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Characterisation of the antibacterial effect of polyethyleneimine nanoparticles in relation to particle distribution in resin composite

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ABSTRACT

Objectives: To characterise the antibacterial effect of resin composite incorporating cross-linked quaternised polyethyleneimine (QPEI) nanoparticles in relation to their distribution in the bulk material.

Methods: The antibacterial effect of resin composite incorporating QPEI nanoparticle was tested against various oral pathogens, including *Enterococcus faecalis*, *Streptococcus mutans*, *Actinomyces viscousus*, *Lactobacilus casei* and whole saliva. Nanoparticle distribution in the modified resin composite was assessed using X-ray photoelectron spectroscopy (XPS). Additionally, the degree of conversion was recorded.

Results: Total bacterial inhibition was detected against all the tested pathogens following direct contact with the outer surface of the modified resin composite. Similarly, the inner surface of the modified resin composite caused total inhibition. Electron microscope images showed bacterial death. XPS revealed surface I^- ions on both the outer and the inner surfaces of the modified composite. No I^- ions were detected in the unmodified composite. Nanoparticle distribution was higher on the inner surface of the modified composite. The composite's degree of conversion was unaffected by nanoparticle addition.

Clinical significance: QPEI nanoparticles represent a new generation of antibacterial nanoparticles which are highly promising in preventing bacterial recontamination when restoring teeth.

1. Introduction

In dentistry, surface coating with antibacterial materials is challenging, as the oral environment offers harsh conditions that may result in detachment or wear of the coating. Thus, enhancement owing to antibacterial properties through full material modification may be more durable.

Studies have shown higher bacterial accumulation on resin composites relative to other materials such as amalgam and glass ionomer.[1,2] Resin composite materials are widely used in tooth restoration, core buildup and in cementation due to their benefits, including aesthetics and adhesion to tooth structure.[3] However, the main drawback of resin composite-based materials is marginal leakage, which may result in secondary caries formation.[4]

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In previous studies it was shown that incorporation of antibacterial quaternised polyethyleneimine (QPEI) nanoparticles in resin composites results in a potent and long-lasting antibacterial effect. The antibacterial compound is stable and does not leach out from the material into the surrounding environment.[5–7] To provide antibacterial properties, QPEI nanoparticles were incorporated into resin composites. These particles most probably disrupt the passage of ions through the bacterial membranes, leading to membrane destruction and death.[5,8,9]

Particle surface area size is an essential component in nanoscale materials.[10] Specifically, particle surface area has a critical role when nanoparticles are used as antibacterial agents. As particle size is reduced, the proportion of the atoms found on the surface interface is enhanced relative to the proportion of the particle volume. This results in nanoscale particles, which are likely to be more reactive than microscale particles, thus generating a more efficient antibacterial effect upon application.[11,12] Unfortunately, alteration of the material surface properties using nanoparticles may be compromised because of the high tendency of the nanoparticles to aggregate,[5] resulting in a less effective surface antibacterial effect.[13] However, to generate an efficient effect, their mode of distribution in the bulk material is critical.[2–6,12]

The purpose of the present study was to characterise the antibacterial activity of QPEI against oral pathogens when incorporated in a resin composite material, in relation to the distribution of the nanoparticles in the bulk material.

2. Materials and methods

2.1. Test materials

Synthesis was as previously described.[7] 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 neutralisation 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 neutralisation 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. The average yield was ≥85% (mol/mol). Then the particles were washed with a 2% solution of N-lauryl-sarcosine surfactant (NLS). A total 20 g of prepared QPEI nanoparticles was placed in a Buchner funnel, using a paper filter and a vacuum source. A 200 ml volume of NLS solution was allowed to pass through the nanoparticles under vacuum conditions. Treated nanoparticles were freeze-dried overnight and a fine powder was obtained.

QPEI nanoparticles were incorporated in resin composite (3M™ ESPE™ Filtek™ Supreme XTE Flowable Restorative, St. Paul, MN, USA). Material curing was preformed according to the manufacturer's instructions.

2.2. Direct contact test (DCT)

The antibacterial effect was evaluated against various oral pathogens (the bacterial species are summarised in Table 1) and against whole saliva bacteria (as approved by the Helsinki Committee for Human Clinical Trials-HMO052511). Bacteria were grown to 10^6 colony forming units (CFU)/ml in brain heart infusion (BHI). The antibacterial effect of modified resin composites incorporating QPEI nanoparticles was tested using the direct contact test.[14] Briefly, triplicate wells in a 96-well microtiter plate were coated with resin composite incorporating QPEI nanoparticles (0%, 1% and 2% wt/wt). The plate was then aged by adding to each well 250 μl PBS, which were replaced every 48 h for 1 month at 37 °C. At the end of the ageing period the plate was dried under sterile conditions and 10 μl of tested bacterial suspension were placed on the surface of each test group to allow direct contact. After an hour, growth medium was added to each well and the plate was placed in a spectrophotometer for 24 h. Optical density readings in each well were recorded continuously every 20 min, with a 5 s mix before each reading. Data were analysed using Kruskal-Wallis One Way Analysis of Variance on Ranks.[15]

Table 1 – Bacterial strains used in the direct contact test.

Microorganisms	Source	Comments
Enterococcus faecalis	Clinically isolated at the Maurice and Gabriela Goldschleger School of Dental Medicine, Tel Aviv University, Israel	Streptomycin-resistant OD_{650} = 1 CFU/ml = 10^6
Streptococcus mutans	ATCC 700610	OD_{650} = ~1 CFU/ml = 10^6
Actinomyces viscousus	ATCC43146	
Lactobacillus casei	ATCC334	
From saliva	Clinical isolate	OD_{650} = 0.3 CFU/ml = 10^6

2.3. Inner and outer surface antibacterial effect

Discs (4.5 mm diameter × 4 mm height) incorporating 0% or 2% wt/wt QPEI nanoparticles were prepared using a silicone template. The discs were then cut in the middle, using a sterile scalpel. The test groups included: #1 – no added nanoparticles (0%, outer surface); #2 – no added nanoparticles (0%, inner surface); #3 – added nanoparticles (2%, outer surface); #4 – added nanoparticles (2%, inner surface).

The discs were inserted in a 24-well microtiter plate (flat bottom plate, Nunclon, Nunc, Denmark) and an *Enterococcous faecalis* (*E. faecalis*) suspension (10 μl; $OD_{650\ nm}$ of 0.5 ≈ 10^5 CFU/ml) was placed on the surface of each disc to test the antibacterial effect of the inner or outer surface of the material. The plate was incubated for 1 h at 37 °C. During this period the suspension liquid evaporated and a thin layer of bacteria was obtained, ensuring direct contact between all the bacteria and the tested surface. *E. faecalis* placed on the surface of the microtiter plate served as control.

After incubation, 1 ml of BHI was added to each well and the microtiter plate was placed on a titermix for 5 min (450 RPM). A 660 μl volume was transferred from each well to a fresh 96-microtiter plate and divided between three wells (220 μl in each well). The plate was then placed in a temperature-controlled microplate spectrophotometer

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