Antimicrobial Effect of Calcium Phosphate Spheres decorated with Ag nanodots on Periodontal Pathogens

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Statement of Purpose:

Dental implants are susceptible to infectious complications accounting for approximately half of the early implant failures[1]. Infection may occur due to contamination of the surgical site or from adjacent periodontally infected teeth, but may also occur due to bacterial persistence in apparently healed bone following tooth extraction[1,2]. Bacteria accumulate on implant surfaces, leading to biofilm formation, chronic inflammation and inhibiting osseointegration processes. Despite aggressive sequential cumulative treatments involving mechanical debridement, antiseptic surface treatment, antibiotic treatment and regenerative or access/resective surgery, many implants require removal[3]. Implant coatings that locally inhibit bacterial attachment and promote healing may address these clinical challenges. For this purpose, a composite coating of chitosan with porous calcium-phosphate (CaP) nanospheres decorated with silver (Ag) nanodots is proposed. The nanospheres will be used as drug-delivery vehicles and as an antimicrobial agent. In this project the antimicrobial effect of the nanospheres on periodontal pathogens was assessed.

Methods: Fabrication of porous CaP nanospheres is based on a hydrothermal process using aqueous D-glucose, biomimetic deposition of calcium-phosphate from simulated body fluid, and heating at 300C for 2hrs.[4] Porous nanospheres are decorated with Ag nanodots by microwaving nanospheres in silver hydroxide solution.[5] Four types of Ca-P nanospheres were tested: Apatite (0%Ag), Apatite + 15% Wt Ag, Apatite + 38% Wt Ag, and Apatite + 50% Wt Ag. Two different amounts (2.5 and 5mg) were added to 0.5 mL of supplemented tryptic soy broth and pre-reduced in an anaerobic chamber for 48 hours. The samples were incubated with 350 µL of bacteria suspension containing $3.5x10^5$ organisms. *P. gingivalis*, *P. intermedia* and *A. actinomycetemcomitans* were incubated for 3 days with the Ag nanoparticles. The supernatants from the samples were collected, aliquoted in 100 µL in triplicate, placed in a 96-well micro titer plate, mixed with 10 µL of MTT label (Roche labs) and incubated for 4 hours anaerobically. The samples were solubilized and incubated over night at 37°C. The reduced MTT crystals in the samples were measured and quantitated spectrophotometrically, to indicate viable bacteria.

Results: The nanospheres exhibit a range from micro to nano scale (Figure 1). The EDS Spectra shows the weight percent of Ag nanoparticles attached to the spheres (Figure 2). Increasing Ag nanoparticles in the nanospheres showed a marked decrease in the viable bacteria at the end of the incubation period (Figure 3). There was a greater response with the higher volume of nanospheres; however, the responses were similar with all three bacteria types tested. The 50% Ag nanospheres showed almost complete bacteria destruction with only 6-20% cells remaining viable after 48 hrs.

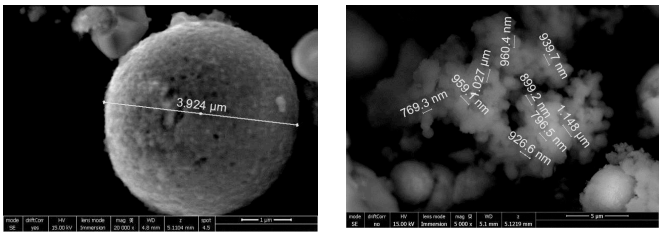

Figure 1: Ca-P nanospheres ranging from micro to nano

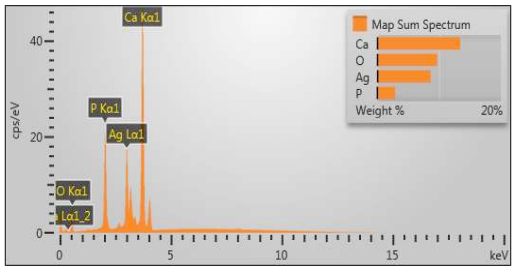

Figure 2: EDS spectrum from 15% Ag Nanospheres

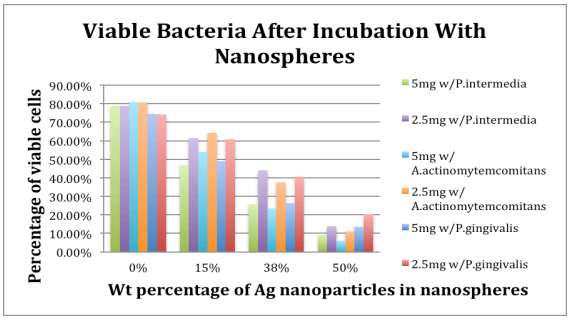

Figure 3: Percentage of viable bacteria after incubation

Conclusions: The Ag laden nanospheres show promise as a broad antimicrobial agent. The antimicrobial ability of the sphere is directly correlated to the amount of Ag present. Future studies should focus on the drug delivery ability of these porous beads to possibly create a dual action antimicrobial bead.

References:

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