Silver-Containing Calcium Phosphate in Mesenchymal Stem Cell-Based Bone Regeneration: Comprehensive Analysis of Antibacterial Efficacy and Osteogenic Potential

Masaya Ueno[1], Hiroshi Miyamoto[2], Sakumo Kii[1,2], Hirohito Hirata[1], Shunsuke Kawano[3], Masaaki Mawatari[1]
[1]Department of Orthopedic Surgery, Faculty of Medicine, Saga University, Saga, Japan, [2]Department of Pathology and Microbiology, Faculty of Medicine, Saga University, Saga, Japan, [3]Research Center of Arthroplasty, Faculty of Medicine, Saga University, Saga, Japan
f8286@cc.saga-u.ac.jp

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INTRODUCTION: Infection of artificial joint implants incurs a severe societal and economic burden. The standard method for the management of infected artificial joints is a two-stage revision arthroplasty, which involves the insertion of an antibiotic spacer and subsequent reimplantation of a new prosthetic device. However, these surgeries are challenging because they require the filling of a huge bone defect following infection and debridement. Advances in mesenchymal stem cell (MSC)-based therapy have made it a promising strategy for bone repair; however, the management of infected sites remains challenging. Silver is an inorganic antimicrobial agent that has been historically used in many medical devices. This study addresses this gap by evaluating the efficacy of silver-containing calcium phosphate (AgCP), an antimicrobial agent, in maintaining the osteogenic capacity of MSCs, potentially revolutionizing the approach to infection-prone bone defects.

METHODS: This study was approved by the Animal Research Ethics Committee at Saga University, Japan (approval number: A2020-008-0). Bone marrow-derived MSCs were isolated from male BALB/c mice and the UOEH6 methicillin-resistant *Staphylococcus aureus* (MRSA) strain, which was isolated from a blood sample of a septic patient, were prepared. AgCP was calcium phosphate powder containing 2.5–3.5 wt% of silver (Apacider-AW; Sangi, Tokyo, Japan; AgCP), and tricalcium phosphate powder (SuperPore; HOYA, Tokyo, Japan; TCP) was used for comparison purposes.
Evaluation of antibacterial activity: In a 24-well transwell co-culture system, 1 wt% of AgCP or TCP was added to the upper compartment and 10^3 CFU/well of MRSA were seeded in the lower compartment within 1 mL of PBS and incubated at 37 °C. After 24 h, 100μL of the supernatant from the lower compartment was inoculated on agar plates and incubated at 37°C for 48 h; thereafter the number of inoculated viable cells was calculated (n = 6).
Cell viability assay: MSCs were seeded in 96-well plates at 10^3 cells per well in 100 μL culture medium with 0.5, 1, 3, and 10 wt% of AgCP and cultured for 1, 3, 7, 14, 21, and 28 days. CellTiter-Blue Cell Viability Assay (Promega, Madison, WI) was performed at each time point (n = 8 for each group).
Evaluation of bone formation in 3D hydrogel: MSCs were encapsulated in a hydrogel scaffold (3D Cell Culture Gel - Col-Tgel; 101 Bio, Mountain View, CA) and molded into a cylindrical shape with a diameter of 3 mm and height of 2 mm. Each scaffold had 2.0×10^6 MSCs/mL. The hydrogels were placed in a 24-well plate and cultured in 1 mL of osteogenic medium, which was changed twice per week. After six weeks of culture, micro-computed tomography (CT) analysis (CT Lab GX130; Rigaku, Tokyo, Japan), hematoxylin and eosin (H&E) staining, and alizarin red staining were performed.

RESULTS: After 24 h of MRSA culture with AgCP, none of the MRSA was detected in the wells with 1% AgCP, whereas in the other groups, MRSA was detected in all wells (Fig.1). The cell viability assay indicated that AgCP was cytotoxic and showed a concentration-dependent effect; however, once the number of MSCs decreased after 28 days, the number of MSCs increased in 0.5% and 1% AgCP (Fig.2).
After six weeks, MicroCT analysis showed calcifications in all groups, with no significant differences in bone volume/tissue volume (BV/TV). H&E staining revealed bone-like structure at superficial layer in all groups. Alizarin red staining showed surface calcification as same as the MicroCT results. (Fig.3).

DISCUSSION: The results of this study showed the potential role of AgCP in enhancing bone regenerative therapies, especially in contexts where infection is a concern. The integration of AgCP in MSC-based treatments offers a novel solution, balancing effective antimicrobial action with the preservation of the natural bone-forming capabilities of MSCs. Further in vivo evaluation is needed to assess the antimicrobial capacity, osteogenic potential, and safety of AgCP.

SIGNIFICANCE/CLINICAL RELEVANCE: The transplantation of hydrogels infused with MSCs and AgCP into infected bone defects suggests a novel therapeutic method, providing local antimicrobial activity along with enhanced proliferation, differentiation, and calcification capabilities.

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Fig.1 10^3 CFU MRSA incubated for 24 hours with TCP or Ag-CP

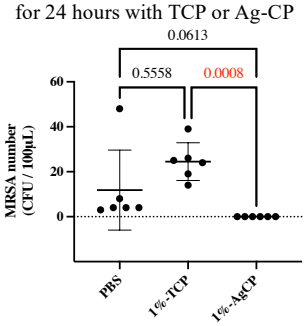


Fig.2 a) The results of CellTiter-Blue Cell Viability Assay at 1, 3, 7, 14, 21 and 28 days. b) Cell viability in all groups at 28 days. **p < 0.01, ***p < 0.001, ****p < 0.0001

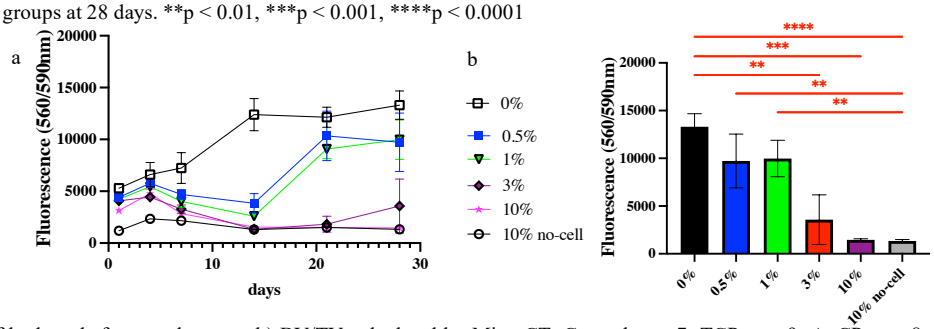
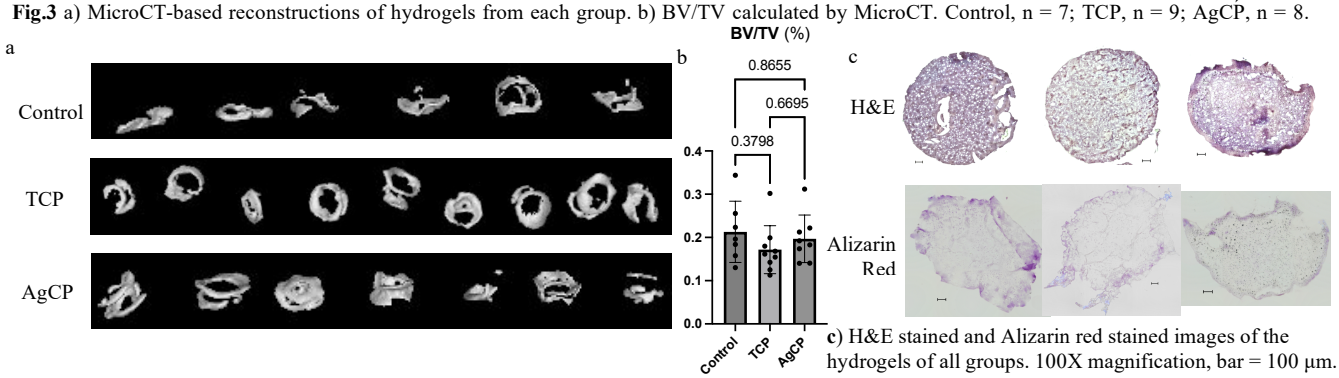


Fig.3 a) MicroCT-based reconstructions of hydrogels from each group. b) BV/TV calculated by MicroCT. Control, n = 7; TCP, n = 9; AgCP, n = 8.



c) H&E stained and Alizarin red stained images of the hydrogels of all groups. 100X magnification, bar = 100 μm.