

Safety and Cell Death Mechanism of Silver Carboxylate When Used as an Antimicrobial Coating for Orthopedic Implants

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INTRODUCTION

The growing burden of antibiotic resistant pathogens warrants rapid attention to developing novel antimicrobial modalities. This problem is coupled with the heavy cost incurred by hospitals and patients due to surgical site infections, with 300k-500k cases occurring annually in the US and a monetary cost of upwards of \$1.5B.¹ Surgically implanted materials in particular provide an optimal environment for bacterial colonization due to sequestration from the immune system by the foreign body response and bacterial biofilm formation.² The aim of this project is to analyze the cytotoxic effect of a novel antimicrobial, silver carboxylate, on human cells involved in deep tissue orthopedic surgical wounds. Silver carboxylate may provide an improvement to antibiotics in that silver induces bacterial death in a multimodal fashion, and the organic moiety improves entry of the silver ion into bacterial cells. As a coating for orthopedic implants, silver carboxylate may be able to prevent bacterial adherence and colony proliferation. It has been shown previously to decrease adherence and prevent biofilm formation in several bacterial species, including *C. acnes*, multi-drug resistant *S. marcescens*, and MRSA, with effects similar to and better than several last resort antibiotics.³⁻⁵ We are specifically investigating the apoptotic versus necrotic mechanism with the long-term goal of inhibiting apoptosis and improving human cell viability while promoting microbial death. We hypothesize that silver carboxylate will induce an apoptotic mechanism of cell death with increasing concentrations, with no induction of necrosis. We do not expect silver carboxylate to impact viability in human cells at the MIC but do expect a decline in cell viability at higher concentrations.

METHODS

Human osteoblasts and skeletal muscle cells (ATCC, Manassas, VA) were selected based on their involvement in deep surgical wounds. These cells were exposed to silver carboxylate in increasing multiples of the MIC (1x, 10x, 30x, 100x, 150x) for 24 hours. Silver carboxylate was delivered in a novel TiO₂-PDMS matrix to induce controlled release via coated inert glass beads in 96-well plates with equal distributions of previously adhered cells (n = 24). Control conditions included media and cell blanks, 10nM and 30nM NanoSilver, 1% Triton-X, TiO₂-PDMS vehicle, and 100% silver carboxylate. Cell viability was measured using a Resazurin assay (Abcam, Cambridge, MA), apoptosis with apoxin dye, and necrosis with 7-AAD dye. The respective dyes were allowed to bind through incubation and the resulting relative fluorescence was measured using a fluorometer. Statistical analysis was performed using Excel and included T-Tests for statistical significance (p < 0.05).

RESULTS

The 1x MIC of silver carboxylate did not impact cell viability, whereas the 10x-150x conditions resulted in increased cell death (p < 0.001). Concurrent and unpublished work has demonstrated a similar relationship between 1x and 10x using an MTT cytotoxicity assay. Preliminary mechanistic results suggest a 39% increase in apoptosis from the 1x MIC to the 10x MIC in skeletal muscle cells (p < 0.001). The 1x MIC did not induce a statistically significant level of apoptosis compared to the cell blank (p = 0.19). A statistically significant induction of necrosis was seen in the 100% silver carboxylate condition in skeletal muscle cells (p = 0.03), with no significant induction in any of the multiples of the MIC. Further exploration is necessary to confirm these results. Initial experimentation did not yield statistically significant results in the apoptosis or necrosis studies of osteoblasts.

DISCUSSION

Characterizing the safety of this antimicrobial silver compound is a very important facet of its development; the material needs to provide bacterial inhibition and death but spare human tissue. Our study goes beyond quantifying human cell death and investigates the mechanism by which cell death occurs in response to silver carboxylate. More specifically, we investigate the effects on cells that will be most adjacent to and affected by surgically implanted orthopedic hardware. Delineating the mechanism of cell death is important because it provides the opportunity to inhibit human cell death and augment bactericidal properties. Apoptosis is a tightly regulated and organized process and can be inhibited using endogenous or synthetic compounds.^{6,7} Conversely, necrosis is a disorganized process which results in local inflammation and cannot be readily intervened upon. Our preliminary results indicate that silver carboxylate may induce apoptosis in favor of necrosis in the 10x and higher concentrations in skeletal muscle cells. The levels of general cell viability are consistent with the trends of cell death in the apoptosis trials. The induction of necrosis at 100% silver carboxylate suggests there may be an upper limit at which point cells are overwhelmed and succumb to necrotic versus apoptotic death. While these results are promising, loss of adherent cells when preparing samples for fluorometry may have contributed to increased variability in our results. Upcoming improvements to this work include optimizing assay protocols and shifting to a flow-cytometric analysis of levels of apoptosis and necrosis in the same cell populations for better acuity and reproducibility.

CLINICAL RELEVANCE

Silver carboxylate in the TiO₂-PDMS matrix is being studied for application as a novel antimicrobial coating for surgically implanted materials, to mitigate the risk of chronic bacterial adherence and proliferation post-operatively. The goal for this coating is widespread use with fracture fixation hardware and other orthopedic implants to prevent localized deep tissue infections.

REFERENCES

- ¹ J. S. Harrop, J. C. Styliaras, Y. C. Ooi, K. E. Radcliff, A. R. Vaccaro, and C. Wu, "Contributing factors to surgical site infections," J. Am. Acad. Orthop. Surg., vol. 20, no. 2, pp. 94–101, 2012.
- ² A. Trampuz and A. F. Widmer, "Infections associated with orthopedic implants," Curr. Opin. Infect. Dis., vol. 19, no. 4, pp. 349–356, 2006.
- ³ D. R. Garcia et al., "Silver carboxylate-doped titanium dioxide-polydimethylsiloxane coating decreases *Cutibacterium acnes* adherence and biofilm formation on polyether ether ketone," Spine J., vol. 22, no. 3, pp. 495–503, 2022.
- ⁴ D. Garcia et al., "Silver carboxylate and titanium dioxide-polydimethylsiloxane coating decreases adherence of multi-drug resistant *Serratia marcescens* on spinal implant materials," Spine Deform., vol. 9, no. 6, pp. 1493–1500, 2021.
- ⁵ D. R. Garcia et al., "Synergistic effects of silver carboxylate and chlorhexidine gluconate for wound care and prevention of surgical site infections by *Cutibacterium acnes* and methicillin-resistant *Staphylococcus aureus*," Surg. Infect. (Larchmt), vol. 23, no. 3, pp. 254–261, 2022.
- ⁶ M. S. Mohamed, M. K. Bishr, F. M. Almutairi, and A. G. Ali, "Inhibitors of apoptosis: clinical implications in cancer," Apoptosis, vol. 22, no. 12, pp. 1487–1509, 2017.
- ⁷ S. D. Barchéath, R. I. Tawatao, M. Corr, D. A. Carson, and H. B. Cottam, "Inhibitors of apoptosis in lymphocytes: Synthesis and biological evaluation of compounds related to pifithrin- α ," J. Med. Chem., vol. 48, no. 20, pp. 6409–6422, 2005.