

Towards antibacterial endodontic sealers using quaternary ammonium nanoparticles

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Abstract

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Aim To change and characterize the antibacterial properties of endodontic sealers by incorporating low concentrations of insoluble antibacterial nanoparticles (IABN).

Methodology The antibacterial effect against *Enterococcus faecalis* was evaluated by (i) agar diffusion test (ADT), (ii) direct contact test (DCT) and (iii) scanning electron microscopy (SEM). IABN were incorporated into AH Plus (Dentsply, DeTrey Konstanz, Germany) and GuttaFlow (Coltène Whaledent, Langenau, Germany) at concentrations of 0.5%, 1% or 2% weight/weight. Bacterial growth rates were analysed using ANOVA followed by Tukey's test.

Results The antibacterial tests demonstrated total bacterial growth inhibition using AH Plus samples incorporating 2% weight/weight IABN after 4 weeks ($P < 0.005$). DCT showed total growth inhibition of up to 6 logs in viable count in AH Plus samples incorporating IABN and up to 4 log in count in GuttaFlow incorporating IABN ($P < 0.005$). Significant differences were found between the unmodified sealers and the experimental groups. No antibacterial effect was observed in the ADT, indicating IABN were not diffusing into the agar. Furthermore, SEM indicated bacterial cell wall damage and lysis.

Conclusions AH Plus and GuttaFlow incorporating low concentrations of IABN exhibited significant and stable antimicrobial properties.

Keywords: direct contact test, *Enterococcus faecalis*, quaternary ammonium, scanning electron microscopy (SEM), sealing materials.

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Introduction

Elimination of bacteria from root canal systems is crucial for successful root canal treatment (Sjögren *et al.* 1997). Despite meticulous mechanical preparation, infection may persist in 20–33% of root canals, even when calcium hydroxide (Ca(OH)₂) dressing is used (Waltimo *et al.* 2005). Bacterial persistence may be

due to (i) ineffective intracanal irrigation, (ii) mechanical preparation that leaves much of the root canal surfaces untouched and (iii) ineffective chemo-mechanical preparation due to anatomical limitations (Ricucci & Siqueira 2010). Treatment failure may also occur as a result of coronal leakage of saliva, nutrients and bacterial re-entry (Moradi *et al.* 2009). A common resistant intracanal pathogen that serves as a gold standard bacterium in endodontic research is *Enterococcus faecalis* (Ørstavik & Haapasalo 1990). *E. faecalis* is a highly resistant pathogen, displaying resistance to various irrigates and medicaments (Ørstavik & Haapasalo 1990, Hancock *et al.* 2001).

It was recognized that following chemo-mechanical preparation of canals, the antimicrobial properties of endodontic sealers, could potentially control infections

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(Ørstavik 1988). Formaldehyde and eugenol are effective against bacterial pathogens but are also cytotoxic and mutagenic (Lai *et al.* 2001). Furthermore, their antibacterial properties are lost rapidly (Neelakantan & Subbarao 2008). For example, AH Plus or AH26 (Dentsply DeTrey, Konstanz, Germany) exhibited no antibacterial activity after several days (Slutzky-Goldberg *et al.* 2008). Furthermore, sealers that before setting had antibacterial activity did not maintain it after setting (Morgental *et al.* 2011).

Apparently, the challenge is to formulate endodontic sealers that possess long-term potent antibacterial properties. One way to secure these properties is to design antibacterial compounds that do not leach into the surroundings. The synthesis of quaternary ammonium polyethylenimine nanoparticles with potent antibacterial properties has been described (Beyth *et al.* 2006). By incorporating 1% insoluble antibacterial nanoparticles (IABN) into resin-based composite, the restorative material gained potent, broad-spectrum and long-lasting antibacterial properties (Beyth *et al.* 2006, 2008, Yudovin-Farber *et al.* 2008). Furthermore, after showing no signs of inflammatory response *in vitro* (Yudovin-Farber *et al.* 2008), an *in vivo* study demonstrated a highly significant reduction in bacterial viability in oral biofilms (Beyth *et al.* 2010).

The objectives of this study were to develop antibacterial endodontic materials incorporating IABN and to investigate their effect on bacterial growth and viability. It was hypothesized that (i) incorporating IABN into endodontic sealers would yield antibacterial properties stronger than those that would be achieved by the non-modified sealers; (ii) The antibacterial properties would not decrease with buffer-ageing time. These properties might set the framework for a new generation of antibacterial endodontic materials.

Materials and methods

Preparation of quaternary ammonium polyethylenimine nanoparticles

Synthesis was performed as previously described (Beyth *et al.* 2006). Polyethylenimine dissolved in ethanol was reacted with dibromopentane under reflux for 24 h. N-alkylation was conducted using octyl. Alkylation was carried out under reflux for 48 h, followed by neutralization with sodium bicarbonate for an additional 24 h under the same conditions. N-methylation was conducted using methyl iodide. Methylation was

continued at 42 °C for 48 h, followed by neutralization with sodium bicarbonate for an additional 24 h. The supernatant obtained was decanted and precipitated in double-distilled water (DDW), washed with hexane and DDW and then freeze-dried. Average yield was 70% (mol/mol). The estimated particle size was approximately 32 nm using scanning electron microscope (SEM) (not shown).

Bacteria and bacterial suspension

A streptomycin-resistant *E. faecalis* strain (clinically isolated at the Maurice and Gabriela Goldschleger School of Dental Medicine, Tel Aviv University, Israel) was cultured overnight in 5 mL of brain-heart infusion (BHI) broth (Difco, Detroit, MI, USA), supplemented with 2 mg mL⁻¹ of streptomycin (streptomycin sulphate salt, Sigma Aldrich) at 37 °C under aerobic conditions. The bacterial suspension was adjusted to an optical density (OD) of 1.0 at A_{650 nm} for the agar diffusion test and of 0.25 at A_{650 nm} (2 × 10⁸ colony-forming units (CFU) mL⁻¹) for both the direct contact test and SEM examination.

Direct contact test (DCT)

Two endodontic sealers were tested: AH Plus (Dentsply DeTrey, Konstanz, Germany) and GuttaFlow (Coltène Whaledent, Langenau, Germany). IABN powder was added at 0.5%, 1% or 2% weight/weight concentration and mixed manually, using a flat dental spatula into the unset sealers according to the manufacturer's instructions. The sidewalls of wells in a polystyrene microtitre plate (96-well flat bottom plate, Nunclon, Nunc, Denmark) were coated with similar amounts of the mixed sealer (surface area approximately 4 mm × 8 mm), 8 wells for each concentration. The materials were allowed to set for 24 h. Three microtitre plates were similarly prepared and tested for each experimental group after 4 weeks. Throughout the ageing process, the plates were kept at 37 °C, and each well was filled with 250 µL of phosphate-buffered saline (PBS), which was replaced every 48 h.

The plate was positioned vertically, and a 10 µL volume of bacterial suspension (2 × 10⁶ CFU mL⁻¹) was placed on the surface of each tested material. The plate was then incubated vertically for 1 h at 37 °C, the suspension liquid evaporated, and direct contact between bacteria and the tested surfaces was ensured. The plate was then positioned horizontally,

and 220 µL of BHI broth were added to each well. Absorbance was measured every 20 min for 14 h, using a temperature-controlled microplate spectrophotometer (VERSAmax, Molecular Devices Corporation, CA, USA), set to at 37 °C, with 5 s of mixing before each reading. Controls included an equal bacterial suspension placed on the sidewall of 8 polystyrene wells.

Calibration

Calibration experiments were performed simultaneously in each plate. Triplicate wells containing 265 µL BHI were inoculated with 10 µL of bacterial suspension. A fivefold dilution was repeated 7 times in triplicates. A gradual and reproducible decrease in OD correlated with serial dilution. This dilution at zero time resulted in a measurable delay in the exponential growth phase. The growth curves obtained from each experimental well were superimposed on the calibration curves, by overlapping the curves, and it was possible to calculate the number of residual viable bacteria on each tested specimen at the end of the 1 h incubation.

Data analysis

Absorbance measurements were plotted, providing bacterial growth curves for each well. The linear portion of the logarithmic growth phase was statistically analysed, with the slope correlating with the bacterial growth rate and the intercept correlating with the total viable bacterial count. The results were analysed using ANOVA followed by Tukey's test ($P < 0.05$). Each experiment was repeated three times.

Agar diffusion test (ADT)

AH Plus and GuttaFlow discs incorporating 0% or 2% weight/weight IABN of equal diameter (4.5 mm) were prepared using silicone moulds and allowed to fully set for a week in an incubator at 37 °C (Binder BD incubator). After complete setting of the AH plus discs, an *E. faecalis* suspension (200 µL; OD of 1.0 at $A_{650\text{ nm}}$) was spread on blood agar plates, followed by placement of the discs as previously described (Shalhav *et al.* 1997). An ampicillin disc (10 µg) served as control. The plates were incubated for 48 h at 37 °C. The inhibition zone at two perpendicular diameters was measured in millimetres after subtracting the disc diameter itself.

Scanning electron microscope (SEM)

AH Plus samples incorporating 0% or 2% weight/weight IABN were prepared. An *E. faecalis* suspension of 10 µL; OD 0.25 at $A_{650\text{ nm}}$ was placed on the surface of each sample for 20 min or 1 h at 37 °C. The specimens were fixed with formaldehyde, glutaraldehyde and osmium tetroxide in cacodylate buffer, followed by dehydration with a graded ethanol and Freon series, and then coated with gold. Specimens were observed with Extra High-Resolution SEM (Magellan™ 400L, The Hebrew University, Jerusalem, Israel, at magnifications of 10K, 40K).

Results

Significant *E. faecalis* bacterial growth inhibition was revealed in the DCT following direct contact with AH Plus samples incorporating IABN ($P < 0.005$). The extent to which bacterial growth was inhibited directly correlated with IABN concentration; complete inhibition of bacterial growth occurring at a 2% concentration ($P < 0.005$) (Fig. 1a).

Based on the linear portion of the logarithmic growth in the calibration curves (Fig. 1b), the number of residual viable bacteria on each tested specimen was determined and analysed. The homogeneity of the variance between the study groups was tested and found to be statistically significant ($P < 0.05$). Data analysis revealed that in AH Plus samples the number of residual viable bacteria decreased 6 logs when IABN were incorporated at 2%. Incorporation of 0.5% and 1% IABN resulted in a 5 log decrease in count. Significant differences were found between the unmodified sealers and the experimental groups ($P < 0.005$). In GuttaFlow samples, the number of residual viable bacteria decreased 4 logs when IABN were incorporated at 1% and 2% concentrations, and 0.5 log in samples with a 0.5% IABN concentration (Fig. 1c). Significant differences were found between the unmodified sealers and the experimental groups ($P < 0.005$). No significant difference was found between the control group and the commercial, unmodified GuttaFlow ($P < 0.05$).

No inhibition halo was observed in the ADT in any of the experimental groups (Table 1).

Following direct contact with a surface without incorporated IABN, SEM micrographs of *E. faecalis* demonstrated the presence of normally dividing cells with evidence of early biofilm formation (Fig 2a). Within 60 min of direct contact between the bacteria

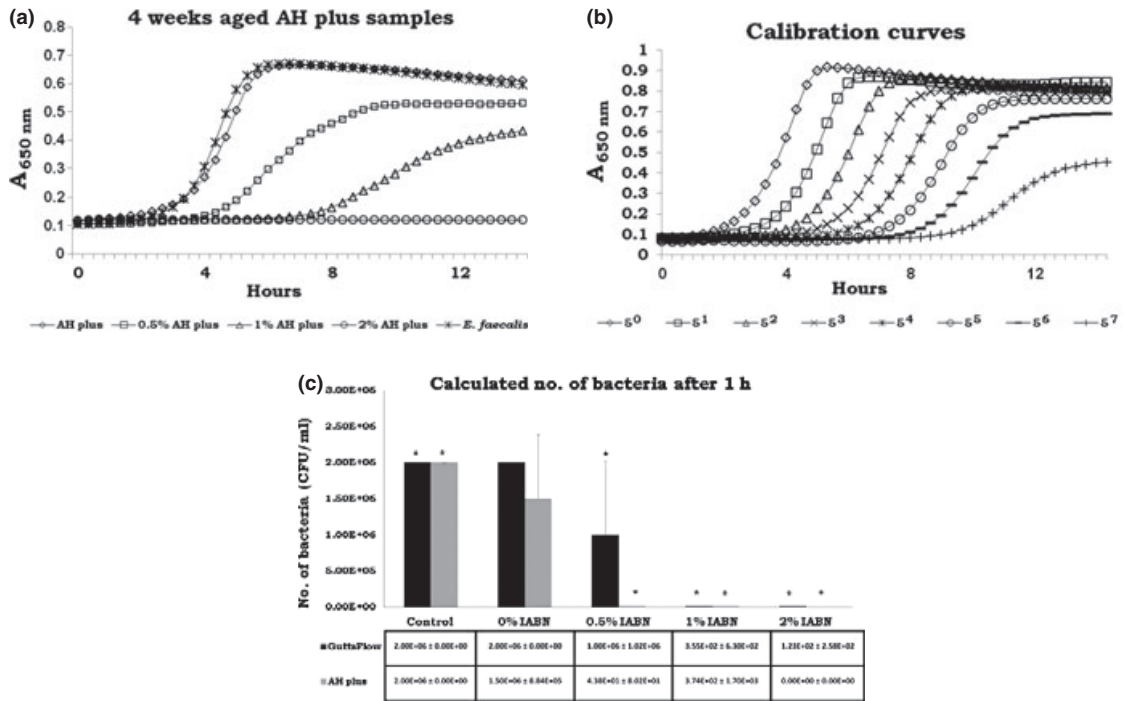


Figure 1 Direct contact test (DCT) depicting anti-*E. faecalis* properties of endodontic sealer samples incorporating insoluble antibacterial nanoparticles (IABN). (a) Bacterial growth measured by temperature-controlled spectrophotometer after direct contact with 4 weeks aged AH Plus samples incorporating 0%, 0.5% or 1% weight/weight IABN. Each point on the curve is the average absorbance measured simultaneously in 8 wells in the same microtitre plate. (b) Calibration experiment. Calibration was performed simultaneously with the experimental set-up in each microtitre plate. Gradual and reproducible decrease in optical density correlated with serial dilution. (c) DCT data analysis depicting viable bacterial counts calculated in three independent experiments following direct contact with two endodontic sealers, AH Plus and GuttaFlow. The linear portion of the logarithmic growth phase taken from wells with bacterial growth in each 96-well microtitre plate was analysed using ANOVA followed by Tukey’s test. Significant differences between the control (*E. faecalis*) and the different sealer groups are indicated by asterisks ($P < 0.005$). Growth curves obtained from each experimental well were superimposed on the calibration curves obtained; that is, the number of residual viable bacteria on each tested specimen was calculated. Viable bacterial number on the surface of the test samples at the end of the 1 h incubation at 37 °C is expressed as the mean ± SD.

Table 1 Agar diffusion test (ADT) of unmodified AH Plus and GuttaFlow samples, and of sealers incorporating 2% weight/weight insoluble antibacterial nanoparticles (IABN)

Tested material	No.	Content of IABN (%)	Inhibition zone (mm)
Ampicillin	1	–	9
AH plus	2	0	0
	3	2	0
Gutta flow	4	0	0
	5	2	0

The inhibition halo diameter was measured after incubation at 37 °C (48 h) at two perpendicular diameters. Zero = no inhibition zone. An ampicillin disc (10 µg) served as control.

and a surface containing 2% weight/weight IABN, distinct morphologic changes were seen in the bacterial cell membrane without visible signs of cell division.

Bacterial aggregation and syncytium-like cell wall fusion were observed (Fig. 2b,c).

Discussion

It is widely accepted that the aetiology of post-treatment disease is associated with bacterial pathogens (Sakamoto *et al.* 2008). Moreover, root canal treatment does not necessarily disinfect root canal walls (Waltimo *et al.* 2005). A promising approach for resolving these infections is to use sealing materials that possess long-term, broad-spectrum antibacterial properties. The activity of most antibacterial agents used in dentistry is dependent on their release from the set material and elution into the surrounding milieu. Inclusion of such components into dental materials

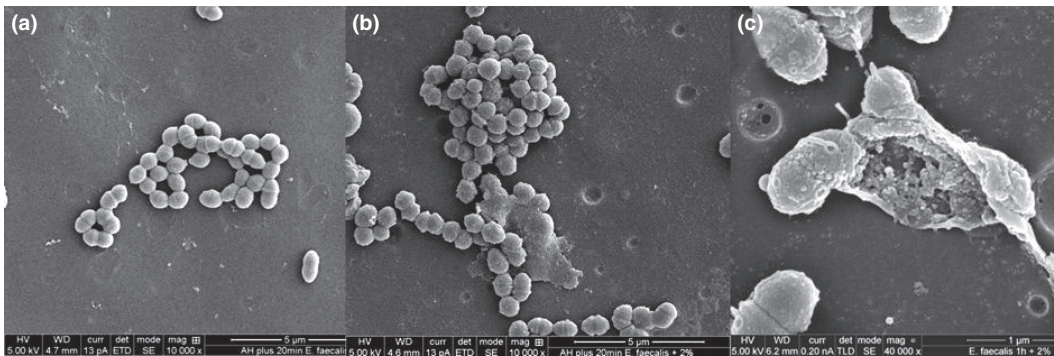


Figure 2 SEM Micrographs of *E. faecalis* on AH plus samples (a) or on AH plus samples incorporating 2% IABN (b, c). Suspension of *E. faecalis* on AH plus samples after 20 min; dividing bacterial cells with intact membranes and early biofilm formation can be observed (a). AH plus samples incorporating 2% IABN after 20 min; morphologic changes in bacterial cell wall, no visible signs of cell division, bacterial aggregation and syncytium-like cell wall fusion can be observed (b). AH plus samples incorporating 2% IABN after 60 min; further morphologic changes include cell wall disruption and lysis (c). Specimens were observed with Extra High-Resolution SEM (MagellanTM 400L, Center for Nanoscience and Nanotechnology, The Hebrew University of Jerusalem, Israel).

will result in continuous dissolution and eventual material degradation over time. Presently available endodontic sealers possess, at best, moderate antibacterial properties, which diminish within days (Neelakantan & Subbarao 2008). Axiomatically, materials containing conventional antibacterial compounds will become less stable and, therefore, will not fulfil the basic requirement of remaining extremely stable over time (Mutal & Gani 2005). It follows that components used for dental materials should preferably act by direct contact and be water insoluble. These properties should ensure the stable, non-degradable properties of the set material.

Various laboratory methods have been used in the past to study the antimicrobial properties of endodontic sealers. Most studies have investigated the antibacterial effect of endodontic sealers utilizing the traditional agar diffusion test and variations of this method. These assay methods for antibacterial activity depend on the ability of the compound being tested to dissolve in the water milieu (Tobias 1988). Unfortunately, as these tests are commonly performed on freshly mixed specimens, they inadequately reflect the clinical conditions and time frame in which the sealers are challenged. Aged dental materials are known to have less antibacterial activity than freshly mixed specimens, the reason being that some of the antibacterial components are washed away in the ageing process or set into the bulk of the materials. Obviously, these methods are inappropriate for screening insoluble antibacterial components used in dental materials. Moreover, it has been stated that

the ability of the commonly used ADT to reflect antimicrobial activity is limited and should not be used to compare and select disinfecting agents for clinical use (Editorial Board of the Journal of Endodontics 2007). For these reasons, the DCT was used to test the endodontic sealers. The DCT used here was developed for assaying solid surfaces for their antibacterial properties (Weiss *et al.* 1996). The DCT was used to determine the bacterial growth following direct contact with the surface of the tested materials. Based on previous findings (Beyth *et al.* 2007) to achieve proximity and contact of the test microorganism and the material surface, which is the first required step in biofilm formation, a small amount of bacterial load was utilized. In the ADT, originally used for evaluating the antibacterial properties of antibiotics, high bacterial loads are commonly used; this test usually accompanies the DCT. Although the ADT is widely used, it is unsuitable for testing materials which are designated to be insoluble, such as endodontic sealers. Alternatively, the DCT was designed to evaluate quantitatively the antibacterial surface properties of materials varying from low to extremely low solubility. In this test, bacteria are allowed to come in direct contact, under controlled conditions, with the tested material in question to study the kinetics of bacterial growth. The DCT suffers from several drawbacks: (i) it measures the growth of surviving bacteria, making it an indirect method and (ii) it requires high manual dexterity.

Previously, it was shown that epoxy resin-based sealers have an antibacterial effect against various

bacteria (Abdulkader *et al.* 1996, Fuss *et al.* 2000, Siqueira *et al.* 2000). In these studies, the materials were subjected to the antibacterial tests immediately following mixing (up to 20 min), resulting in the antibacterial effect of the freshly mixed materials. A possible explanation for the antibacterial effect described in these studies is the release of antibacterial components from the unset sealer into the surrounding milieu prior to final fixation. In the present study, the sealers were tested following full setting of the material, taking into consideration that sealing materials remain in their set form in the root canal for long periods of time. A possible explanation for the absence of an antibacterial halo in the ADT is that the set sealers did not release antibacterial components into the agar. Thus, it can be speculated on the basis of previous studies and the present results that endodontic sealers possess antibacterial properties, which are lost after setting of the material. Nonetheless, according to the DCT and SEM, the set materials surface caused bacterial inhibition when nanoparticles were incorporated. Based on these *in vitro* results, it can be speculated that modified sealers can prevent re-infection caused by bacterial leakage or by residual bacteria in the dentinal tubules, as these bacteria will be exposed to the modified sealers surface at the sealer–dentine interface and eradicated. Nevertheless, bearing in mind that the antibacterial works in direct contact only, the effect of the antibacterial nanoparticles will have within the dentinal tubules or the accessory canals should be further tested.

In moving towards antibacterial sealers, IABN were incorporated into two commercial products. The antibacterial effect differed between sealers, depending on the material used and the IABN concentration. Incorporation of nanoparticles into AH Plus resulted in a stronger antibacterial effect when compared with GuttaFlow. This may be attributed to the different composition of the sealers, epoxy resin-based versus silicone-based, respectively. A different interaction with the IABN of the sealers should be considered and further tested. The evaluation of samples of these sealers using ADT demonstrated no inhibition zone. It can be concluded that the modified sealers remained stable for 48 h without leakage. In contrast, when samples containing IABN were aged in large volumes of buffer for 4 weeks, mimicking prolonged challenge of the materials to liquids, allowing soluble compounds to elute, the results of the DCT showed total growth inhibition.

The unmodified AH plus exhibited limited antibacterial properties, compared with GuttaFlow, which showed no antibacterial properties. For clinical usage, sealers with antibacterial properties may be beneficial, although safety is a crucial issue that needs to be established. Recently, the subject of IABN cytotoxicity when incorporated in endodontic sealers was discussed and published (Abramovitz *et al.* 2012). The cytotoxic effect of three commercially available sealers (AH plus, GuttaFlow and Epiphany), before and following incorporation of IABN, was tested and compared. The results showed that IABN incorporation did not impair the sealers' biocompatibility.

The mechanism by which quaternary ammonium compounds distress bacteria was previously described (Gao *et al.* 2007). In the present study using SEM, we observed dramatic cell surface changes in *E. faecalis* that came in contact with the modified sealer. Syncytium-like aggregates and disrupted cells were observed, indicating cell wall destabilization that can be attributed to IABN immobilized on the AH Plus surface. The findings show that bacteria which come in contact with the modified sealers surface are eradicated within one hour. To demonstrate the process that the bacterial cells go through, scanning electron micrographs were taken at various time points during this first hour of direct contact, one of these time points was 20 min.

Quaternary ammonium compounds, similarly to IABN, have a positive surface charge and a hydrophobic moiety. The first step in the cationic disinfectants' lethal action is considered to be their adsorption to the bacterial cell surface (Kawabata & Nishiguchi 1988). IABN are bactericidal through a proposed mechanism of adsorption and penetration through the bacterial cell wall, after which they combine with the protein and analogous fat layer of the cell membrane. This causes blocks in the normal exchange of ions and substances and leakage of intracellular contents, leading to cell death (Gao *et al.* 2007). Other antimicrobial agents against endodontic pathogens, such as chlorhexidine show controversial results (Sena *et al.* 2006, Arias-Moliz *et al.* 2010). Although 12-methacryloyloxydodecylpyridinium bromide (MDPB) demonstrated a strong antibacterial effect, it was not tested when incorporated into endodontic sealers (Izutani *et al.* 2010).

It appears that the antibacterial mechanism of the nanoparticles is limited to direct contact. In accordance with previously published data (Kawabata & Nishiguchi 1988, Gao *et al.* 2007), it can be speculated that

when contact between the negatively charged bacteria and the positively charged IABN is established, bacterial cell wall disintegration may occur as observed by SEM. This study constitutes the basis for the performance of clinical studies using endodontic sealers containing antibacterial nanoparticles.

Conclusion

Commercially available endodontic sealers modified using IABN demonstrated an antibacterial effect against *E. faecalis*, which was maintained for 4 weeks. Incorporation of IABN into endodontic sealers reduced significantly *E. faecalis* bacterial counts. These durable antibacterial properties, plus the non-cytotoxic effect previously reported, may be useful in designing endodontic sealing materials. To establish clinical relevance, further investigation of the antibacterial potential against various microorganisms and their ability to function in different environmental situations is necessary.

Acknowledgement

Declaration: A patent entitled 'Antimicrobial Nanoparticulate Additives Forming Non-Leachable Sustained Antimicrobial Polymeric Compositions' is pending approval. We thank DENTSPLY Company for supplying commercial materials. The authors declare no other conflict of interests.

References

- Abdulkader A, Duguid R, Saunders EM (1996) The antimicrobial activity of endodontic sealers to anaerobic bacteria. *International Endodontic Journal* **29**, 280–3.
- Abramovitz I, Beyth N, Weinberg G et al. (2012) *In vitro* biocompatibility of endodontic sealers incorporating antibacterial nanoparticles. *Journal of Nanomaterials* **2012**, Article ID 858073, doi:10.1155/2012/858073.
- Arias-Moliz MT, Ferrer-Luque CM, Gonzalez-Rodriguez MP, Valderrama MJ, Baca P (2010) Eradication of *Enterococcus faecalis* biofilms by cetrimide and chlorhexidine. *Journal of Endodontics* **36**, 87–90.
- Beyth N, Yudovin- Farber I, Bahir R, Domb A, Weiss EI (2006) Antibacterial activity of dental composites containing quaternary ammonium polyethylenimine nanoparticles against *Streptococcus mutans*. *Biomaterials* **27**, 3995–4002.
- Beyth N, Domb AJ, Weiss EI (2007) An *in vitro* quantitative antibacterial analysis of amalgam and composite resins. *Journal of Dentistry* **35**, 201–6.
- Beyth N, Hourri- Haddad Y, Baraness- Hadar L, Yudovin- Farber I, Domb AJ, Weiss EI (2008) Surface antimicrobial activity and biocompatibility of incorporated polyethylenimine nanoparticles. *Biomaterials* **29**, 4157–63.
- Beyth N, Yudovin- Farber I, Perez-Davidi M, Domb AJ, Weiss EI (2010) Polyethylenimine nanoparticles incorporated into resin composite cause cell death and trigger biofilm stress *in vivo*. *Proceedings of the National Academy of Sciences of the United States of America* **107**, 22038–43.
- Editorial Board of the Journal of Endodontics (2007) Wanted: a base of evidence. *Journal of Endodontics* **33**, 1401–2.
- Fuss Z, Charniaque O, Pilo R, Weiss E (2000) Effect of various mixing ratios on antibacterial properties and hardness of endodontic sealers. *Journal of Endodontics* **26**, 519–22.
- Gao B, Zhang X, Zhu Y (2007) Studies on the preparation and antibacterial properties of quaternized polyethylenimine. *Journal of Biomaterials Science. Polymer Edition* **18**, 531–44.
- Hancock Hh III, Sigurdsson A, Trope M, Moiseiwitsch J (2001) Bacteria isolated after unsuccessful endodontic treatment in a North American population. *Oral Surgery, Oral Medicine, Oral Pathology Oral Radiology and Endodontics* **91**, 579–86.
- Izutani N, Imazato S, Noiri Y, Ebisu S (2010) Antibacterial effects of MDPB against anaerobes associated with endodontic infections. *International Endodontic Journal* **43**, 637–45.
- Kawabata N, Nishiguchi M (1988) Antibacterial activity of soluble pyridinium-type polymers. *Applied and Environmental Microbiology* **54**, 2532–5.
- Lai CC, Fm Huang, Yang HW et al. (2001) Antimicrobial activity of four root canal sealers against endodontic pathogens. *Clinical Oral Investigation* **5**, 236–9.
- Moradi S, Naghavi N, Rohani E, Javidi M (2009) Evaluation of microleakage following application of a dentin bonding agent as root canal sealer in the presence or absence of smear layer. *Journal of Oral Science* **51**, 207–13.
- Morgental RD, Vier-Pelisser FV, Oliveira SD, Antunes FC, Cogo DM, Kopper PM (2011) Antibacterial activity of two MTA-based root canal sealers. *International Endodontic Journal* **44**, 1128–33.
- Mutal L, Gani O (2005) Presence of pores and vacuoles in set endodontic sealers. *International Endodontic Journal* **38**, 690–6.
- Neelakantan P, Subbarao CV (2008) An analysis of the antimicrobial activity of ten root canal sealers—a duration based *in vitro* evaluation. *The Journal of Clinical Pediatric Dentistry* **33**, 117–22.
- Ørstavik D (1988) Antibacterial properties of endodontic materials. *International Endodontic Journal* **21**, 161–9.
- Ørstavik D, Haapasalo M (1990) Disinfection by endodontic irrigants and dressings of experimentally infected dentinal tubules. *Endodontics and Dental Traumatology* **6**, 142–9.

- Ricucci D, Siqueira Jf Jr (2010) Fate of the tissue in lateral canals and apical ramifications in response to pathologic conditions and treatment procedures. *Journal of Endodontics* **36**, 1–15.
- Sakamoto M, Siqueira JF, Rôças IN, Benno Y (2008) Molecular analysis of the root canal microbiota associated with endodontic treatment failures. *Oral Microbiology and Immunology* **23**, 275–81.
- Sena NT, Gomes BP, Vianna ME et al. (2006) *In vitro* antimicrobial activity of sodium hypochlorite and chlorhexidine against selected single-species biofilms. *International Endodontic Journal* **39**, 878–85.
- Shalhav M, Fuss Z, Weiss EI (1997) *In vitro* antibacterial activity of a glass ionomer endodontic sealer. *Journal of Endodontics* **23**, 616–9.
- Siqueira JF Jr, Favieri A, Gahyva SM, Moraes SR, Lima KC, Lopes HP (2000) Antimicrobial activity and flow rate of newer and established root canal sealers. *Journal of Endodontics* **26**, 274–7.
- Sjögren U, Figdor D, Persson S, Sundqvist G (1997) Influence of infection at the time of root filling on the outcome of endodontic treatment of teeth with apical periodontitis. *International Endodontic Journal* **30**, 297–306.
- Slutzky-Goldberg I, Slutzky H, Solomonov M, Moshonov J, Weiss EI, Matalon S (2008) Antibacterial properties of four endodontic sealers. *Journal of Endodontics* **34**, 735–8.
- Tobias RS (1988) Antibacterial properties of dental restorative materials: a review. *International Endodontic Journal* **21**, 155–60.
- Waltimo T, Trope M, Haapasalo M, Ørstavik D (2005) Clinical efficacy of treatment procedures in endodontic infection control and one year follow-up of periapical healing. *Journal of Endodontics* **31**, 863–6.
- Weiss EI, Shalhav M, Fuss Z (1996) Assessment of antibacterial activity of endodontic sealers by a direct contact test. *Endodontics and Dental Traumatology* **12**, 179–84.
- Yudovin-Farber I, Beyth N, Nyska A, Weiss EI, Golenser J, Domb AJ (2008) Surface characterization and biocompatibility of restorative resin containing nanoparticles. *Biomacromolecules* **9**, 3044–50.