Assessment of the Safety and Application of Silver Carboxylate as a Pre-Surgical Dressing

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INTRODUCTION

The incidence of surgical site infections (SSIs) in the United States is linked to 300k-500k cases annually and imposes monetary costs exceeding $1.5 billion to our healthcare system. [1] The accelerating rise of antimicrobial resistance necessitates innovative approaches to combat this increasingly challenging problem. A significant challenge in preventing SSIs is the limited efficacy of current pre-surgical sterilants.[2] Silver carboxylate (AgCar) is a novel organometallic antimicrobial compound which achieves predictable pharmacokinetics of loading and release via a TiO_2/polydimethylsiloxane (TiO_2/PDMS) matrix. Previous studies have provided evidence of AgCar's efficacy against drug-resistant pathogens in prosthetic liners, sutures, spinal implants and as a pre-surgical skin prep in combination with chlorhexidine gluconate (CHG) prep.[3,4] The unique attributes of AgCar chemistry, including penetration into the pilosebaceous pores, controlled release, and broad-spectrum antimicrobial efficacy, position it as a noteworthy option for infection prophylaxis. AgCar has also demonstrated comparable antimicrobial efficacy to last-resort antibiotics against bacteria commonly encountered in SSIs. This study explores AgCar's cytotoxic profile on human-derived primary endothelial cells (ECs) and keratinocytes in the context of superficial wound healing, and its potential application as an alternative antimicrobial pre-surgical dressing.

METHODS

MTT Assay

After 24 hours, primary cell lines involved in wound healing were exposed to conditions of 1x silver carboxylate, 10x silver carboxylate, vancomycin (5 µg/ml), vancomycin (50 µg/ml), tobramycin (5 µg/ml), tobramycin (50 µg/ml), linezolid (2 µg/ml), linezolid (20 µg/ml), and polymyxin E (2 µg/ml). 10 nM silver nanoparticles, 30 nM silver nanoparticles, 100 nM colloidal silver, and 300 nM colloidal silver, cell blank and a 95% TiO_2 /PDMS matrix only conditions were used as negative controls and 100% silver carboxylate and 1%Triton X were used as positive controls. Silver carboxylate conditions, TiO2/PDMS matrix only, and 100% silver carboxylate positive control were added by dip coating 2 mm inert glass beads. After 24 hours, cell viability was measured by Promega Cell Titer 96 Non-Radioactive Cell Proliferation Assay protocol (MTT assay) and spectrophotometry at a wavelength of 570 nm. Optical density of each condition was then compared to optical density of the cell blank to determine percent cell viability. Replicates of n=15 were performed for each condition. The MTT assay served as the foundation for investigating cell viability and proliferation at increasing multiples of minimal inhibitory concentration (MIC) to determine the cytotoxicity profile of silver carboxylate above 1X MIC via cell viability assay.

Resazurin Assay

Endothelial cells and keratinocytes were subjected to increasing multiples of the MIC (1x, 10x, 30x, 100x, and 150 x) for 24 hours. Resazurin assay and fluorometry measured cell viability after a 4-hour incubation period. Treatment with silver carboxylate was delivered via coated inert glass beads of the same area as 96-well plates with respective TiO2-PDMS matrix ratios (n = 15). Media and cell blank were our control conditions, 10nM and 30nM Nanosilver, TiO2-PDMS matrix only, 1% Triton-X, and 100% silver carboxylate were our negative and positive controls, respectively. After 24 hours, cell viability was estimated via Resazurin assay and fluorometry at a wavelength of 550/590nm Optical density. Each condition then underwent subtractions of the optical density of the media blank with dye as well as the optical density of each relative condition with dye to account for background. Replicates of n=15 were performed for each condition. Statistical analysis was completed using Excel for T-Tests with statistical significance ($p < 0.05$).

RESULTS

Silver carboxylate at 10x MIC demonstrated significant cytotoxicity across all cell lines per the MTT assay. 1x MIC silver carboxylate demonstrated comparable or lower cytotoxicity than conventional silver formulations and certain concentrations of last-resort antibiotics. Cytotoxicity at 10x silver carboxylate concentrations approximated that of positive controls. Several higher antibiotic concentrations commonly employed in wound care exhibited cytotoxicity comparable to positive controls. ECs treated with 1X Silver Carboxylate showed higher viability compared to all tested antibiotics and crude silver conditions. Per Resazurin assay, 1x MIC silver carboxylate exhibited statistically insignificant impact on cell viability for endothelial cells, whereas concentrations of 10x to 150x resulted in statistically significant decreased cell viability in ETs and keratinocytes ($p < 0.001$).

DISCUSSION

Ongoing research is investigating the molecular mechanisms of AgCar as well as exploring methodologies to decrease cytotoxicity beyond the 1x MIC. Overall, our research underscores the potential for silver carboxylate in pre- and post-operative surgical wound healing, particularly when combined with current preparations. Further research will reveal its antimicrobial potential, especially in surgical skin preparation and wound care synergy, offering promise as an additional tool for preventing surgical site infections.

CLINICAL RELEVANCE

Silver carboxylate embedded within the TiO_2-PDMS matrix holds promise as a versatile antimicrobial option for orthopedic surgical sites. Its potential use as a stand-alone or synergistic pre-operative sterilant could overcome antibiotic limitations and antibiotic resistance. The concept of enhanced combined efficacy presents intriguing possibilities in augmenting the capabilities of currently utilized antimicrobial therapies.

REFERENCES

1. Al-Mulhim FA, Baragbah MA, Sadat-Ali M, Alomran AS, Azam MQ. Prevalence of surgical site infection in orthopedic surgery: a 5-year analysis. Int Surg. 2014 May-Jun;99(3):264-8. doi: 10.9738/INTSURG-D-13-00251.1. PMID: 24833150; PMCID: PMC4027911.
2. Dockery, Dominique & Allu, Sai & Vishwanath, Neel & Li, Troy & Berns, Ellis & Glasser, Jillian & Spake, Carole & Antoci, Valentin & Born, Christopher & Garcia, Dioscaris. (2021). Review of Pre-Operative Skin Preparation Options Based on Surgical Site in Orthopedic Surgery. Surgical Infections. 22. 10.1089/sur.2021.085.
3. Haglin, Jack M. BS; Garcia, Dioscaris R. PhD; Roque, Daniel L. BS; Spake, Carole S.L. MSc; Jarrell, John D. PhD; Born, Christopher T. MD. Assessing the Efficacy of a Silver Carboxylate Antimicrobial Coating on Prosthetic Liners. Journal of Prosthetics and Orthotics 32(4):p 251-257, October 2020. | DOI: 10.1097/JPO.0000000000000271
4. Dioscaris R. Garcia, Neel Vishwanath, Sai Allu, Dominique M. Dockery, Ellis M. Berns, Carole S.L. Spake, Troy Li, Caitlin Barrett, Valentin Antoci, and Christopher T. Born. 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. Surgical Infections. Apr 2022.254-261.

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