Antibacterial activity of bone cement containing quaternary ammonium polyethyleneimine nanoparticles

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A bone cement mantle is commonly used in orthopaedic surgery, and creates an ideal environment for bacterial growth. Bacterial infection following joint replacement surgery is a catastrophic complication. It is estimated that infections occur in up to about 2% of primary hip and knee replacements, whereas revision surgery carries a 2–3-fold higher risk.¹ The most common bacteria isolated from infected joints are Gram-positive cocci (Staphylococcus epidermidis, Staphylococcus aureus and Streptococcus spp.) followed by Gram-negative bacteria and, less frequently, mixed and fungal infections.² Addition of antibiotics to the cement has been advocated, especially in revision surgery.³ However, several studies describe the decreasing potency of common antibiotics in treating the infecting organisms and a parallel rise in the prevalence of resistant strains.⁴ As the implant itself lacks a blood supply, it is inherently susceptible to colonization by bacteria, which are less likely to be eradicated than in viable tissue. Therefore, efforts are focused on prevention of implant colonization. However, attempts to protect the implants using an antibiotic coating as well as the addition of antibiotics to the cement in the cemented implant have not proved successful.

A novel strategy involves the use of antibacterial molecules that are bound to the implant or to the surrounding cement and are not released but remain functional for long periods of time.⁵ Quaternary amine residues proved to have excellent antibacterial properties.⁶–⁷ The purpose of this study was to modify a commonly used bone cement to obtain a safe and long-lasting antibacterial effect using quaternary ammonium polyethyleneimine (QPEI) nanoparticles.

QPEI nanoparticles were synthesized as previously described.⁶ The tested materials were prepared by adding the synthesized powder to clinically available bone cement [Simplex™ P Bone Cement: 75% methyl methacrylate-styrene copolymer, 15% polymethylmethacrylate (PMMA) plus 10% barium; Stryker, Kalamazoo, MI, USA]. QPEI nanoparticles were added at 0%, 1%, 2% or 3% (w/w) to the bone cement and homogeneously mixed according to the manufacturer’s instructions. An antimicrobial effect against S. aureus ATCC 8325-4 and Entercoccus faecalis (a clinical isolate from the Maurice and Gabriela Goldschleger School of Dental Medicine at Tel Aviv University, Israel) was tested using the direct contact test (DCT)⁸ and agar diffusion test (ADT). Biocompatibility was tested on human primary polymorphonuclear cells as previously described.⁵ Cell viability was measured using the XTT assay (Biological Industries) and levels of tumour necrosis factor-α (TNF-α) in the supernatant were measured using an ELISA kit (Biolegend, San Diego, CA, USA). Additionally, physical properties of the cements were evaluated. Testing was performed using a Controlled Teststore 25 Tons MTS Device (Minneapolis, MN, USA) and results were analysed using MPT software (Multi-Purpose Testware 793.10, MTS System Corporation, Eden Prairie, MN, USA). Strain (ε) and Young’s modulus (E) were calculated for each specimen.

A strong antibacterial effect after an ageing period of 4 weeks was evident (P < 0.05) in all the bone cement samples in which the QPEI nanoparticles were incorporated compared with bone cement samples with no additives, which showed no antibacterial effect. The DCT showed significant antibacterial activity against both bacteria for at least 4 weeks (Figure 1); the ADT revealed no inhibition halo in the agar plates for both tested bacteria, indicating that the nanoparticles are retained in the PMMA and do not diffuse into the agar. Moreover, QPEI nanoparticles did not change the biocompatibility properties of PMMA. Addition of the nanoparticles at all tested percentages did not result in a significant change in cell viability compared with that of the bone cement group; incorporation of 0%, 1%, 2% and 3% QPEI.
nanoparticles resulted in relative viabilities of 58.3 ± 4.7%, 57.4 ± 7.4%, 44.6 ± 1.1% and 44.6 ± 2%, respectively. Similarly, TNF-α levels did not show significant changes compared with non-modified bone cement; 0%, 1%, 2% and 3% QPEI nanoparticles resulted in TNF-α levels of 3.2 ± 0.3, 2.5 ± 0.3, 2.6 ± 0.3 and 2.7 ± 0.3 pg/mL, respectively.

The Young’s modulus of bone cement incorporating 0%, 1%, 2% and 3% QPEI nanoparticles was 2.31 ± 0.1, 2.3 ± 0.2, 1.9 ± 0.2 and 1.8 ± 0.2 GPa respectively. No significant differences in Young’s modulus were found between the modified bone cement and bone cements with 1% and 2% QPEI nanoparticles.

The strategy of incorporating non-eluting nanoparticles in bone cement may be advantageous over antibiotic-loaded bone cement and presents an option for a significant reduction in prosthetic joint infection rate. Our results indicate that incorporation of QPEI nanoparticles in bone cements has a long-lasting antibacterial effect without compromising the cement’s biocompatibility and physical properties. Thus, early and late prosthetic joint infections may be prevented and the clinical performance of the implants may be prolonged.

As our study is based on in vitro assays, it is limited and cannot yield clinical recommendations. It is therefore suggested that, following safety tests and FDA/Conformite Europeene approval, incorporation of QPEI nanoparticles in bone cement could become an option when performing primary cemented joint replacement or revision. Future applications may include incorporation of antibacterial nanoparticles into prosthetic coatings and into formulations of orthopaedic and other implants.

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Figure 1. Bacterial outgrowth of S. aureus and E. faecalis following direct contact with bone cement incorporating 0%, 1%, 2% or 3% (w/w) QPEI nanoparticles. S. aureus and E. faecalis outgrowth served as control. Samples were aged for 4 weeks before testing. The results are expressed as mean ± SD.

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Transparency declarations

References