SNEP- Silver Nanoparticles Embedded in Polymers: A New Class of Implant Coatings to Prevent Biofilm Formation

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Statement of Purpose: Failure of orthopedic implants due to microbial colonization is frequently increasing with the use of total-joint replacements. In addition to this, the emergence of antibiotic-resistance conferred by the microbiota and other opportunistic pathogens has exacerbated implant-failure. Hitherto, prevention of microbial colonization has been addressed by coating implants with small molecules. Nevertheless, the high implant replacement index dictates the need for effective alternatives [1]. We have developed an alternative strategy to use silver nanoparticles in implant coatings to prevent microbial colonization. The antimicrobial property of silver nanoparticles against gram-negative and gram-positive bacteria, including drug-resistant strains, namely methicillin-resistant *S. aureus* is very well documented. Further, the effects of geometry of silver nanoparticles and its role as antibacterial agents on implants are being actively investigated. Considering the potential of silver nano particles and the deficit in implant coatings; we bridge this gap by developing an implant coating comprised of silver nanoparticles. This new class of SNEP is comprised of silver nanoparticles (AgNP) encapsulated in dextran-chitosan co-polymer. We have assessed i) the antimicrobial effects associated with the geometry of AgNP, ii) release of AgNP from co-polymer coating, iii) microbial colonization of coating; and iv) cytotoxicity.

Methods: Silver nanoparticles (AgNP) of spherical (s), triangular (t) and cuboid (c) shapes were synthesized by chemical reduction methods [2, 3]. Briefly, tAgNP were synthesized by adding sodium borohydride ($NaBH_4$) to a suspension of silver nitrate ($AgNO_3$), trisodium citrate, poly(vinyl pyrrolidone) (PVP) and hydrogen peroxide and stirred until the colloid turned blue indicating the formation of triangular AgNP. sAgNP were synthesized by the same method but without the addition of hydrogen peroxide and trisodium citrate. cAgNP were synthesized by combining silver nitrate and PVP in ethylene glycol over heat to produce AgNP of cuboid shape. Particles were then isolated and washed by centrifugation. The antibacterial property of these AgNP against *S. aureus* and methicillin resistant *S. aureus* (MRSA) over a period of 7 days was determined using a colorimetric assay. Further, the cytotoxic effects of AgNP against human fetal osteoblast (hFOB) were assessed using LIVE/DEAD cytotoxicity/viability assay. Minimal cytotoxic concentration of AgNP preventing biofilm formation was carefully chosen to encapsulate in the implant coating. The coating is a co-polymer of chitosan and dextran. The release kinetics of the AgNPs from the coating was determined over a period of 7 days. Briefly, the AgNP-co-polymer mix was coated onto a Ti64 disk (15mm diameter) and sterilized by ethylene oxide (ETO) for 12 hours. The coated disks were then submerged in 2ml 1xPBS and incubated at 37^{o}C for 7 days. AgNP was measured daily using a colorimetric assay employing a PAN reagent silver determination kit. Subsequently, the antibacterial property of the AgNP-loaded co-polymers against *S. aureus* and MRSA was evaluated using XTT assay that monitors metabolic activity. Moreover, the cytotoxic effects of the AgNP-loaded co-polymers against hFOB cells were evaluated using MTT assay, a viability assay. All experiments were tested for statistical significance using one-way ANOVA.

Results: Despite the geometry, AgNPs at a concentration of 25µg/ml conferred 50% and 20% reduction in *S. aureus* and MRSA cultures with no concomitant cytotoxic effects. Furthermore, tAgNP conferred the greatest activity against *S. aureus* and MRSA (Fig 1). When loaded into the chitosan-dextran co-polymer coating, the AgNP of all geometries displayed a burst release followed by a slow but continuous release. Microbial studies on AgNP-doped co-polymers exhibited greater antibacterial activity than the AgNP in suspension or the co-polymer alone. Further, the tAgNP toxicity toward hFOB at the same concentrations was reduced when compared to sAgNP (Fig 2).

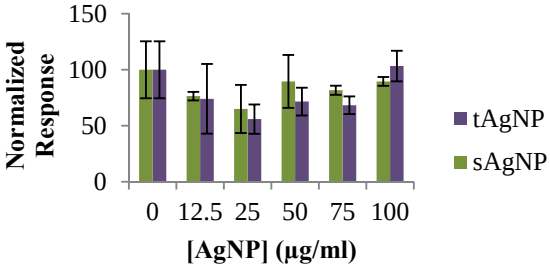

Figure 1: Susceptibility profile of *S. aureus* against silver nanoparticles

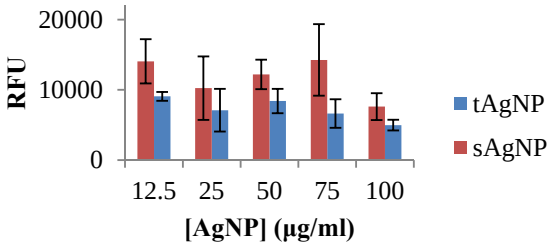

Figure 2: Toxicity profile of hFOB against silver nanoparticles

Conclusions: The preliminary studies indicate the favorable use of tAgNP against *S. aureus* and MRSA. Based on the results, SNEPs portrayed higher antimicrobial property with minimal cytotoxic effects towards hFOB[4]. Thus SNEPs might serve as a new class of alternatives to implant coatings.

References: **[1]** Mahendra R, et al., Biotechnol Adv, 2009, 27, p. 76-83. **[2]** Metraux G, et al. Adv Mater, 2005, 17, p. 412-415. **[3]** Sun Y., et al, Science, 2002, 298, p. 2176-2179. **[4]** Kong M, et al., Int J Food Microbiol, 2010, 144, p. 51-63.