Applied Physics Letters

RESEARCH ARTICLE | MAY 22 2024

On-demand release of silver from composite hydrogel by cold atmospheric plasma jet for wound infection control

Naing Tun Thet ; Bethany L. Patenall; Robert D. Short ; A. Toby A. Jenkins

Appl. Phys. Lett. 124, 211902 (2024)
https://doi.org/10.1063/5.0206812

AIP Publishing

Applied Physics Letters ARTICLE pubs.aip.org/aip/apl

On-demand release of silver from composite hydrogel by cold atmospheric plasma jet for wound infection control

Cite as: Appl. Phys. Lett. **124**, 211902 (2024); doi: 10.1063/5.0206812
Submitted: 4 March 2024 · Accepted: 12 May 2024 ·
Published Online: 22 May 2024

View Online Export Citation CrossMark

Naing Tun Thet,[1] Bethany L. Patenall,[1] Robert D. Short,[2] and A. Toby A. Jenkins[1,a)]

AFFILIATIONS

[1]Department of Chemistry, University of Bath, Bath BA2 7AU, United Kingdom
[2]Department of Chemistry, The University of Sheffield, Dainton Building, Sheffield S3 7HF, United Kingdom

[a)]Author to whom correspondence should be addressed: a.t.a.jenkins@bath.ac.uk

ABSTRACT

Silver is an antimicrobial commonly used within wound care chiefly in advanced dressings or in a topical cream form, such as silver sulfadiazine (SSD). Although silver is effective at controlling the growth of many common wound bacteria, it can be cytotoxic and can build up in tissue, stalling the healing process. Here, we demonstrate the development of an on-demand release system for delivery of silver from a composite hydrogel comprising sodium polyacrylate particles dispersed in a cryo-crosslinked polyvinyl alcohol carrier gel. Application of cold atmospheric plasma (CAP) jet to the silver loaded hydrogel resulted in controlled release of silver. This release is thought to occur due to the formation of nitrous acids in the hydrogel by the CAP, resulting in protonation of carboxylate groups in the hydrogel and subsequent gel de-swelling due to the reduction in interchain charge repulsion. The location of silver within the sodium polyacrylate particles was probed using scanning electron microscopy and EDX imaging. The released silver inhibited the growth of *Enterococcus faecalis, Pseudomonas aeruginosa*, and *Staphylococcus aureus* and significantly reduced the viable cell count of the *P. aeruginosa* biofilm.

Silver ions (Ag^+) and silver nanoparticles (NPs) exhibit broad-spectrum antifungal and bactericidal effects and show efficacy against various human wound pathogens.[1–5] Silver binds to DNA and vital proteins in the bacterial cell[6,7] and may make the target bacteria susceptible to oxidative stress by binding to glutathione.[8,9] Such multiple modes of action of silver make it an effective antimicrobial agent and it is used in many wound dressings.[10,11]

However, there are issues around systemic and localized cytotoxicity of silver: studies have shown that excessive use of silver in a non-infected wound not only slows/interrupts the healing but also causes cytotoxicity in fibroblasts, epithelial, and dermal cells.[12,13] Systemic overdose of silver can cause agyria.[14,15] The cytotoxic threshold of silver in wounds is ≥ 33 ppm, yet, some commercially available wound dressings containing silver sulfadiazine show high uncontrolled release of silver, up to 100 times higher than the cytotoxic threshold.[12,16] To decrease silver cytotoxicity, nanocrystalline silver has been introduced and impregnated into wound dressings, these dressings have been marketed and sold by leading wound care product companies for several decades. Nonetheless, the release of silver into wounds, regardless of infection state, is still unavoidable, as the silver release by current wound dressing products is driven by passive diffusion. Most wounds heal without intervention, provided they are covered and kept clean; however, clinicians will often use silver-based dressings prophylactically, based on concern for the potential of infection (or hospital policy in some cases). Ideally a wound dressing would contain an antimicrobial agent which would only be released on demand and only if needed, for example, based on clinical suspicion of infection or detected by a wound infection diagnostic test.[17] However, silver is not without problems: there are also clinical concerns around the potential of silver to slow healing in non-infected wounds.[13] It is recommended that use of silver dressings be restricted to only seriously infected wounds.[18]

Over the past decade, there has been a significant growth in the research and the clinical application of the application of cold atmospheric plasma (CAP) to living tissue.[19] Direct CAP devices work upon the basis of the interaction of high-energy plasma with oxygen and nitrogen molecules in ambient air, and this interaction produces a mixture of reactive oxygen species (ROS) and reactive nitrogen species

(RNS), collectively known as RONS. The use of CAP with hydrogels in the context of wound healing is logical as hydrogels provide a moist wound environment that supports healing. Hydrogels exhibit excellent absorptive and water storage properties (important in exudating wounds) as well as having proven biocompatibility, important when placed in direct contact with an open wound.[20] Using CAP with a hydrogel has three important advantages: the hydrogel prevents CAP dehydration of the wound; it can be used as a reservoir of therapeutic molecules which can be released by CAP application; and the hydrogel screens out potentially hazardous components of the CAP jet.[27]

In this work, we have loaded silver ions into a polyacrylic acid (PAA)/polyvinyl alcohol (PVA) composite hydrogel prototype dressing and demonstrated that the silver can be released on demand by the application of an argon CAP jet to the topside of the dressing (Fig. 1). The silver loading and release was quantified by EDX and ICP-MS analysis. The inhibitory efficacy of the released silver was quantified by a modified Kirby–Bauer method and analysis of biofilm inhibition using three species of pathogenic bacteria commonly found in wounds.

P. aeruginosa (PAO1), *S. aureus* (H560), and *E. faecalis* (JH2-2) were from the Jenkins and Gebhard collection from the University of Bath. Cross-linked sodium polyacrylate particles (CAS 27599-56-0) and high molecular weight poly(vinyl alcohol), M_w 146 000–186 000, 99+% hydrolyzed (PVA), and silver nitrate ($AgNO_3$) were purchased from Sigma, UK.

Details of the single argon cold atmospheric plasma (CAP) jet are reported in previous publications.[21] Briefly, argon gas, flowing at 1 standard liter per minute (SLPM) through a thin quartz tube (0.6 mm inner dia.) was electrically excited by a high voltage needle electrode with two separate ground electrodes and 7 kV, 23.5 kHz using the electrical power supplied (Fig. 1 and supplementary material Fig. S1).

100 mg of silver nitrate was dissolved in 100 ml of de-ionized water and 1 g of partially crosslinked sodium polyacrylate (PAA) particles (Sigma UK, CAS Number: 27599-56-0) was added to a round bottom flask. The flask was left for ca. 1 h to allow the complete swelling of the PAA particles, the gel was washed with 2×100 ml de-ionized water on a Büchner funnel, before heating under vacuum on a rotary evaporator to around 95 °C to remove most of the water. The remaining particles were then freeze dried using liquid nitrogen. After three cycles of freeze/thaw, 10 ml of ethanol was added to the almost dry gel to form a water–ethanol azeotrope, and two further freeze/thaw cycles applied to yield a final dry product of silver loaded PAA. The silver concentration in the PAA particles was optimized by measuring the PAA swelling as a function of silver nitrate concentration, with a minimum 50% of swelling (compared to PAA particle swelling in pure water) taken as the maximum silver concentration for effective PAA loading. [supplementary material Figs. S2(a) and S2(b)]. Experience has shown that to ensure sufficient loading of silver, a minimum 50% PAA swelling was required.

100 mg of the silver loaded PAA particles was ground in a pestle and mortar to a particle size between 500 and 100 μm, determined by sieving and dry mixing with 1 g PVA, then dissolved in 20 ml of de-ionized water, and heated at 95 °C for 1 h. The molten gel was cast into a Petri dishes (90 mm dia.) before freezing (at −20 °C) for 2 h and thawing (at 40 °C). Three freeze/thaw cycles in total were performed to facilitate the cryo-cross-linking of PVA, giving the silver loaded composite hydrogel. A slight brown coloration of the gel was observed, which disappeared on application of the CAP (supplementary material Fig. S4).

For the modified Kirby–Bauer assay, three bacterial strains (10 ml each) were individually cultured in Falcon tubes in the respective liquid culture media (Luria broth for *P. aeruginosa* and *E. faecalis*, and tryptic soy broth for *S. aureus*) at 37 °C, 200 rpm for 18 h.[22] The bacterial culture was centrifuged at 10 000 rpm for 5 min and the supernatant was discarded, the cell pellet was resuspended in phosphate buffer saline (PBS), optical density (OD) was adjusted to 0.5 absorbance units at $l = 600$ nm, and 100 μl of OD adjusted bacteria cells in PBS was evenly spread on each Mueller–Hinton agar (MHA). The silver loaded composite gel was cut into 10 mm diameter disks in triplicate, placed on a bacterial inoculated agar surface (ca.10^5 CFU ml^{-1}), and CAP treated at 1.5 cm distance (from quartz tube orifice to gel surface) for 3 min

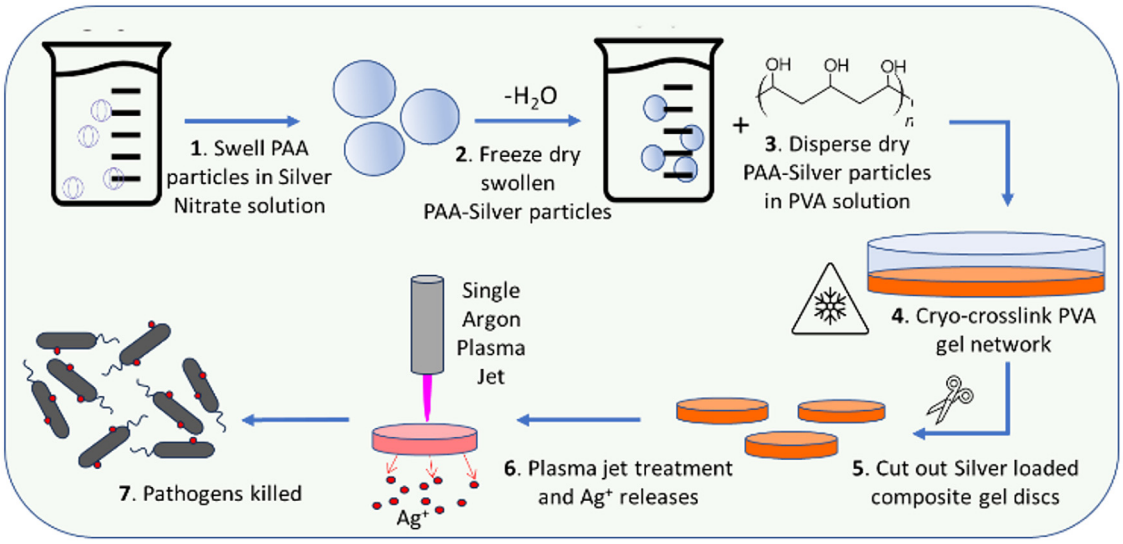

FIG. 1. The schematic procedure for preparing silver loaded PAA/PVA composite gels, depicting the eradication of wound pathogens by CAP-triggered released silver ions.

(supplementary material Fig. S1). The agar plates were incubated at 37 °C for 24 h and the zone of inhibition (ZOI) measured (Fig. S9).

For the biofilm cell reduction assay, a modified colony biofilm model was used.[23] A nanoporous polycarbonate membrane in triplicate was placed on MHA, and the membranes were disinfected under UV light (254 nm) for 10 min. 20 ml of sterilized artificial wound fluid (AWF) was pipetted, thinly spread, and allowed to dry on each membrane. AWF was aseptically prepared by mixing an equal volume of fetal calf serum (FCS) and MilliQ water containing 0.85% NaCl (%w/v) with 0.1% peptone (%w/v). 30 μl of OD adjusted *P. aeruginosa* (PAO1) cells in PBS was pipetted on each membrane and incubated at 37 °C for 8 h. Composite hydrogels (with controls) in triplicate were placed on each growing young colony biofilm and CAP treated for 3 min, followed by incubation under the same condition for another 16 h, before stripping each biofilm in PBS buffer, performing serial dilution, plating, and colony counting.

For the silver release test, 18 mm diameter gel disks were CAP treated for 5 min in a 12 well plate, and each gel was incubated in 2.5 ml of MilliQ water (pH 7.3) incubated at 37 °C, for 22 h. The concentration of released silver ions was quantified using an inductively coupled plasma mass spectrometer (ICP-MS) and an Agilent 710 simultaneous spectrometer at the University of Birmingham. The same gel was treated with CAP 24-h later and the release of silver ions quantified. Silver release was quantified using a standard calibration curve [supplementary material Fig. S4(a)]. For SEM/EDX analysis, a silver loaded PAA particle was cut in half using a razor blade, and cross-sectional SEM imaging and EDX elemental analysis were performed using JOEL JSM-7900F Field Emission SEM (supplementary material Fig. S5). Gel pH change was measured by loading the gels with bromothymol blue at 0.1 mg ml^{-1}.

The composite hydrogels were translucent gels of 2 mm thickness with good mechanical strength and rheological properties: they could be handled with tweezers and subjected to light static load (ca. 100 g cm^{-2}) without irreversible deformation, which is important if the gel is to be used as a wound dressing, where it might be left *in situ* for several days. Loading of PAA particles into the PVA did not negatively affect the rheological properties of the composite hydrogel and increased both the storage and loss moduli. (supplementary material Fig. S6).

Two key questions related to the interaction of the silver nitrate with the PAA particles are: how far into the particle did the silver penetrate, and was there any evidence of Ag^+ reduction/nanoparticle formation? The EDX mapping in Fig. 2 suggests that silver does not penetrate the core of the PAA particles but is located up to around 50 μm from the particle surface. The silver could potentially be in two primary forms: Ag^+ or Ag nanoparticles. Ag^+ could be reduced by acetate, taking up electrons and potentially forming Ag NPs. The brown coloration of the silver loaded gel (supplementary material Fig. S3) may suggest partial reduction of ionic silver to nanoparticles.

SEM and EDX elemental analysis of silver loaded PAA particles showed the extent of silver penetration into the PAA (Fig. 2). Most of the silver was found on or near the surface, with silver concentration in the core of the particle relatively low. This inhomogeneous distribution could be explained by the process of silver loading. When PAA particles were swollen in solution containing silver ions, the positively charged silver ions (Ag^+) were attracted by Coulombic forces to negatively charge carboxylic acid chains ($COOH^-$) of PAA and reduced to Ag (0), thus initiating further silver reduction and NP formation and creating a silver loading gradient from high to low from the surface to the core of the particle. Silver acetate is known to be photosensitive, with reduction to Ag(0) observed under light. It is likely that the intense UV produced by the plasma will reduce Ag^+ during treatment, in addition to any reduction occurring on exposure to ambient light during gel preparation. EDX color mapping indicated approximately 2.2 atomic % of silver at the particle surface (supplementary material Fig. S5).

Silver release from the composite gels was measured with/without CAP treatment followed by 22 h incubation in water. The silver release was quantitatively measured by ICP-MS initially following the formation of the composite hydrogel and then daily for 4 days. Measurement of CAP triggered silver release suggested was 2.8 μg ml^{-1} was released following 3 min plasma application; repeat application of CAP 24 h later afforded a near identical concentration of released silver which was largely maintained over the 4 days of test. Passive release from the gel

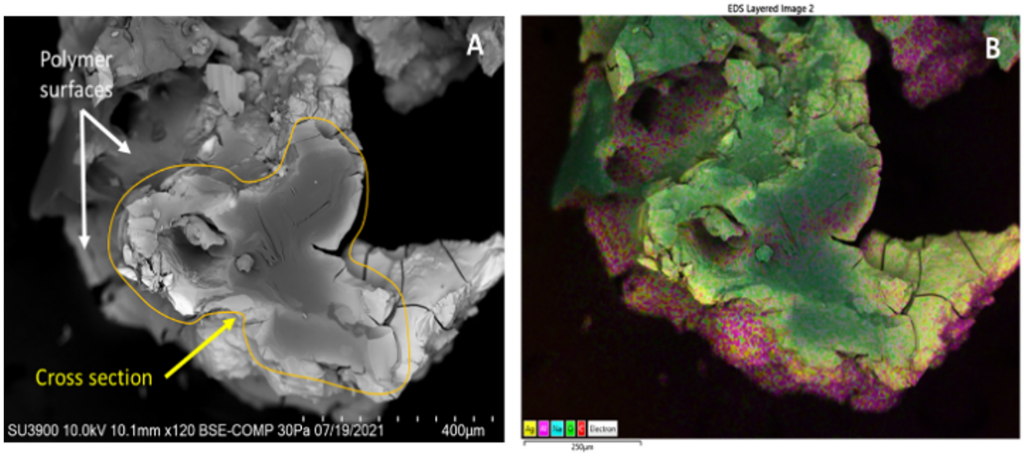

FIG. 2. Cross section of silver nitrate loaded PAA particle: (a) SEM and (b) EDX analysis [Ag (yellow), aluminum (pink), sodium (blue), oxygen (green), and carbon (red)]. Aluminum is used to externally cross-link the outside of the PAA particles.

(where the gel was not exposed to CAP) was also measured: initially this was relatively high at 1.3 μg ml^{-1}, but 24 h later had decreased to 0.2 μg ml^{-1} (supplementary material Fig. S4). This suggests that following gel formation, there is a significant concentration of silver in the PVA component of the composite hydrogel, but this diffuses away over time, as evidenced by the lower passive release of silver on day 2. However, it appears that the PAA particles provide an effective reservoir of silver, with only a fraction being lost with a short (5 min) application of CAP as evidenced by a near identical release of silver on day 2 following CAP application.

A modified Kirby–Bauer (KB) assay was used to measure the efficacy of CAP-induced silver release from the composite hydrogel in inhibiting bacterial growth. The ZOI (in millimeters) was measured in Figs. 3(a)–3(c) for *P. aeruginosa, S. aureus*, and *E. faecalis*, respectively). The ZOI was calculated by measuring the diameter of the zone of bacterial clearance subtracting the diameter of the hydrogel. ZOIs for the different bacterial species varied on the application of CAP to the silver loaded gels (supplementary material Fig. S8). CAP activated silver gels were able to inhibit growth of all bacterial three bacterial species. Our data show that *P. aeruginosa* was more susceptible to CAP activated silver gels than *S. aureus* and *E. faecalis*. This finding is supported by the MIC data for silver where *P. aeruginosa* was found to be most susceptible; the measured MIC_{50} values of $AgNO_3$ were: 11 μg ml^{-1} for *E. faecalis* (JH2-2), 8.2 μg ml^{-1} for *S. aureus* (H560), and 0.78 μg ml^{-1} for *P. aeruginosa* (PAO1), see supplementary material Fig. S7. There is evidence that in the case of *P. aeruginosa*, CAP mediates the antibiofilm efficacy of various antimicrobials via the oxidative stress response from the CAP produced RONS (reactive oxygen nitrogen species).[24] Inhibition of *S. aureus* and *E. faecalis* was observed [Figs. 3(b) and 3(c)] even though the silver concentration released was below the MIC of these species. As the silver diffuses away from the hydrogel, a locally high concentration adjacent to the gel will be expected and combined with the inhibitory effect of the high energy oxygen species created by the CAP creates an inhibitory zone around the gel.

The effect of CAP generated reactive species such as RONS on direct microbial inhibition without the presence of silver was observed in some of the negative control gels (PVA/PAA Gel + CAP). *P. aeruginosa* showed no growth inhibition from CAP produced RONS alone [Fig. 3(a)] compared with *S. aureus* [Fig. 3(b)] and *E. faecalis* [Fig. 3(c)], which showed ZOI of 4 and 8 mm, respectively. In contrast, CAP treated silver loaded composite hydrogels showed statistically significant increase in ZOIs for all three bacterial species strains compared with the silver loaded gel without application of CAP. Silver loaded gels without applied plasma all showed some bacterial

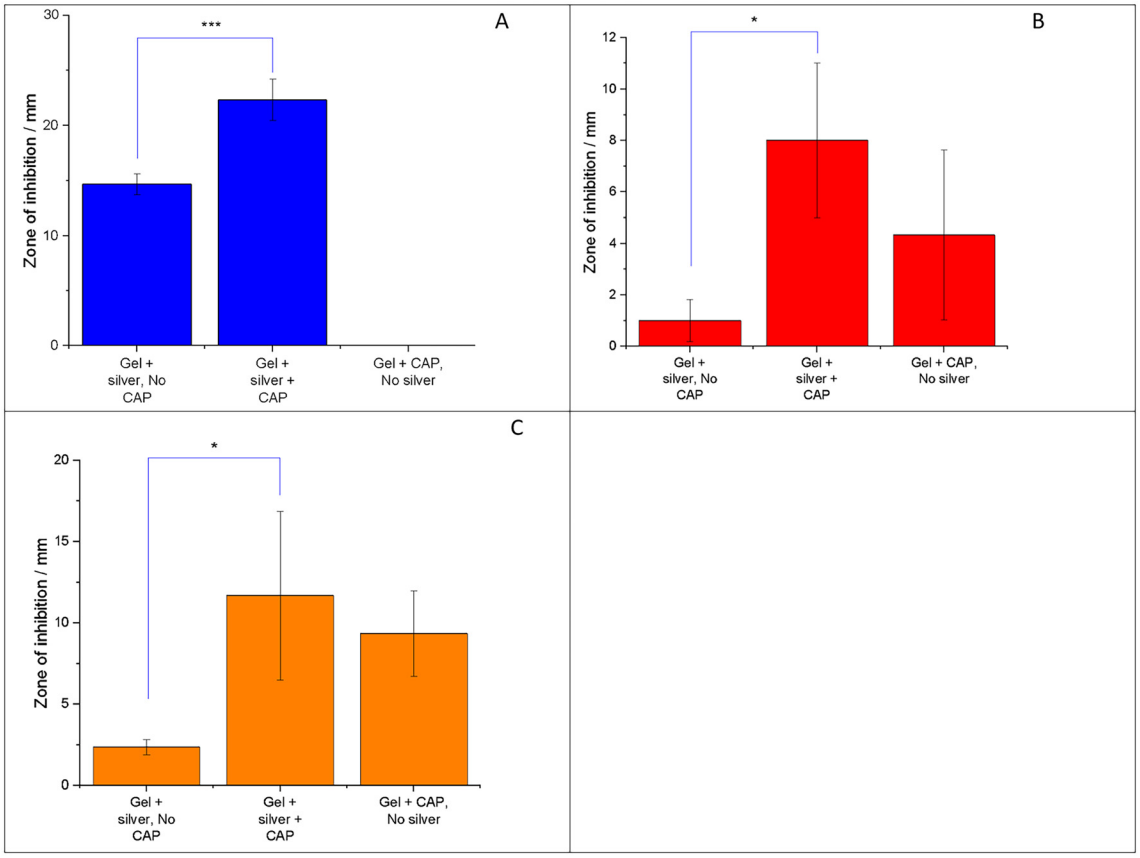

FIG. 3. ZOI of silver loaded composite hydrogels with (CAP Ag + PAA) and without (Ag + PAA) CAP treatment compared to non-loaded composite gels with (PVA/PAA Gel + CAP) CAP treatment. (a) *P. aeruginosa*, (b) *S. aureus*, and (c) *E. faecalis*. Error bars represent the standard deviation of the mean and a 2-sample unpaired t test: $^{*}p < 0.05$; $^{***}p < 0.001$.

inhibitory activity due to a degree of passive leaching, although this effect was not measurable after 24 h.

P. aeruginosa is a common wound pathogen and is often found in burns and chronic/hard to heal wounds where it forms biofilms which are relatively resistant to antimicrobials.[25] In this respect the result in Fig. 4 is most exciting: biofilms are a much more rigorous test for antimicrobial efficacy. The effect of released silver onto a growing colony biofilm of *P. aeruginosa* is shown in Fig. 4. *P. aeruginosa* was chosen for this study for its clinical relevancy, as it is often found colonizing chronic wound and burns. The difference in biofilm cell reduction between the released silver by CAP treatment and four controls was clearly seen. The combined effects of silver ions with RONS gave a >1.5 log reduction of viable biofilm cells: a 98% reduction.

The actual mechanism of action, i.e., why the plasma jet initiates silver release is still under investigation, but recent studies have shown that the CAP jet causes a local significant decrease in pH on the hydrogel. Composite hydrogels containing the pH indicator bromothymol blue, showed a pH drop of around 2–3 pH units where CAP interacts with the gel (supplementary material, Fig. S8). It is hypothesized that the CAP produced RONS creates nitrous acids in the gel (as well as significant oxygen and hydrogen peroxide), which results in the observed pH drop.[26] This decrease in pH, below the pK_a of the carboxylate groups in the PAA ($pK_a = 4.7$) causes their protonation reducing the electrostatic repulsion of the charged carboxylate groups and the displacement of the associated silver ions (or possibly nanoparticles) from the gel matrix. This causes a physical deswelling of the PAA particle, with a reduction in physical size and, thus, a likely "pumping out" action of the silver payload.

Silver is an effective antimicrobial, but there are clinical and regulatory concerns about its toxicity. Current silver dressings release silver passively. There is a global concern about silver stewardship and its use only when really needed. The system presented in this communication only releases silver on demand, i.e., based on the clinical suspicion of wound infection. Since silver can slow heal non-infected wounds, a dressing prototype that utilizes this technology should allow healthy wounds to heal without intervention but with rapid delivery of silver if required. Moreover, using cold plasma to effect silver release is potentially advantageous as it would be straightforward to deploy in a clinical setting, and as well as delivering the silver payload, it also delivers hydrogen peroxide and oxygen into the wound.

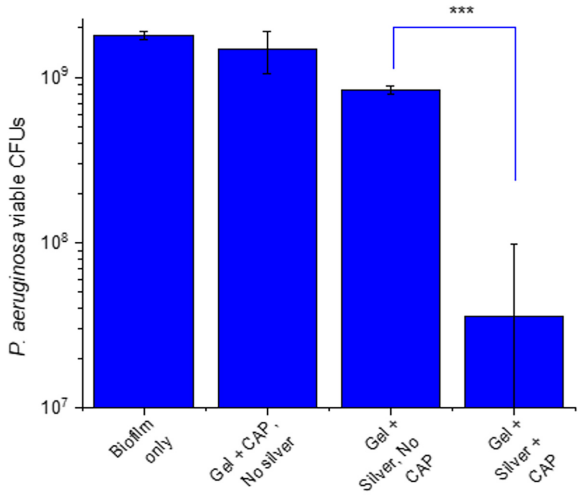

FIG. 4. Viable cell count of 8 h *P. aeruginosa* biofilms after 18 h of incubation with control composite hydrogels with no silver (H_2O gel), with (CAP + H_2O Gel) silver loaded gels without CAP and silver loaded gels with CAP (CAP + Ag + Gel), and without (Ag + Gel) CAP activation relative to untreated control biofilms. Error bars represent the standard deviation of the mean and a 2-sample unpaired t test: $^{***}p < 0.001$.

See the supplementary material for information on the cold atmospheric plasma jet (photo and parameters) S1; information on sodium polyacrylate gel swelling as a function of silver nitrate concentration S2; photographs of composite gel color change following plasma activation S3 and S4; EDX mapping of silver in sodium polyacrylate particles S5; composite hydrogel rheology data S6; MIC data for three bacterial species for silver nitrate **S7**; evidence of PH change in composite hydrogels following plasma application S8; exemplar Kirby–Bauer assay for Pseudomonas aeruginosa with composite hydrogel S9.

We acknowledge funding from the UK Engineering and Physical Sciences Research Council: EP/V00462X/1 and EP/R003939/1.

AUTHOR DECLARATIONS

Conflict of Interest

The authors have no conflicts to disclose.

Author Contributions

Naing Tun Thet: Formal analysis (equal); Investigation (equal); Methodology (equal); Writing – original draft (equal). **Bethany L. Patenall:** Funding acquisition (equal); Investigation (equal); Methodology (equal). **Robert D. Short:** Conceptualization (equal); Methodology (equal); Project administration (equal); Writing – review & editing (equal). **A. Toby A. Jenkins:** Conceptualization (equal); Formal analysis (equal); Project administration (equal); Writing – original draft (equal); Writing – review & editing (equal).

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

[1]R. Salomoni, P. Leo, A. F. Montemor, B. G. Rinaldi, and M. F. A. Rodrigues, "Antibacterial effect of silver nanoparticles in *Pseudomonas aeruginosa*," Nanotechnol. Sci. Appl **10**, 115–121 (2017).
[2]K. R. Halkai, J. A. Mudda, V. Shivanna, V. Rathod, and R. Halkai, "Antibacterial efficacy of biosynthesized silver nanoparticles against *Enterococcus faecalis* biofilms: An *in vitro* study," Contemp. Clin. Dent. **9**, 237–241 (2017).
[3]R. Behdad, M. Pargol, A. Mirzaie, S. Z. Karizi, H. Noorbazargan, and I. Akbarzadeh, "Efflux pump inhibitory activity of biologically synthesized silver nanoparticles against multidrug-resistant Acinetobacter baumannii clinical isolates," J. Basic Microbiol **60**, 494–507 (2020).
[4]S. M. Mousavi, S. M. A. Mousavi, M. Moeinizadeh, M. Aghajanidelavar, S. Rajabi, and M. Mirshekar, "Evaluation of biosynthesized silver nanoparticles

effects on expression levels of virulence and biofilm-related genes of multidrug-resistant Klebsiella pneumoniae isolates," J. Basic Microbiol. **63**, 632–645 (2023).
[5]H. Li, A. You, X. Feng, C. Zheng, X. Zeng, and H. Xu, "Effective treatment of *Staphylococcus aureus* infection with silver nanoparticles and silver ions," J. Drug Delivery Sci. Technol. **80**, 104165 (2023).
[6]A. Kramer, J. Dissemond, S. Kim, C. Willy, D. Mayer, R. Papke, F. Tuchmann, and O. Assadian, "Consensus on wound antisepsis: Update 2018," Skin Pharmacol. Physiol. **31**, 28–58 (2018).
[7]W. K. Jung, H. C. Koo, K. W. Kim, S. Shin, S. H. Kim, and Y. H. Park, "Antibacterial activity and mechanism of the action of the silver ion in *Staphylococcus aureus* and *Escherichia coli*," Appl. Environ. Microbiol. **74**, 2171–2178 (2008).
[8]M. L. Reniere, A. T. Whiteley, K. L. Hamilton, S. M. John, P. Lauer, R. G. Brennan, and D. A. Portnoy, "Glutathione activates virulence gene expression of an intracellular pathogens," Nature **517**, 170–175 (2015).
[9]L. Wang, L. Liu, Y. Liu, F. Wang, and X. Zhou, "Antimicrobial performance of novel glutathione-conjugated silver nanoclusters (SGH@AgNCs) against *Escherichia coli* and *Staphylococcus aureus* by membrane-damage and biofilm-inhibition mechanisms," Food Res. Int. **160**, 111680 (2022).
[10]Wounds UK, *Best Practice Statement: Use of Silver Dressings in Wound Care* (Wounds UK, London, 2021).
[11]S. L. Percival, P. G. Bowler, and D. Russell, "Bacterial resistance to silver in wound care," J. Hosp. Infect **60**, 1–7 (2005).
[12]V. K. M. Poon and A. Burd, "In vitro cytotoxicity of silver: Implication for clinical wound care," Burns **30**, 140–147 (2004).
[13]I. Khansa, A. R. Schoenbrunner, C. T. Kraft, and J. E. Janis, "Silver in wound care—Friend or Foe?: A comprehensive review," Plast. Reconstr. Surg. **7**(8), e2390 (2019).
[14]H. B. Kwon, J. H. Lee, S. H. Lee, A. Y. Lee, J. S. Choi, and Y. S. Ahn, "A case of argyria following colloidal silver ingestion," Ann. Dermatol. **21**, 308–310 (2009).
[15]J. J. Kim, K. Konkel, L. McCulley, and I.-L. Diak, "Cases of argyria associated with colloidal silver use," Ann. Pharmacother. **53**, 867–870 (2019).
[16]R. Warriner and R. Burrell, "Infection and the chronic wound: A focus on silver," Adv. Skin Wound Care **18**, 2–12 (2005).
[17]N. T. Thet, J. Mercer-Chalmers, R. J. Greenwood, A. E. R. Young, K. Coy, S. Booth, A. Sack, and A. T. A. Jenkins, "*SPaCE* Swab: Point-of-care sensor for simple and rapid detection of acute wound infection," ACS Sens. **5**, 2652–2657 (2020).
[18]K. D. Verma, F. Lewis, M. Mejia, M. Chalasani, and K. A. Marcus, "Food and drug Administration perspective: Advancing product development for non-healing chronic wounds," Wound Repair Regener. **30**, 299–302 (2022).
[19]S. Sabrin, S. H. Hong, and S. Kumar KC, "Electrochemically enhanced antimicrobial action of plasma-activated poly(vinyl alcohol) hydrogel dressings," Adv. Funct. Mater 2314345 (2024).
[20]H. Fang, J. Wangb, L. Li *et al.*, "A novel high-strength poly(ionic liquid)/PVA hydrogel dressing for antibacterial applications," Chem. Eng. J. **365**, 153–164 (2019).
[21]N. Gaur, B. L. Patenall, B. Ghimire, N. T. Thet, J. E. Gardiner, K. E. Le Doare, G. Ramage, G. Short, R. A. Heylen, C. Williams, R. D. Short, and T. A. Jenkins, "Cold atmospheric plasma-activated composite hydrogel for an enhanced and on-demand delivery of antimicrobials," ACS Appl. Mater. Interfaces **15**, 19989–19996 (2023).
[22]A. W. Bauer, W. M. M. Kirby, J. C. Sherris, and M. Turck, "Antibiotic susceptibility testing by a standardized single disk method," Am. J. Clin. Pathol. **36**, 493–496 (1966).
[23]N. T. Thet, D. R. Alves, J. E. Bean, S. Booth, J. Nzakizwanayo, A. E. R. Young, B. V. Jones, and A. T. A. Jenkins, "Prototype development of the intelligent hydrogel wound dressing and its efficacy in the detection of model pathogenic wound biofilms," ACS Appl. Mater. Interfaces **8**, 14909–14919 (2016).
[24]J. A. Maybin, T. P. Thompson, P. B. Flynn, T. Skvortsov, N. J. Hickok, T. A. Freeman, and B. F. Gilmore, "Cold atmospheric pressure plasma-antibiotic synergy in *Pseudomonas aeruginosa* biofilms is mediated via oxidative stress response," Biofilm **5**, 100122 (2023).
[25]J. M. Andrews, "Determination of minimal inhibitory concentrations," J. Antimicrob. Chemother. **48**(suppl 1), 5–16 (2001).
[26]J.-S. Oh, E. J. Szili7, N. Gaur, S.-H. Hong, H. Furuta, H. Kurita, A. Mizuno, A. Hatta, and R. D. Short, "How to assess the plasma delivery of RONS into tissue fluid and tissue," J. Phys. D **49**, 304005 (2016).
[27]S.-H. Hong, E. J. Szili, M. Fenech, N. Gaur, and R. D. Short, "Genotoxicity and cytotoxicity of the plasma jet-treated medium on lymphoblastoid WIL2-NS cell line using the cytokinesis block micronucleus cytome assay," Sci. Rep. **7**, 3854 (2017).