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Bacterial inhibition by chitosan coatings loaded with silver-decorated calcium phosphate microspheres

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ABSTRACT

Porous calcium phosphate microspheres have been modified to contain nanoparticles of silver to provide both osteoconductive and antimicrobial components to implant coatings. These microspheres have been mixed with chitosan and bonded to titanium via alkyloxysilane reaction. Silver concentration on calcium phosphate microspheres was varied from 0 to 50% and microspheres were loaded at 30 wt.% within chitosan coatings. Increasing concentrations of silver loaded on calcium phosphate microspheres within the chemically bound coating reduces bacterial viability by up to 90% in both anaerobic and aerobic pathogenic microorganisms, including *Staphylococcus aureus*, *Prevotella denticola*, and *Porphyromonas gingivalis*. This novel coating could reduce the incidence of infection in orthopaedic and dental implant applications.

1. Introduction

Biofilm occurs when microorganisms attach to a surface [1,2]. Many different types of microorganisms, including bacteria and fungi, are capable of forming biofilm [3,4]. Up to 80% of clinical infections are biofilm-based [2,5]. For implanted devices, such as dental implants and total joint arthroplasty devices, the presence of metal biomaterials increases the susceptibility to biofilm formation and subsequent infection [6,7]. Because biofilm bacteria enter a senescent state of non-division, communicate within multi-organism communities, and produce an exopolymeric substance, they become resistant to traditional antibiotic therapy, immune cell attack, and other treatment methods [8]. Further, biofilm can form from polymicrobial communities conferring increased virulence and presenting difficult diagnostic and treatment challenges [3,4,9]. Often the only viable course of action is to remove the implant, thoroughly debride and wash the area, place local antibiotic delivery devices as an adjunct to systemic antibiotic therapy, and wait for the infection to clear before revision and reconstruction procedures [10].

Local antibiotic delivery has been increasingly researched to prevent or treat biofilm-based infection [10–12]. Advantages of local delivery are that high doses of antimicrobials can be delivered directly to the site, which may overcome treatment obstacles such as limited vascularity and poor diffusion of antimicrobials to affected tissue [13,14]. Silver is a known antimicrobial with broad spectrum activity against multiple different types of microorganisms [15–17], but in some forms has been found to cause toxic reactions in cells and tissues [18,19]. In order to effectively deliver antimicrobials to the injured site as well as to prevent high levels of systemic release, surface coatings and modifications are an advantageous local delivery strategy for implants [20].

Antoci et al. have developed methods to chemically bond the antibiotic vancomycin to titanium and bone graft implants [21], however vancomycin is only effective against Gram-positive microorganisms. Implants dipped in the anti-biofilm antimicrobial farnesol and dried inhibited immediate biofilm formation in an in vitro model [22], however, long term release without binding the antimicrobial to the surface limits long term activity. Researchers have achieved coatings of silver on titanium through physical vapor deposition [23], ion implantation [24], and co-sputtering with hydroxyapatite [25] to achieve reduction in bacterial attachment and viability in vitro. Among the concerns with silver-modified implant surfaces are host-tissue response including osseointegration and silver ion release.

In this study a titanium coating of the biodegradable natural polymer chitosan and silver-loaded calcium phosphate microspheres has been evaluated for its antimicrobial activity against common oral and bone pathogens. Both the chitosan and calcium phosphate components are

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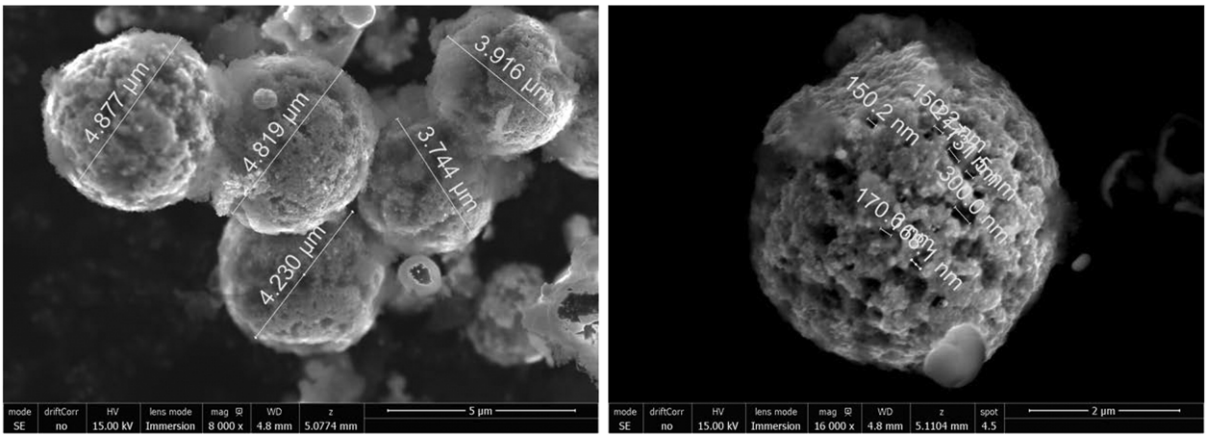

Fig. 1. SEM micrographs of silver-decorated microparticles showing representative particle and pore sizes.

known to aid in osseointegration of implants [26,27]. Coatings are expected to degrade over the course of 3–4 months [28,29], during which calcium phosphate becomes incorporated into new bone in direct apposition to the implant [30,31]. Silver released into tissues would be minimal and removed via biliary excretion [32]. Chemical bonding of the natural biopolymer chitosan to a titanium surface allows for incorporation of microparticles with antimicrobial silver to provide a barrier to bacterial attachment at the surface [15,29,33–35].

2. Experimental

Microsphere fabrication.

Porous calcium phosphate were fabricated using a carbon nanosphere template derived from hydrothermal processing of aqueous D-glucose, biomimetic deposition of calcium phosphate from simulated body fluid on carbon nanospheres, and then heating at 400 °C for 2 h to yield hollow hydroxyapatite microspheres ~4000 nm in diameter with 150 nm diameter pores randomly distributed (Fig. 1).

Porous microspheres were decorated with ~17 nm Ag nano-dots by microwaving microspheres in silver hydroxide solution. The concentration of silver hydroxide solution was varied to produce microspheres with 0, 15, 37, and 50% silver. Particles were imaged using Nova NanoSEM 650 scanning electron microscopy (FEI, Hillsboro, OR) to determine size distribution and morphological features. Electron Dispersive Spectroscopy (EDS) was then performed for elemental mapping using an Oxford EDS system and an X-MaxN50 detector.

Chitosan coating fabrication.

Coatings were created by mixing 30 weight % calcium phosphate microspheres into solutions of 2 wt% chitosan in acetic acid. This solution was cast onto titanium squares pretreated with triethoxsilylbutyraldehyde to chemically bond chitosan to titanium [36].

After drying overnight, coatings were neutralized by dipping into a solution of 0.05 M sodium hydroxide in 80% ethanol and washing thoroughly with sterile water [1]. Chitosan coatings without calcium phosphate and uncoated titanium were also prepared as controls.

Bacterial viability assay.

Coated and uncoated Ti squares were placed in 6-well culture dishes, and then incubated with 3.0 ml of pre-reduced culture media containing 5×10^6 bacteria (*Porphyromonas gingivalis*: ATCC BAA-308; *Prevotella denticola*: ATCC 35308) for 3 days in an anaerobic chamber. *Staphylococcus aureus* (ATCC 25923) was grown for 24 h in trypticase soy broth under aerobic conditions at 37 °C and 5×10^6 bacteria were added to each well containing coated and uncoated disks and incubated for 24 h under aerobic conditions. At the end of the incubation period the test materials were rinsed twice with PBS and then incubated with a tetrazolium dye (MTT) label (Roche Life Sciences) (30 μl in 3.0 ml of PBS) for 4 h. After incubating with MTT label, a solubilizing agent was added and incubated overnight.

Supernatants were collected from each test material, centrifuged briefly and the absorbance of the supernatant was measured at 550 nm (according to the protocol). Percent viability was calculated based upon the absorbance value obtained from the total number of bacteria added to each sample. One-way ANOVA with Holm–Sidak post hoc testing was performed to determine significant differences in bacterial viability between groups.

3. Results and discussion

Coatings were formed with uniform consistency on titanium materials. Porous calcium phosphate nanospheres formed were black in color, with increasing levels of silver changing the color of coatings. EDS spectroscopy confirmed the presence of silver on the calcium phosphate microspheres (Fig. 2). The porosity of the hollow mineral microparticles may allow for incorporation and extended release of therapeutics such as antibiotics, anti-inflammatory agents, and growth factors.

Chitosan-coated titanium and coatings loaded with calcium phosphate without silver nanoparticles had minimal effect on bacterial viability or attachment. As the concentration of silver nanoparticles on calcium phosphate microparticles increased, bacterial viability was decreased to 42.8% for 15% Ag and 9.6% for 50% Ag loading (Fig. 3). Percent reduction in viability for different silver levels was uniform across different strains of bacteria. Uncoated titanium had lower attachment and viability than

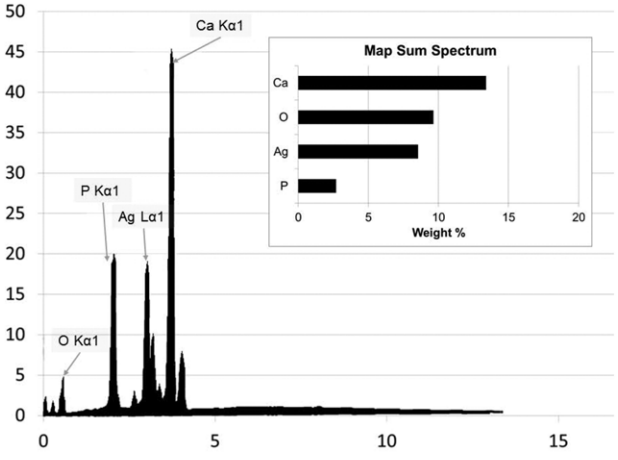

Fig. 2. Representative energy dispersive X-ray spectrum of silver-decorated microparticle samples showing the presence and relative amounts of calcium, phosphorus, silver, and oxygen.