The use of Ag_5IO_6 as an antibiofilm agent in wound dressings

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ABSTRACT

The novel antibiofilm activity of Ag_5IO_6 was explored. Wound dressings impregnated with Ag_5IO_6 were compared to commercially available silver, chlorhexidine, and PHMB dressings. The dressings were tested against *P. aeruginosa*, *S. aureus*, and *C. albicans* for their ability to prevent microorganism adherence, eliminate planktonic species, and eliminate mature biofilms within 24h. Only the Ag_5IO_6 dressings were able to prevent adherence and eliminate surrounding planktonic microorganisms for all species tested for ≥28 days of elution with log reductions >4. Moreover, only these dressings were able to generate >4 log reductions against all mature biofilms. Thus, Ag_5IO_6 has superior activity to a number of antimicrobials, with long-term prevention of microbial adherence, rapid kill of planktonic microorganisms, and the ability to eliminate mature biofilms. Ag_5IO_6 may be a valuable antimicrobial agent for use in a number of medical device applications, including catheters, and implants.

Keywords: Silver periodate, antimicrobial, wound dressing, infection, biofilm

1 INTRODUCTION

Silver agents as topical antimicrobials have been used for hundreds of years in wound care [1]. Silver has been used to prevent or manage infection in its solid elemental form, as solutions of silver salts used to cleanse wounds, and more recently as creams or ointments containing a silver-antibiotic compound. However, these applications of silver have come with considerable challenges that include complex methods for incorporation/coating of silver into/onto materials, lack of efficacy against planktonic microorganisms and biofilms in part due to inactivation by bodily fluid components [2, 3], cost effectiveness, and questions about safety [1]. Recently, numerous new silver compounds/products have been developed and added to medical devices in efforts to overcome these obstacles, mostly as antibiotic alternatives in the face of limited antimicrobial spectrum and growing microbial resistance. Bacteria exist predominantly in biofilms [4]. Biofilms are responsible for 80% of human infections [5], and are typically 100-1000x more resistant to treatment than planktonic microorganisms [6]. Therefore, when testing new silvers for potential clinical relevance, they need to demonstrate efficacy against biofilm phenotypes.

Ag_5IO_6 (pentasilver hexaoxoiodate) possesses promising properties for efficacy against biofilms, and simplicity for coating onto/incorporating into medical devices with approriate release characteristics. These properties include the presence of silver in both cation ($[Ag_3]^{3+}$), and anion-complexed with highly oxidized iodine ($[Ag_2IO_6]^{3-}$) [7]. This may enhance penetration of the biofilm relative to Ag^+, which binds to negatively charged surface components of biofilms. The periodate structure around the anionic silver may slow its inactivation by bodily fluid components. Combining silver and iodine may increase antimicrobial mechanisms of action, reducing the likelihood of developing resistance to Ag_5IO_6. The synthesis method used here combines small grain size with large particle size, resulting in polycrystallinity on a large surface area which may improve its antimicrobial activity relative to other Ag-compounds. Ag_5IO_6 has demonstrated excellent stability (storage; thermal; and in the presence of light, water, saline, organic solvents, autoclaving, ethylene oxide, etc.), and can be easily synthesized with high purity in a form that is simple to deposit onto metals or incorporate into wound dressings, gels, polymers, etc. [8]

This study examined whether Ag_5IO_6 could prevent biofilm formation on surfaces and eliminate mature biofilms, with comparison to commercially available antimicrobials to determine whether its activity was novel.

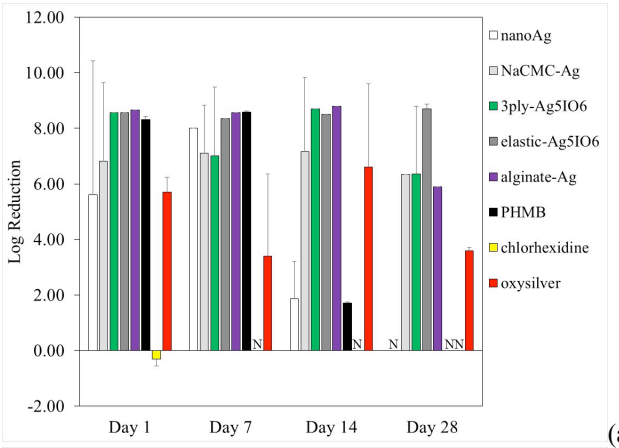
2 EXPERIMENTAL SECTION

Materials. Ag_5IO_6 was synthesized in-house [8]. Ag_5IO_6 was coated onto two bandages - a dressing with two outer layers of high-density polyethylene (HDPE) and a rayon/polyester core (3ply-Ag_5IO_6); and a rayon/cotton elastic adhesive bandage (elastic-Ag_5IO_6) [9]. Commercial dressings included in testing: nanoAg, alginate-Ag, NaCMC-Ag, oxysilver, chlorhexidine, and PHMB (polyhexamethylene biguanide). **Microorganisms.** *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 6538, *S. aureus* ATCC 29213, and *Candida albicans* ATCC 18804. **Materials for tests.** 0.9% NaCl was used in pre-treatments. Human plasma (Human Male AB; SeraCare Life Sciences Inc., Gaithersburg, MD) was used

in challenge medium. The recovery/neutralization buffers used as appropriate were STS [0.1% (w/v) sodium thioglycolate (Fisher Scientific, Ottawa, ON, Canada) and 0.5% (w/v) Tween 80 (Fisher Scientific) in phosphate-buffered saline), and Difco Dey/Engley (DE) neutralizing broth (Becton Dickinson, Mississauga, ON, Canada) plus 5 g/L each of L-cysteine and L-glutathione. Tryptic soy agar and broth were used for plating and medium, respectively, unless otherwise indicated. For *C. albicans*, Sabouraud dextrose broth supplemented with 500 mM galactose was used as needed. **Method.** The methodology followed for this study comprises a) Anti-adherence: pre-treatment of the wound dressing, challenge, planktonic recovery, and adherent microorganism recovery [10]. b) Mature biofilm: biofilm growth, challenge, and recovery [10, 11]. c) Planktonic log reduction: organism growth, challenge, and recovery [3, 12].

3 RESULTS AND DISCUSSION

The anti-adherence method simulated a scenario in which an antimicrobial wound dressing is used to prevent a biofilm infection. Dressings were exposed to a wound-like environment for different time periods (1, 7, 14, and 28 days) followed by exposure to microorganisms under conditions that would promote biofilm formation, allowing for determination of use-times for devices. Control dressings were used to account for material effects. Overall, only the Ag_5IO_6 dressings were active up to 28 days against all three microorganisms (Fig.1). The alginate-Ag and NaCMC-Ag reached 28 days with *P. aeruginosa* only, likely because *P. aeruginosa* is more sensitive to silver than the other micro-organisms tested [12], and PHMB reached 28 days with *C. albicans* only. It was interesting that nanoAg had a shorter life, given its superior anti-planktonic kinetics compared with alginate-Ag and NaCMC-Ag [10, 12]. Possible reasons include that the active agent is coated onto the outer surface rather than impregnated in the dressing, meaning that uncoated layers may act as surfaces for biofilm growth. As well, the active agent was likely inactivated by the saline pre-soaks [13], given the precipitate observed. Furthermore, the manufacturer's recommended dressing usage is 3 days. An observed change in color of the oxysilver suggested deactivation/depletion of the active agent resulting in its use time of <14 days. The chlorhexidine showed virtually no activity despite manufacturers' claims of 3-4 days activity [14].



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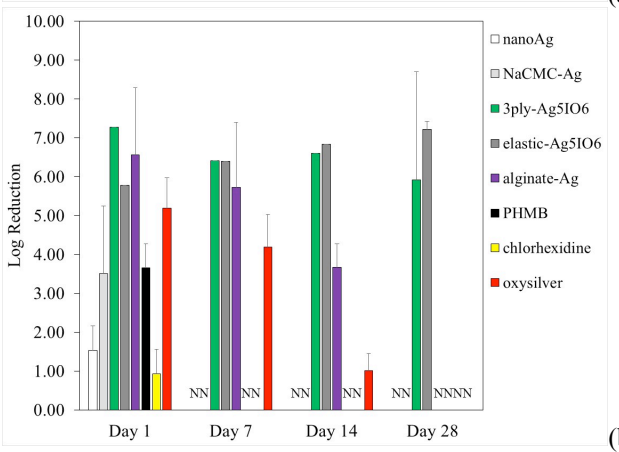

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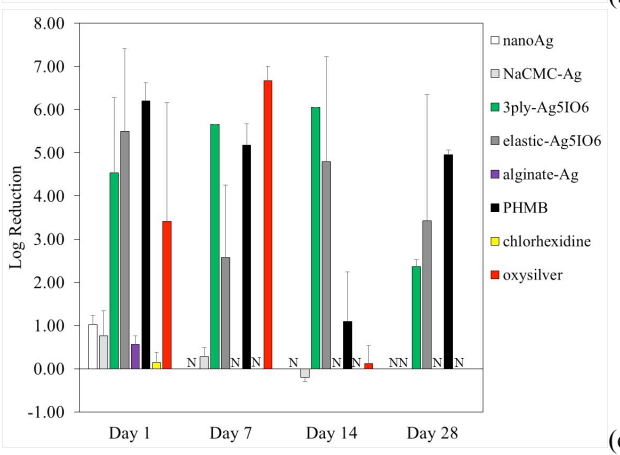

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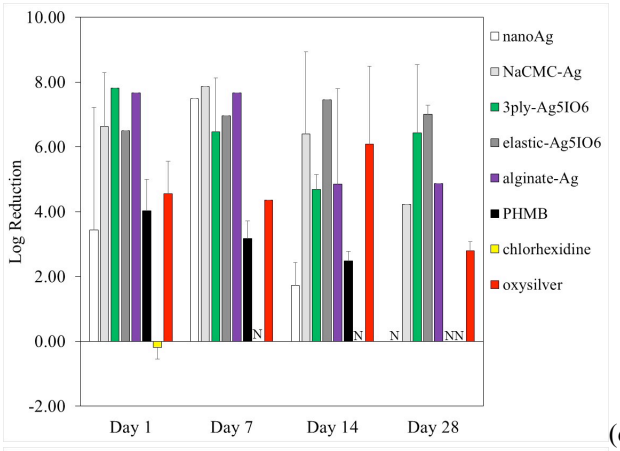

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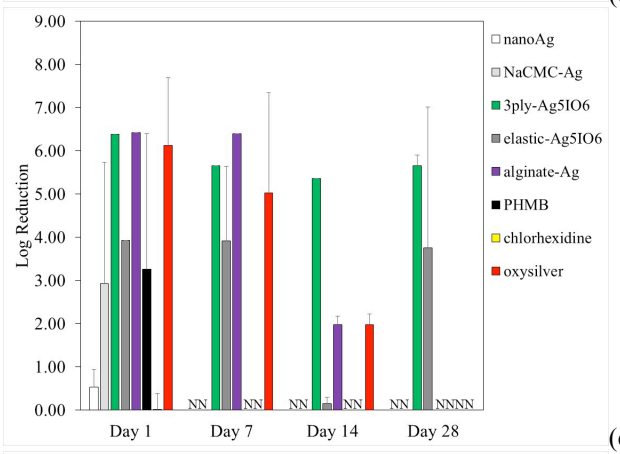

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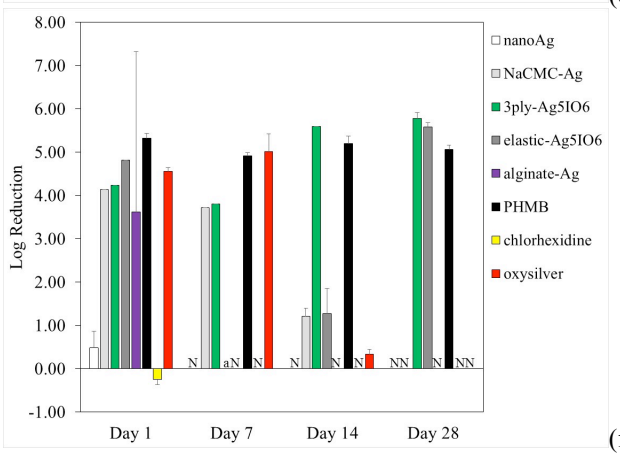

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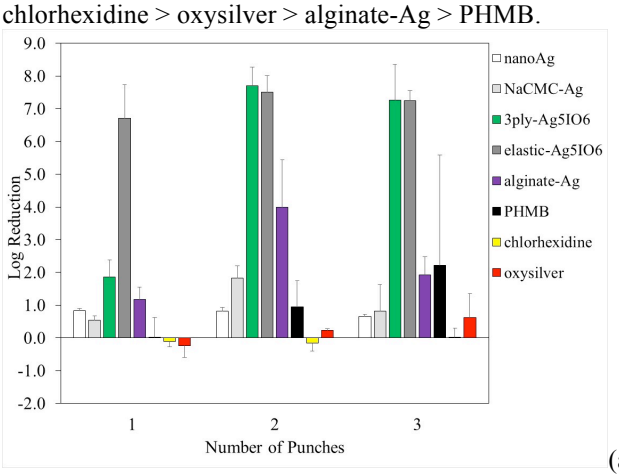
Figure 1. 28-day anti-adherence data. Mean log reductions on each test day, calculated relative to control dressings, are shown along with the standard deviations (SD). SD of zero indicate total kill of the microorganisms. When a dressing generated a log reduction <4 both for planktonic microorganisms and adhered biomass, the dressing was eliminated from the study after that time point (indicated by 'N'). (a) Planktonic *P. aeruginosa*, (b) planktonic *S. aureus*, (c) planktonic *C. albicans*, (d) adhered *P. aeruginosa*, (e) adhered *S. aureus*, (f) adhered *C. albicans*. For data point 'a', there was no growth of the microorganism on the test or control dressings.

Comparing commercially intended concentrations (ca. 1 punch for most dressings, ca. 3 punches for PHMB, Fig. 2), the relative activity of the dressings against mature biofilms was as follows: for *P. aeruginosa*, elastic-Ag_5IO_6 > PHMB > 3ply-Ag_5IO_6 > alginate-Ag > nanoAg > NaCMC > chlorhexidine > oxysilver; for *S. aureus*, elastic-Ag_5IO_6 > PHMB > 3ply-Ag_5IO_6 > chlorhexidine > NaCMC-Ag > nanoAg > oxysilver > alginate-Ag; and for *C. albicans*, 3ply-Ag_5IO_6 > elastic-Ag_5IO_6 > NaCMC-Ag > nanoAg > chlorhexidine > oxysilver > alginate-Ag > PHMB.



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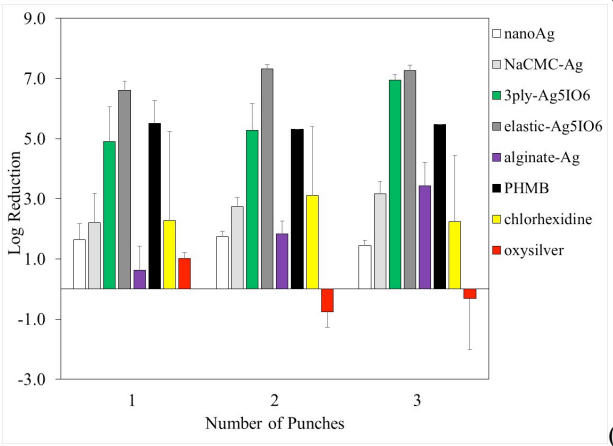

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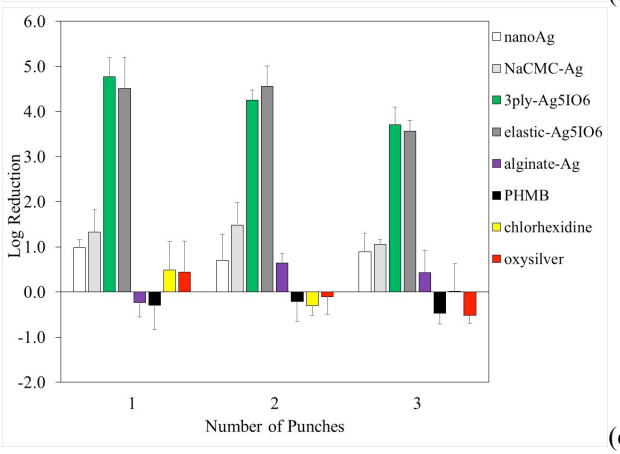

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Figure 2. Ability of dressings to eliminate mature biofilm in 24h. One, two, or three dressing punches were exposed to mature biofilm for 24h. Mean log reductions, calculated relative to control dressings, are shown along with the SD. SD of zero indicate total kill of the microorganisms. (a) *P. aeruginosa*, (b) *S. aureus*, (c) *C. albicans*.

None of the commercial silver and chlorhexidine dressings disrupted/killed mature biofilms. The charge (Ag^+) and structure (chlorhexidine) of these materials may cause them to bind to extracellular biofilm components prior to interaction with microorganisms. Some species-specific activity was seen with PHMB.

In the standardized planktonic log reduction assay, rapid kill of planktonic microorganisms (total kill in 30 min) was achieved with Ag_5IO_6. These results were comparable to nanoAg and PHMB, and significantly better than NaCMC-Ag and alginate-Ag (0 log reduction). Rate of kill at medical device surfaces is important both for preventing biofilm formation by eliminating planktonic microorganisms before biofilms are started, and microorganism translocation and subsequent systemic infection.

4 CONCLUSION

The application of Ag_5IO_6 in wound dressings has shown efficacy with combined abilities to rapidly kill planktonic micro-organisms, prevent adherence of micro-organisms for ≥28 days in-use, and disrupt/eliminate mature biofilms. This makes Ag_5IO_6 an excellent candidate for use in a variety of medical devices, including those that need a coating to prevent microbial adherence both for short- and longer-term applications, and in treatment of mature biofilms. These applications could include use in wound dressings and gels, catheters, needles, connectors, ports, and implants.

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