**INTRODUCTION**

Implant-associated infection, although rare, has been one of main complications accompanied by orthopedic implant surgeries including prosthetic joint replacement and fracture fixation. The number of such infections has continuously increased with growing demands for surgical implantation due to population aging and increased participation in outdoor activities. The established infections may not be effectively treated with systemic chemotherapy with antibiotics, often necessitating additional surgical procedures such as simple debridement and reimplantation. Even for systemic antibiotic treatment, there still remain several concerns, for example, systemic toxicity, low efficiency and hospitalization issue. Therefore, there have been continuing need to deliver antibacterial agent to the surgical site in a sustained manner.

Calcium phosphate (CaP) biomaterials including hydroxyapatite (HAP), β-tricalcium phosphate (β-TCP) and biphasic calcium phosphate (BCP) composed of HAP and β-TCP, are widely used as a bone substitute and a coating material on various orthopedic implants. On the other hand, silver ions have demonstrated broad antibacterial spectrum including gram-positive and gram-negative pathogens. In view of clinical relevance of CaP materials and well-known antibacterial activity of silver, we hypothesized that the silver ions gradually released from CaP-based orthopedic implants would efficiently suppress postsurgical infections. Here, we present a facile method to grow antibacterial silver particles on CaP biomaterials and subsequently release silver ions for a controllable period of time. Our strategy includes two steps; i) binding a reducing agent, citrate, on CaP surface and ii) reducing silver ions (Ag+) into silver (Ag0) locally on the CaP surface. Our goal in this study is the formation of silver particles on CaP-based biomaterials and the sustained release of silver ions with controllable dosage.

**METHODS**

We tested three different types of CaP materials, HAP, β-TCP, and bone-like mineral (BLM) coating. HAP slabs were generously provided by Prof. Amy Waggoner-Johnson at University of Illinois at Urbana-Champaign. The BLM coatings were prepared on poly(lactide-co-glycolide) (85:15) films using modified simulated body fluid (mSBF) as described in our previous studies. To create silver particles, CaP materials were first incubated in sodium citrate solution (1, 5 and 10 mM) for predetermined time periods (0.5, 1 and 4 hour). After washing, citrate-bound CaP materials were transferred and incubated in silver nitrate solution (1, 5 and 10 mM) for different times (0.5, 1 and 4 hour). The treated CaP materials were washed and freeze-dried. The resultant CaP materials were imaged by scanning electron microscope (SEM), and chemical compositions of their surface were examined using energy dispersive x-ray spectroscopy (EDS). The silver ion release was monitored in water using inductively coupled plasma optical emission spectrometry (ICP-OES).

**RESULTS**

Silver particles could be created on all CaP biomaterials tested (Figure 1). SEM images showed that the size of silver particles was in the range of tens nanometers to several micrometers, depending on the preparation conditions (representative images shown in Figure 1a-c). Specifically, the size of silver particles was increased with longer incubation time and higher concentration of silver nitrate solution, whereas incubation time and concentration of sodium citrate solution did not affect the size of silver particles. In all conditions, the particles on CaP substrate uniformly covered on the entire surface. Appearance of silver peak in EDS spectrum also confirmed the formation of silver particles (Figure 1d). It should be noted that we could not see any indication (i.e., color change) of silver particle formation in the bulk solution. This implies that the silver particles were likely nucleated and grown selectively on CaP surface.

Once we confirmed broad applicability of the method to various CaP materials, we chose BLM coatings to further examine the effect of preparation parameters (i.e., concentration and incubation time of silver nitrate solution and sodium citrate solution). When the incubation time is increased to 4 hour in sodium citrate solution, the silver ion release rate was significantly increased (Figure 2a). The sodium nitrate concentrations tested did not influence the release behavior of silver ions (Figure 2b). In all groups treated with sodium citrate, the silver ions were released without initial burst release, while the groups untreated with sodium citrate show much higher initial release during the first two days.

![Figure 2](image2.png)

**DISCUSSION**

We demonstrated that antibacterial silver particles could be grown on various CaP biomaterials including HAP, β-TCP and BLM. The process was simple, and likely to be applicable to a broad range of orthopedic devices. The release kinetics and the quantity of released silver ions were controlled by changing the concentration and incubation time of sodium citrate and silver nitrate. The time period of silver ion release was in the range of 3 days to over 30 days, depending on preparation conditions. Pretreatment using sodium citrate led to linear silver release kinetics without initial burst release.

**SIGNIFICANCE**

Long-term release of antimicrobial silver ions from CaP-based orthopedic implants could efficiently prevent implant-associated infection. This method is expected to be readily applicable to orthopedic devices in current clinical setting, which makes clinical translation easier.