Silver-zinc EDTA complexes demonstrate strong antibiofilm/antibacterial activity in an *in vitro* artificial wound slough (AWS) biofilm model

Rui Chen, PhD, Marcus Swann, PhD, Jeanne Saint Bezard, BSc, Fergus Watson, PhD, Steven L. Percival, PhD

5D Bioscience, 5D Health Protection Group Ltd, Accelerator Building, 1 Daulby Street, Liverpool. L7 8XZ, UK

BACKGROUND

The presence of slough, which serves as a reservoir for microorganisms, damaged/devitalized cells and inflammatory chemokines, is a hallmark of chronic wounds. Slough attracts microbes to the wound bed and form biofilms, which are particularly hard to treat with conventional topical antimicrobials. Multifunctional metal complexes (MMC) have shown strong antibacterial and antibiofilm efficacy during *in vitro* testing using standardized tests, such as MIC/MBC assays and the CDC bioreactor biofilm model. However, it is also important to understand the activity of MMC using clinically relevant wound models. Previously, we demonstrated an artificial wound slough (AWS) model capable mimicking *in vivo* wound eschar and slough, could be used to determine the efficacy during wound debridement. In this study, the AWS model has been further developed to incorporate biofilm formation under the slough layer to produce a new cost-effective AWS-biofilm model. The validity of the AWS-biofilm model has been demonstrated by measuring silver penetration and antibiofilm efficacy of silver-containing complexes (Ag-Zn MMC).

METHODS

Microorganism: *Pseudomonas aeruginosa* ATCC 15442

Test samples: Ag-Zn MMC complex was prepared by mixing $AgNO_3$, $Zn(NO_3)_2$ and EDTA into a solution, and then diluted to 3.0% for test. PBS and 1.1% $AgNO_3$ were used as negative and positive control groups.

Method: An *in vitro* AWS-biofilm model was developed using a pre-formed *P. aeruginosa* biofilm under a layer of slough composed of collagen, elastin and fibrin - key constituents of eschar and slough. The test antimicrobials (Ag-Zn MMC and $AgNO_3$ solutions) were added onto the AWS-biofilm substrate to validate the AWS-biofilm model at varying thicknesses. After 24 hours contact time, the viability of the biofilm was determined by aspirating the culture from the surface and enumerating the resultant sample. The penetration of the antimicrobial agents (e.g. silver ions) through the AWS membrane was monitored electrochemically using a printed carbon sensor. The disruption cause to the *P. aeruginosa* biofilm by the treatments was imaged by LSM 780 Zeiss confocal microscope; using fluorescence stains.

RESULTS

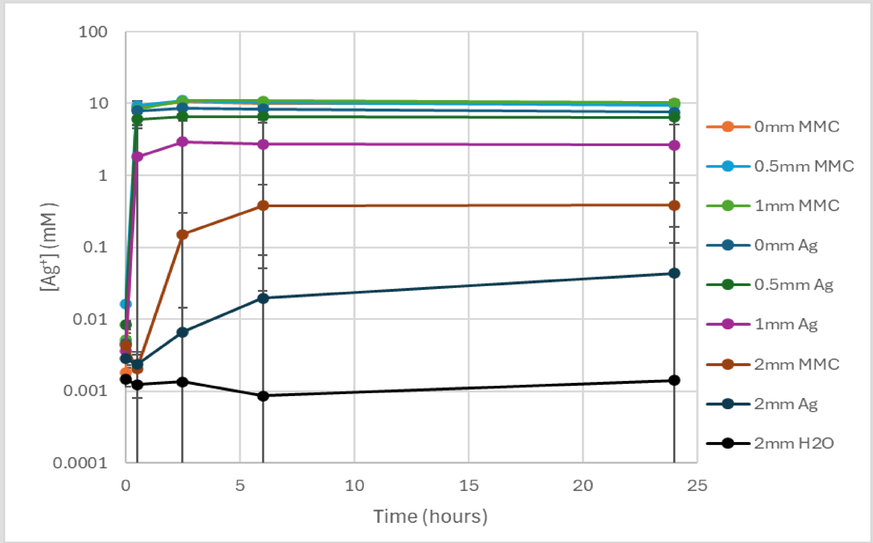

Figure above. Silver penetration vs. time (hours) with the silver concentration determined using the calibration curves. Curves correspond to samples with 0.0 mm, 0.5 mm, 1.0 mm and 2.0 mm thick AWS samples with 1.1% $AgNO_3$ and 3% Ag-Zn MMC test solutions (n=3). The control data using water as a test solution at 2.0 mm has been included.

Table below. The average cell density (Log_{10} CFU/mL) for P. aeruginosa AWS-biofilms after treatment with $AgNO_3$ and MMC test solutions for 24 hours. The error is representing the standard deviation of the mean. Cell density at 0 hour of treatment was 9.06±0.02, 8.97± 0.04, and 8.99± 0.07 respectively for 0.5mm AWS, 1.0mm AWS and 2.0mm AWS.

	0.5mm AWS	1.0mm AWS	2.0mm AWS
Control (PBS)	9.24±0.08	9.15±0.08	9.27±0.02
$AgNO_3$	0.00±0.00	0.00±0.00	3.18±0.00
Ag-Zn MMC	0.00±0.00	0.00±0.00	0.00±0.00

RESULTS

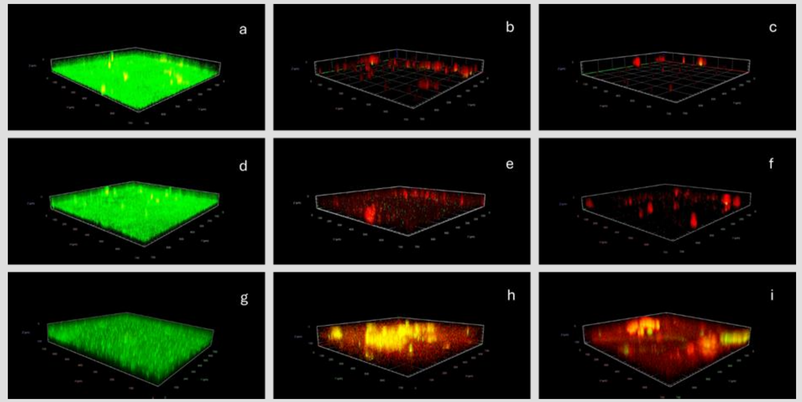

Figure above. 3D representative images of the biofilm disruption and inactivation of P. aeruginosa biofilm after treatment with PBS, $AgNO_3$ and MMC for 24 hours. a, b, and c: biofilm under 0.5mm AWS; d, e, and f: biofilm under 1.0mm AWS; g, h, and i: biofilm under 2.00mm AWS. a, d and g: Control (PBS); b, e, and h: treatment with 1.1% $AgNO_3$; c, f, and I : treatment with 3% Ag-Zn MMC.

CONCLUSIONS

The AWS-biofilm was employed to investigate the antibiofilm and antibacterial efficacy of a new patented smart next generation antibiofilm technology composed of silver-zinc EDTA complexes and designed as a family of multifunctional metal complexes referred to as MMCs. In a liquid format we determine both the performance and penetration through AWS to control and manage biofilms. The results demonstrated the potential for the proprietary EDTA multifunctional metal complexes to be used for the disruption of biofilms, such as those that form in chronic wounds. The results also demonstrated the ability of the AWS-biofilm model to be employed for the evaluation of the efficacy of a new anti-biofilm and antimicrobial next generation smart technology by simulating a more complex and clinically relevant wound environment.

REFERENCES

1. Chen R, Salisbury AM, Percival SL. Int Wound J. 2020 Feb;17(1):73-82. doi: 10.1111/iwj.13234.

2. Swann MJ, Freeman NJ, Watson F, Law SJ, Percival SL. Surgical Technology International. 2023 Jul 26;42:sti42-1692.