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Development of nanosized silver-substituted apatite for biomedical applications: A review

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Abstract

The favorable biocompatibility of hydroxyapatite (HA) makes it a popular bone graft material as well as a coating layer on metallic implant. To reduce implant-related infections, silver ions were either incorporated into the apatite during co-precipitation process (AgHA-CP) or underwent ion-exchange with the calcium ions in the apatite (AgHA-IE). However, the distribution of silver ions in AgHA-CP and AgHA-IE was different, thus affecting the antibacterial action. Several studies reported that nanosized AgHA-CP containing 0.5 wt.% of silver provided an optimal trade-off between antibacterial properties and cytotoxicity. Nevertheless, nanosized AgHA and AgHA nanocoatings could not function ideally due to the compromise in the bone differentiation of mesenchymal stem cells, as evidenced in the reduced alkaline phosphatase, type I collagen and osteocalcin. Preliminary studies showed that biological responses of nanosized AgHA and AgHA nanocoatings could be improved with the addition of silicon. This review will discuss on nanosized AgHA and AgHA nanocoatings.

Key words: Antibacterial; Apatite; Nanosized; Silver

Hydroxyapatite (HA) is a synthetic bone alternative, which possesses a chemical similarity to the bone mineral. Since HA is biocompatible when implanted *in-vivo*, it exhibits bioactive behavior by forming a direct bond between the implants and bones (osseointegration). Therefore, HA is commonly used as a bone graft to fill the defects or deposited as a coating layer on orthopedic implant, to promote bone regeneration in order to facilitate bone healing process. However, due to the lack of protection from the body immune system, HA is susceptible to immediate and delayed infections, leading to implant-related infections. Despite the use of perioperative antimicrobial prophylaxis and laminar flow operating rooms, implant-related infections were common.[1] According to the U.S. Centers for Disease Control and Prevention (CDC), the risk of acquiring serious infections during medical treatments had risen over 35% in the last 20 years.[2] In 1990s, implant-related infections accounted for about half of all hospital-acquired infections.[3] Today, with the increasing use of implants, the cases of implant-related infections are expected to increase rapidly, particularly in sub-populations comprising of immune-compromised, chronically ill, and elderly patients. Besides pain and suffering, implant-related infections often incurred huge medical costs.[4] For example, an estimated average direct cost associated with an infected case was reported to range between US$15 K and US$30 K, which was approximately three times of the initial intervention.[5] Although the rate of infections was observed to be only 5% for the primary cases, it could be greatly increased to 43% for previously infected cases.[6] Thus, the issue of implant-related infections is a major problem affecting the service life of the medical implants. As such, it is equally important to reduce the intrinsic vulnerability of the biomaterial so as to minimize bacterial colonization.

Upon implantation, a competition exists between the integration of material into the surrounding tissues and the adhesion of bacteria onto the implant surfaces.[7] For a successful implantation, tissue integration must occur prior to appreciable bacterial adhesion as host defenses are often not capable of

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Table 1
Various proposed mechanism of antibacterial action by silver ions.

Proposed mechanism	Ref.
Silver inhibited the uptake of phosphate and caused the efflux of intracellular phosphate	98
Silver ions bound to sulfhydryl groups of the many important metabolic enzymes of the bacterial electron transport and respiration. For example, silver bound to the thiol groups (sulfhydryl, S-H) presented in the cysteine residues of the transport proteins, which induced a massive proton leakage though the bacterial membrane, resulting complete de-energization, and ultimately leading to cell death.	1, 35, 99-102
Silver ions entered into the bacterial cells by penetrating through the cell wall, and turned the DNA into condensed form. As a result, DNA lost its replication ability and led to cell death.	103
Silver ions bound to microbial DNA by interacting with nucleic acids, and changed DNA structure, which consequently prevented bacterial replication.	104
Silver ions were observed to increase the DNA mutation frequencies during polymerase chain reactions.	105
Silver ions generated reactive oxygen species (ROS) and damaged the cell membrane.	106-109
Bacterial cell suffered from morphological changes such as cytoplasm shrinkage and detachment contents such as potassium ion.	103, 110

preventing further colonization if bacterial adhesion occurs before tissue integration. Implant-related infections are caused by the consequence of bacterial adhesion and subsequent biofilm formation at the implantation site. Biofilm formation proceeds as a four-step process: (1) initial attachment of bacterial cells; (2) accumulation in multiple cell layers; (3) biofilm maturation; and (4) detachment of cells from the biofilm into a planktonic state to initiate a new cycle of biofilm formation elsewhere.[8] Bacteria living in a biofilm are highly resistant to antibacterial agents, and biofilm shields them from the influence of host's defense.[9] Thus, even antibiotic therapies remain ineffective against biofilms.

Previous works on loading biomaterials with antibiotics for orthopedic applications were carried out using bone-filler materials and bone cements.[10-12] However, the removal of pathogen in the open fracture injury and periapical lesions of jaws (dental) was not easily resolved by systematic antibiotic. Moreover, bacteria would develop resistance against antibiotic over time. In some worst cases, when biomaterial responded poorly to the antibiotic therapy, the removal of the infected implant was necessary to cure it.[13] Thus, implant-related infections complicate bone healing, and can also lead to the failure of orthopedic surgery. Nevertheless, effective results of antibacterial activities of implant materials designated with antibacterial properties were reported in recent commercially available silver-impregnated dressing and catheters.[14-16] Hence, this shows the potential of incorporating antibacterial properties to the implant material, which will become the upcoming strategies to treat implant-related infections.

Therefore, it will be beneficial if HA-based bone grafts or HA coatings are incorporated with antibacterial properties as this will aid in reducing the occurrence of implant-related infections. To overcome the infection issues encountered in HA, substitution of functional ion such as silver has emerged as a preventative approach. Silver ions were reported to interfere with the integrity of the bacterial cell, or bind to the enzymes and proteins within the bacteria, which severely damaged the cell and its major functions such as permeability, regulation of enzymatic signaling activity, cellular oxidation and respiratory processes, resulting in the bacteria death. Hence, this review paper will firstly discuss on the antibacterial action of silver. Nanosized silver-substituted HA (AgHA) will be featured in two forms — bulk and nanocoatings. Silver ions were either incorporated into the apatite structure during the co-precipitation process (AgHA-CP) or underwent ion-exchange with calcium ions in the apatite (AgHA-IE), which would be discussed in detail in the synthesis section. However, the distribution of the silver ions in AgHA-CP and AgHA-IE was different, which in turn affected the antibacterial action. This would be discussed through summarizing the chemical and physical characterization, antibacterial properties and biological responses of AgHA-CP and AgHA-IE, which were reported from numerous experimental studies. For the second part of the review, AgHA-CP and AgHA-IE were deposited using various techniques onto the substrate to form nanocoatings. Similarly, the different distribution of the silver ions in AgHA-CP and AgHA-IE nanocoatings affected the chemical and physical characterization, antibacterial properties and biological responses, and these properties would also be discussed. Lastly, preliminary work on the introduction of silicon to improve the biological responses of bulk AgHA and AgHA nanocoatings will be presented.

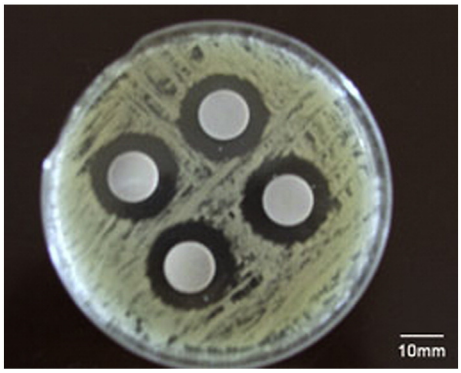

Figure 1. Photograph of antibacterial test results of AgHA-IE samples against *E. coli*.[28] Reprinted from applied surface science, 257, Stanic V., Janackovic D., Dimitrijevic S., Tanaskovic S. B., Mitric M., Pavlovic M. S., Krstic A., Jovanovic D., Raicevic S., Synthesis of antimicrobial monophase silver-doped hydroxyapatite nanopowders for bone tissue engineering, p. 4510, Copyright (2011), with permission from Elsevier.

Silver as an antibacterial agent

One common and accepted strategy to prevent implant-related infections is to create antibacterial properties for the implant. Implants with antibacterial properties reduce the treatment duration,[17] and decrease the side effects of systemic treatments,[18] thereby improving its efficacy. Inorganic antibacterial agents generally have a low resistance against bacteria, and are comparatively stable than organic ones, thus they become an