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Original Article

Antibacterial silk fibroin/nanohydroxyapatite hydrogels with silver and gold nanoparticles for bone regeneration

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Abstract

The rapid emergence of antibiotic resistance is becoming an imminent problem in bone tissue engineering, and therefore biomaterials must be modified to promote the tissue integration before bacterial adhesion. In this work, silk fibroin/nanohydroxyapatite hydrogel was modified with *in situ* synthesized silver and gold nanoparticles (AgNPs and AuNPs), taking advantage of the tyrosine amino acid. The presence of AgNPs and AuNPs in the hydrogels was characterized by UV spectrophotometer, transmission electron microscopy and thermogravimetric analysis. *In vitro* antimicrobial studies revealed that hydrogels with AgNPs and AuNPs exhibited significant inhibition ability against both gram-positive and gram-negative bacteria. Cytocompatibility studies carried out using osteoblastic cells revealed that up to 0.5 wt% of AgNPs, and for all concentrations of AuNPs, the hydrogels can be effectively used as antimicrobial materials, without compromising cell behavior. On the basis of the aforementioned observations, these hydrogels are very attractive for bone tissue engineering.

Key words: Silk fibroin; Nanohydroxyapatite; Silver nanoparticles; Gold nanoparticles; Antimicrobial activity

The ideal approach for bone tissue engineering is that the tissue integration occurs prior to bacterial adhesion, thereby preventing material colonization for certain bacterial species. Adhesion of bacteria to human tissue and implanted biomaterials is the first critical step in the pathogenesis of infection, whereby the bacteria can divide and colonize the surface. After adhering to the surface, some bacterial strains, particularly *Staphylococcus epidermidis*, secrete a layer of slime, which serve to anchor the bacterial cells. Bacterial biofilms are particularly problematic because sessile bacteria can often withstand host immune response and antibiotic therapies. Additionally, these sessile biofilms can give rise to nonsessile individuals, planktonic bacteria that can rapidly multiply and disperse.[1–4] A very large proportion of biomaterial-associated infections in orthopedics are caused by *S. aureus* and *S. epidermidis*, the main responsible agents for the two major types of infection affecting bone, septic arthritis and osteomyelitis, with consequent devastating effects on bone and surrounding soft tissues. Treatment for *S. aureus* infections is often complex, namely due to the emergence of methicillin-resistant*S. aureus* (MRSA) strains and resistance to other classes of antibiotics.[1,5]

The increase in antibiotic-resistant bacterial strains has prompted a renewed interest in the development of new antimicrobial approaches.[6–8] Metallic elements have garnered prominent consideration as they present antimicrobial properties. Moreover, these metallic elements in the form of nanoparticles are excellent candidates for antimicrobial applications due to their large surface area to volume ratio, providing better contact with microorganisms, and these nanoparticles are also an

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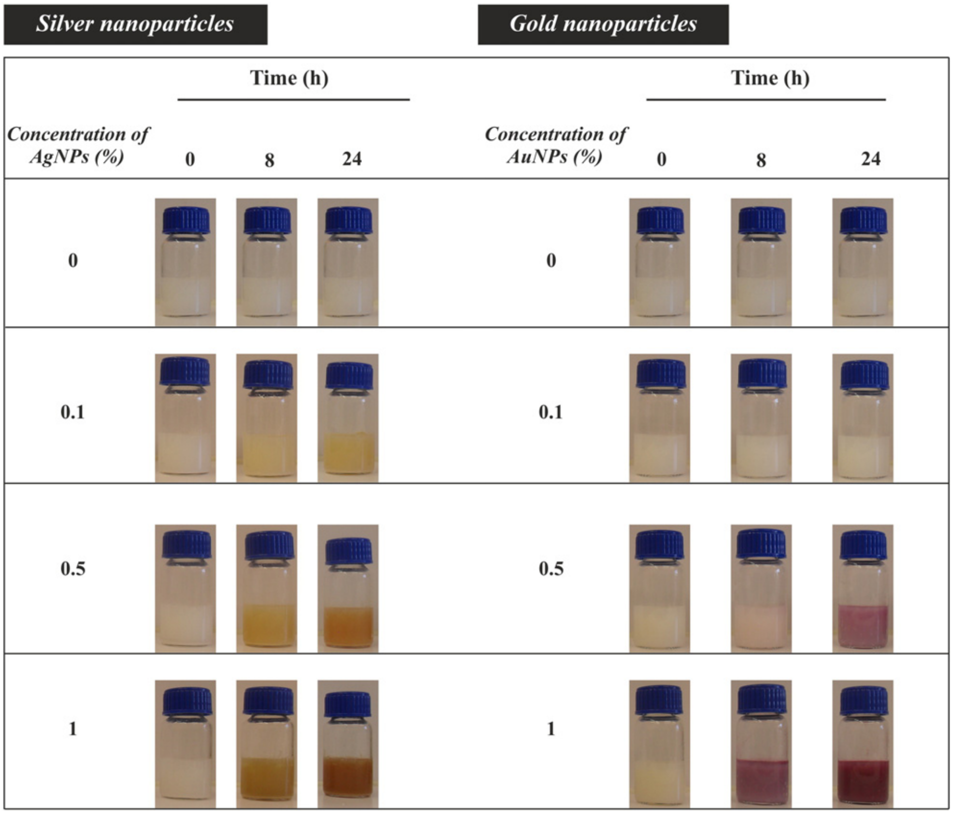

Figure 1. Photographs illustrating SF/nanoHA hydrogels with different AgNPs and AuNPs concentrations over different periods of time.

encouraging approach concerning their chemical stability, long life, and heat resistance.[9,10] In recent years, silver nanoparticles (AgNPs) have attracted much attention for a range of biomedical applications owing to their potent antimicrobial activity against a large number of bacteria, including antibiotic-resistant strains.[11–13] The antibacterial activity of AgNPs has been related to inhibition of enzymatic activities, prevention of DNA replication and disruption of bacterial cell membranes.[9,14] Gold nanoparticles (AuNPs) are also getting huge attention since their antimicrobial activity has been recently reported.[15,16] The exact mechanism of bacterial growth inhibition has not been elucidated yet, however some reports present the bacterial wall damage as the cause of the bacterial cell death.[17]

Silk fibroin (SF) is a natural, biocompatible, biodegradable and low-cost polymer obtained from the cocoons of *Bombyx mori* with 5263 amino acids residues composed of glycine, alanine, serine, tyrosine, valine, and only 4.7% of the other 15 amino acid types.[18,19] The tyrosine residues in SF has strong electron donating properties making this polymer an appealing template for Ag and Au nanoparticles biosynthesis as both reducing and stabilizing agents.[20]

In a previous work, the incorporation of nanosized HA particles (nanoHA) into porous SF hydrogels showed promising physicochemical performance with improved osteoblastic induction characteristics.[21] Thus, the results of this previous work supported the potential application of this composite as a bone graft substitute for clinical situations when local bone formation is needed. Therefore, providing antimicrobial properties to this composite with nanoHA could greatly improve the current bone tissue engineering strategies. In this context, our work was designated to produce a hydrogel containing SF and nanoHA for bone tissue engineering with antimicrobial properties by forming silver or gold nanoparticles *in situ*, an approach that has been poorly explored.

Methods

Preparation of Silk Fibroin Solution

Cocoons of *Bombyx mori* silkworm (supplied by Bratac, São Paulo, Brazil) were degummed in 1 g/L of Na_2CO_3 solution at 85 °C for 1 h 30 min, with Na_2CO_3 changes every 30 min, to remove the sericin of the cocoons and obtain pure SF fibers. Then, SF fibers were dried and dissolved in a ternary solvent of $CaCl_2$:CH_3CH_2OH:H_2O, at 85 °C until total dissolution, to a SF salt solution of 10% (w/v). The SF salt solution was then dialyzed (cellulose membrane, Viscofan 22 EU– 20) against distilled water for 3 days, with water changes every 24 hours.

In situ Synthesis of AgNPs and AuNPs in the SF/nanoHA Hydrogels

The synthesis of silver nanoparticles was realized by simply mixing different concentrations of silver nitrate ($AgNO_3$, Sigma) with SF solution, in order to prepare four types of hydrogels with