Functionalization of Polyurethane with Antimicrobial Nanoparticles, for Central Venous Catheters with Prolonged Anti-Biofilm Efficacy

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Statement of Purpose: Central Venous Catheters (CVCs) are indwelling intravenous devices which are essential in the management of critically ill and immunocompromised patients. Long term CVCs, which can remain *in situ* for >4 months, are associated with bacterial colonization and subsequent bloodstream infection, which is a major cause of patient morbidity.[1] The increasing prevalence of antibiotic-resistant bacteria highlights the urgency to address CVC-related infections via alternative methods. Hence there is a considerable drive to design and fabricate new antimicrobial CVC surfaces, to reduce initial colonization by microorganisms and eventual biofilm formation, without the need for conventional antibiotics. The purpose of this study is to apply recently developed, novel, antimicrobial nanoparticles (NPs) based on a hexametaphosphate (HMP) salt of chlorhexidine (CHX)[2] to the development of an antimicrobial polyurethane (PU) CVC with long-lasting anti-biofilm efficacy.

Methods: *Nanoparticle synthesis and characterization*
Colloidal NP suspensions were prepared from a 1:1 ratio of 5 and 50 mM aqueous CHX: aqueous HMP at room temperature and pressure. CHX-NPs were characterized by dynamic light scattering (DLS), atomic force microscopy (AFM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM) combined with energy dispersive X-Ray analysis (EDX).

PU sample functionalization
PU specimens (12 x 15 mm) were immersed in rapidly stirred CHX-NP suspensions for 30 s then in deionized water for 10 s before drying in air.

CHX elution studies
Functionalized PU specimens were individually immersed in 2.5 mL of deionized water in semi-micro cuvettes and agitated on an orbital shaker at 150 rpm. UV absorption at 255 nm was used to determine the release of soluble CHX from the 5 mM NP-coated specimens as a function of time[2] in comparison with control specimens which were immersed in aqueous 25 µM CHX, the residual concentration of aqueous CHX in 5 mM CHX-NPs.

Antimicrobial efficacy of CHX-NPs
Antimicrobial efficacy against methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae* was investigated using total viable counting (TVC) and bacterial viability assays. These are all microorganisms associated with biofilms on the surface of CVCs and causing CVC-related bloodstream infections.

Results: The CHX-NP colloids are composed of negatively charged nanoparticles (-55 mV) ranging from 45-80 nm in size. EDX analysis confirmed the presence of both chlorine and phosphorus in the NPs. NP shape can be tuned from spherical to rod-like, by increasing the concentration from 5 mM to 50 mM (Figure 1).

Functionalized PU specimens exhibited a continuous release of soluble CHX over an 86 day period, while the 25 µM CHX control did not show significant release of CHX (Figure 2).

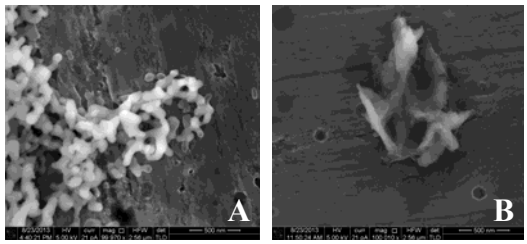

Figure 1. SEM micrographs. (A) Spherical 5 mM NPs. (B) Rod-like 50 mM NPs. Scale bars 500 nm.

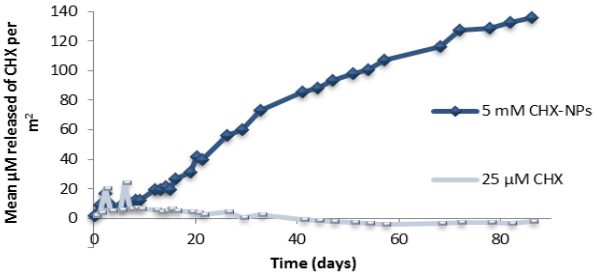

Figure 2. CHX release from NP-functionalized PU.

TVCs showed complete inhibition of MRSA, *P. aeruginosa* and *E. coli* when treated with 5 and 50 mM CHX-NPs. It was also observed from Live/Dead viability assays that 5 and 50 mM CHX-NPs prevented biofilm formation of MRSA, *P. aeruginosa*, *E. coli* and *K. pneumoniae*.

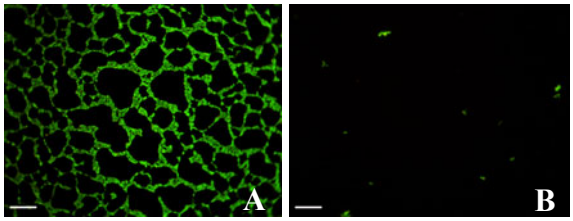

Figure 3. Live/Dead images of untreated MRSA (A) and MRSA treated with 5 mM NPs (B). Scale 13 µM.

Conclusions: 5 and 50 mM CHX-NPs were deposited onto PU surfaces representative of CVCs. 5mM CHX-NP specimens exhibited an extended release of soluble CHX over 86 days. Due to experimental design limitations, these results, in addition to 50 mM CHX-NP specimens, will be repeated over a longer period in future studies. The NPs prevented biofilm formation of MRSA, *P. aeruginosa*, *E. coli* and *K. pneumoniae*. Incorporation of novel antimicrobial CHX-NPs into a PU-CVC could provide a promising new approach to the prevention of catheter-related infections.

References: 1. Walz JM. J Intensive Care Med. 2010;25:131–8. **2.** Barbour ME. Int J Nanomedicine 2013;8:3507–3519.