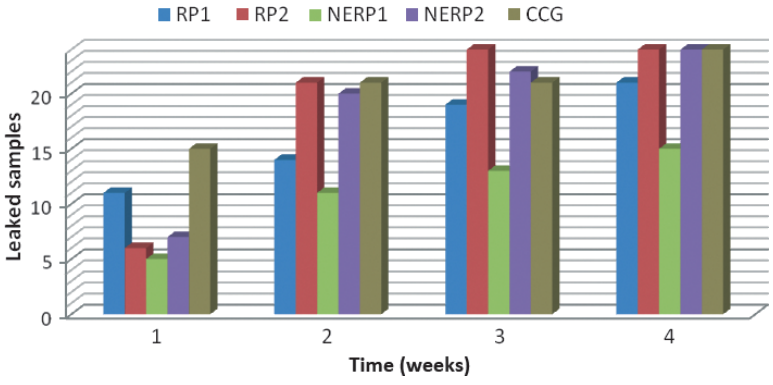

Figure 1 Comparison bar chart for cumulative turbidity versus time.

Table 2 Chi-square comparison of leaked samples for NERP1 group

Day	RP1 versus NERP1	RP2 versus NERP1	NERP2 versus NERP1	CCG versus NERP1
1	0.1092	0.9999	0.0496	0.1092
2	0.0971	0.6085	0.1882	0.0983
3	0.1867	1.0000	0.9999	0.0599
4	0.1246	0.9999	0.7416	0.0687
5	0.1246	0.9999	0.7416	0.0171*
6	0.1246	0.9999	0.7416	0.0077
7	0.0335*	0.5171	0.3451	0.0077
8	0.0421	0.7516	0.5515	0.0189
9	0.0421	0.7516	0.0687	0.0189
10	0.0421	0.7516	0.0171*	0.0189
11	0.0421	0.7516	0.0012	0.0189
12	0.0845	0.0001*	0.0003	0.0004

NERPs, nanoparticle-enhanced root-end filling polymers.
*Beginning of significance.

NERP versus CCG

Nanoparticle-enhanced root-end filling polymers 1 displayed significantly fewer leaked samples than CCG ($P = 0.0171$) starting at day 5 as seen in Table 2. The number of leaked samples for NERP1 remained significantly different in comparison with CCG throughout the experiment. In week 1, odds ratio analysis indicated that CCG was 6.33 times more likely to show apical turbidity than NERP1 (Table 3). This figure doubled in week 2 when CCG became 12.14 times more likely to show a leaked sample. Interestingly, even RP1 displayed significantly fewer leaked samples than CCG but only after the 13th day ($P = 0.0490$). Conversely, NERP2 was not significantly different from CCG, and RP2 demonstrated even higher leaked samples than CCG ($P = 0.0392$).

NERP groups and non-NERP groups:

Nanoparticle-enhanced root-end filling polymer 1 displayed significantly less leaked samples than RP1 and RP2 ($P = 0.0335$ and $P < 0.0001$, respectively). There were no significant differences between the NERP2 group and RP1 and RP2. Within the NERP groups, NERP1 demonstrated consistently fewer leaked samples than NERP2, with a significant difference emerging on day 10 ($P = 0.0171$).

Discussion

Addition of the nanoparticle C18 organoclay to an optimum concentration of Bis-GMA, TEGDMA and HEMA (NERP1) yielded significantly fewer samples with apical leakage of *E. faecalis* compared to all other groups. Geristore® (CCG) was selected as the benchmark root-end filling material for comparison because of the similarity in consistency, method of application and setting conditions to NERP groups. Although the antimicrobial activity of set NERPs has not yet been determined, this characteristic would play a significant role in the outcome of leakage of viable bacterial cells leading to turbidity in the apical broth. Geristore® has demonstrated *in vitro* antibacterial activity for *E. faecalis* (Eldeniz *et al.* 2006). However, in the present study, it failed to prevent bacterial leakage into the apical broth. It is also worth noting that the RP2 and NERP2 groups showed similar leaked samples to the CCG group with inhibitory effect of C18 on leakage. It may be the monomer combination of HEMA and UDMA itself rather than the addition of C18 that helped contribute to dimensional shrinkage and leakage.

Within the NERP groups, NERP1 had significantly fewer leaked samples compared to NERP2. NERP2 consisted of one diluent monomer (UDMA) and one hydrophilic monomer (HEMA), whereas NERP1 consisted of one diluent (TEGDMA) and two monomers (HEMA and BisGMA). This may have potentially reduced the amount of flowability or fluid nature of the NERP2 material resulting in inadequate adaptation to dentinal walls.

Another possible explanation for NERP1 outperforming NERP2 may be related to the degree of dispersion of

Table 3 Odds ratios comparing NERP1 to other groups

NERP1 comparison	Week 1		Week 2		Week 3		Week 4	
	Odds ratio (CI)	*P* value	Odds ratio (CI)	*P* value	Odds ratio (CI)	*P* value	Odds ratio (CI)	*P* value
RP1	3.21 (0.94, 12.32)	0.072	3.40 (1.06, 11.81)	0.045	5.92 (1.52, 29.99)	0.016	4.12 (1.05, 21.41)	0.055
RP2	1.27 (0.33, 5.10)	0.732	17.00 (4.26, 90.53)	0.0002	–	–	–	–
NERP2	1.56 (0.42, 6.18)	0.507	9.23 (2.62, 37.91)	0.001	9.31 (2.09, 66.56)	0.008	–	–
CCG	6.33 (1.85, 29.91)	0.005	12.14 (3.29, 54.90)	0.0004	8.88 (1.99, 63.64)	0.010	–	–

NERPs, nanoparticle-enhanced root-end filling polymers.

the C18 organoclay in the polymer matrix. To enhance the physical properties of a nanocomposite, the reinforcing phase must be dispersed at a nanoscale (exfoliated) within the polymer matrix (Fig. 2). This will result in a very high surface-area-to-volume ratio and substantially increase the interfacial area between the reinforcing phase and the matrix (Ray *et al.* 2003, Fu & Qutubuddin 2005). Factors that determine the dispersion of the nanoparticles are functional groups present on the host monomer/polymer, the mode of dispersion used and the nature of surfactant used to modify the clay (Krishnamoorti & Vaia 2002, Ray *et al.* 2003, Fu & Qutubuddin 2005). Depending on the degree of dispersion, the property enhancements could be substantial or none at all. This may also explain the similar leakage patterns between RP2 and NERP2. A possible lack of adequate dispersion of C18 into the RP2 matrix could have resulted in no significant improvement in NERP2 properties. Future studies will look more closely into this relationship. The dispersion of organoclay through various polymer matrices will also need to be characterized using X-ray diffraction (XRD). In addition, tests such as atomic force microscopy, scanning electron microscopy (SEM), transmission electron microscopy, differential scanning calorimetry and dynamic mechanical analysis (DMA) on MM1 + C18 are being conducted to help further elucidate the enhanced physical properties.

In the present study, all root-end filling material groups demonstrated some amount of bacterial leakage including the best group NERP1. This may result in questioning the ultimate benefit of NERP as a root-end filling material. Continued development of the NERP material may promise further improvement in apical sealing and the addition of drug elution capabilities. One aspect currently under investigation is the level of polymerization of the monomer matrix. In any known polymerization reaction, the level of conversion of monomer to polymer may be close to but never reach 100%. A significantly lower conversion will negatively impact adaptability, sealing ability of the material and biocompatibility as well. Currently, the NERP materials are being tested for levels of unpolymerized or residual monomers within the matrix using UV spectrophotometer and nuclear magnetic resonance analysis. A modification to the initiator system is also being explored to further increase the level of polymerization over time. A recently concluded cytotoxicity study on gingival fibroblasts by the authors has shown favourable results for NERP1 (Modareszadeh *et al.* 2011).

Pending further development and investigations comparing other popular root-end filling materials like MTA and superEBA, it is conceivable that a reduction in leakage and antimicrobial elution capacity would enable NERP to counter a primary, residual or secondary microbial challenge against the surgerized tooth

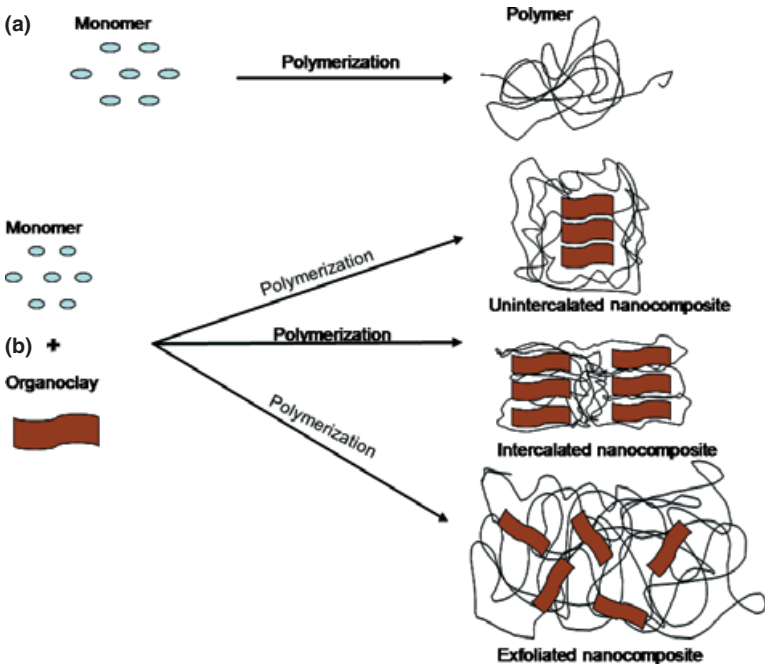

Figure 2 Incorporation and Levels of dispersion of Organoclay in a polymer matrix. (a) Polymerization of monomer to polymer. (b) Incorporation of Organoclay into monomer and the following polymerization resulting in no dispersion (unintercalated), some dispersion (Intercalation) or high dispersion (exfoliation).

and surrounding periradicular tissue. This may be especially relevant in cases where the clinician could not precede apical surgery with nonsurgical retreatment. The combined effects of minimal leakage and drug elution could potentially increase outcomes of periradicular surgery.

Conclusion

The addition of C18-nanoparticles to a monomer matrix significantly reduced apical microleakage in an *in vitro* environment. Although the authors feel the results are promising, these preliminary findings need to be further investigated to better understand and enhance the effect of nanotechnology and drug elution in developing superior endodontic materials.

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