COMPARISON OF VARIOUS ULTRA-SMALL AND NANOTECHNOLOGY-BASED PRODUCTS FOR WOUND CARE

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ABSTRACT

Background: Nanostructured wound care products have gained traction over the past 30 years. Nanocrystalline silver dressings that release Ag^0 clusters, Ag^+, and higher-oxidation-state Ag are indicated for use with burns, chronic ulcers, & surgical wounds as bacterial barriers. Colloidal/nanoparticle Ag is indicated for uses in skin/wound care, including restoring skin integrity. A micro-catalytic wound gel containing SiO_2, TiO_2, and Cu is indicated for the management of chronic, acute, and minor wounds; burns; and minor skin infections and irritations by maintaining a moist environment, supporting autolytic debridement, reducing inflammation, and providing broad-spectrum antimicrobial/biofilm protection.This study compared the antimicrobial activity of various nanostructured materials.

Methods: Antimicrobial activity of the catalytic microtitania/silica wound gel, the nanocrystalline Ag dressing, and 40 ppm colloidal Ag was compared to $CuCl_2$ (0.1 ppb, 100 ppm) and $AgNO_3$ (40 ppm Ag^+), against *S. aureus* and *P. aeruginosa* for 5, 15, 30, 45, and 60 min challenges at RT (n=3). 16h cultures were inoculated in 25 mL calf serum and incubated for 18-20h at 37°C & 110 RPM to produce 7.3-7.6 log CFU (colony forming unit) inocula; 5.6-5.8 log CFU for $CuCl_2$ & $AgNO_3$. 1"x1" nanocrystalline Ag dressings received 200 µL inoculum. For $CuCl_2$, 4.90 mL dH_2O, 50 µL inoculum, & 50 µL stock solution were combined (4.90 mL, 50 µL, & 20 µL, respectively, were used for $AgNO_3$). For colloidal Ag, 240 µL was added to 30 µL dH_2O & 30 µL inoculum. For the gel, 100 µL of inoculum was mixed into 100 µL gel in a 96-well plate. After the challenges, Cu- or Ag-containing samples were neutralized with ethylenediaminetetraacetic acid (EDTA) or Na-thioglycolate/NaCl/Tween-80, respectively, followed by serial dilution in PBS, spot plating, incubation at 37°C for ~24h, and CFU counts.

Results: The catalytic gel and colloidal Ag showed no antimicrobial activity against *S. aureus* (log survivors between 7.5-9.1 and 7.5-8.0, respectively) or *P. aeruginosa* (log survivors ≥9.18 and 6.70-8.1, respectively). $CuCl_2$ at 0.1 ppb generated total kill at all times against *P. aeruginosa* (log survivors were below the detection point; log reductions >5.6), and at 100 ppm was nearly bactericidal by 30 min (2.63 log reduction; 3.0 log survivors), with subsequent total kill (log reductions >5.6) on *S. aureus*. Nanocrystalline Ag generated total kill against *S. aureus* (log reductions >7.8) and was bactericidal against *P. aeruginosa* (log reductions of 3.5 at 5 min with log survivors of 5.1 at 15 min, and >7.6 – total kill – thereafter). $AgNO_3$ generated a 2.7 log reduction against *S. aureus* at 5 min, followed by total kill (log reductions >5.8) thereafter; and consistent total kills against *P. aeruginosa* (log reductions >5.6).

Conclusion: The catalytic gel had no antimicrobial activity when used with high organic matter (serum), while $CuCl_2$ was effective at 100 ppm against *S. aureus*, and at 0.1 ppb against *P. aeruginosa*. Colloidal Ag did not exhibit antimicrobial activity, whereas the nanocrystalline Ag dressing was more effective than $AgNO_3$, achieving rapid, total kill of both microorganisms.

INTRODUCTION

Nanostructured wound care products have gained traction over the past 30 years. Nanocrystalline silver dressings that release Ag^0 clusters, Ag^+, and higher-oxidation-state Ag are indicated for use with burns, chronic ulcers, & surgical wounds as bacterial barriers. Nanocrystalline silver also releases higher-oxidation-state silver, which is thought to be in an anion complex that protects it from chloride in the wound environment. It is believed that silver's higher oxidation state is responsible for the enhanced antimicrobial activity of the nanocrystalline material. While Ag^+ is released from the nanocrystalline silver dressing, its rapid consumption by chloride in the test media, or in serous exudate, results in it contributing very little to the antimicrobial activity.

Ag^+ can be used effectively when high concentrations are used and reapplied frequently, as with silver nitrate. In a burn unit, silver nitrate is applied every 2-6 hours as a 0.5% solution (3176 µg/mL Ag^+) (1). At this concentration and addition frequency, there is sufficient free silver to maintain antimicrobial control. Products such as silver sulfadiazine deliver silver in a controlled-release formulation up to 3000 µg/mL. While these concentrations are far higher than typical MBCs, between 1-10 µg/mL, they do not cause appreciable cytotoxic effects.

Colloidal/nanoparticle Ag is indicated for uses in skin/wound care, including restoring skin integrity. Colloidal silver was first used in the early part of the 20th century when it was approved by the FDA as an antimicrobial agent. However, the FDA withdrew this approval shortly thereafter due to insufficient evidence of antimicrobial activity. Currently, the literature is rife with papers claiming that colloidal silver has antimicrobial properties, as well as those disputing this (2, 3, 4, 5).

A micro-catalytic wound gel containing SiO_2, TiO_2, and Cu is indicated for the management of chronic, acute, and minor wounds; burns; and minor skin infections and irritations by maintaining a moist environment, supporting autolytic debridement, reducing inflammation, and providing broad-spectrum antimicrobial/biofilm protection. Copper is known as a good antimicrobial agent with particularly strong anti-fungal activity. It was first used as an antifungal in the 1800s in a solution known as Bordeaux mixture (6). But its use in humans was curtailed due to allergic reactions which occur in approximately 4% of the population (7).

Reactive oxygen species (ROS) are produced by titania catalysts in dark reactions (see Table 1 for common forms of ROS). They are potent antimicrobials, as they attack organic matter. The non-specific nature of their chemical activity limits their usefulness, as they are often associated with an inflammatory response and the attack on pro-healing proteins, such as cytokines and chemokines.

Table of common reactive oxygen species and their basic sources

Name	Notation	Comments and Basic Sources
Molecular oxygen (triplet ground state)	O_2	Common form of dioxygen gas
Singlet oxygen (1st excited singlet state)	O_2	Photoinhibition; UV irradiation; e- transfer reactions in chloroplasts.
Superoxide anion	$O_2^{\bullet-}$	Formed in many photooxidation reactions; Mehler reactions in chloroplasts, mitochondrial e- transfer reactions; glyoxysomal photorespiration; peroxisomal activity; nitrogen fixation; reactions of O_3 and $OH^\bullet$ in apoplastic space; defense against pathogens; oxidation of xenobiotics.
Hydrogen peroxide	H_2O_2	Formed by $O_2^{\bullet-}$ dismutation; photorespiration; β oxidation; proton-induced decomposition of $O2^{\bullet-}$ defense against pathogens.
Hydroxyl radical	$OH^\bullet$	Decomposition of O_3 in apoplastic space; defense against pathogens; reaction of H_2O_2 with $O2^{\bullet-}$ (Haber-Weiss); reactions of H_2O_2 with Fe^{2+} (Fenton); highly reactive with all macromolecules.
Perhydroxyl radical	$O_2H^\bullet$	Protonated form of $O_2^{\bullet-}$ reactions of O_3 and $OH^\bullet$ in apoplastic space.
Ozone	O_3	UV radiation or electrical discharge in stratosphere; reactions involving combustion products of fossil fuels and UV radiation in the troposphere.

Table 1. Table adapted from Scandalios J. G. (2005). Oxidative stress: molecular perception and transduction of signals triggering antioxidant gene defenses. Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas, 38(7), 995–1014. https://doi.org/10.1590/s0100-879x2005000700003

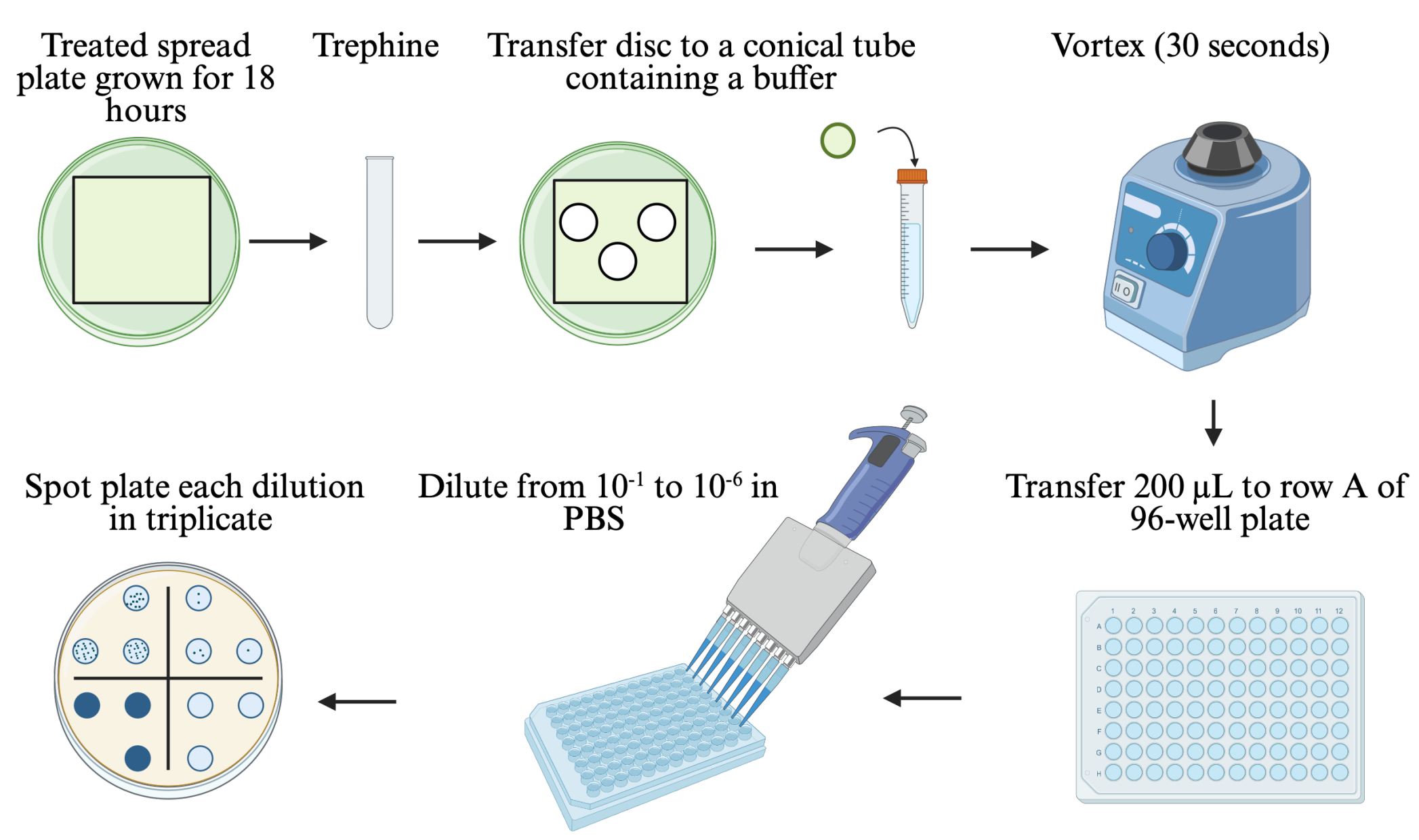
METHODS

Standard time survivor curves were generated for $CuCl_2$ (100 ppm for *S. aureus* and 0.1 ppb for *P. aeruginosa*) , 40 ppm $AgNO_3$, Acticoat 7™, NanoSALV (a microtitania/silica-based gel), and 40 ppm colloidal silver. Survivor curves were generated using methods similar to those of Gallant et al. (9). A 40 ppm $AgNO_3$ concentration was used, as that is the amount of silver present in the same unit area of Acticoat 7™. The $CuCl_2$ concentration used was determined using previously generated concentration curves.

Further testing was performed using NanoSALV at 0.06 g/25.8 cm² to match patient use instructions, Acticoat 7™ as provided, 4% CMC (a gel control for the NanoSALV), and untreated controls. The testing was performed by spreading 100 µL of either *P. aeruginosa* or *S. aureus* on TSA and treating a defined area of the TSA with the different treatments. Organic matter concentrations were 60 g/L serum and 20 g/L TSA. After incubation for 18 hours, a 15 mm trephine was used to cut a disc for enumeration on TSA plates.

An experiment to look at the effect of the ratio of medium to efficacy of the NanoSALV gel. It was performed by adding either a 1:1 or 1:5 ratio of organics (serum) to NanoSALV with a 4% CMC gel as a control. An incubation period of 1 hour was utilized, followed by serial dilutions and spot plating. In all instances, CFU/mL counts were determined on the dilution whose 20 µL spots contained between 2-30 colonies.

METHODS



Treated spread plate grown for 18 hours

Trephine

Transfer disc to a conical tube containing a buffer

Vortex (30 seconds)

Transfer 200 µL to row A of a 96-well plate

Dilute from 10^{-1} to 10^{-6} in PBS

Spot plate each dilution in triplicate

Figure 1. Method for the determination of efficacy of NanoSALV on bacterial lawns grown at 37°C for 18 hours. The spread plate was made by spreading a lawn of 100 µL bacteria and then immediately treating with 0.06 g/25.8 cm² of NanoSALV or CMC gel, or 3 Acticoat 7™ 1" x 1" pieces as purchased, with untreated controls included, then incubated for 18 hours.

RESULTS

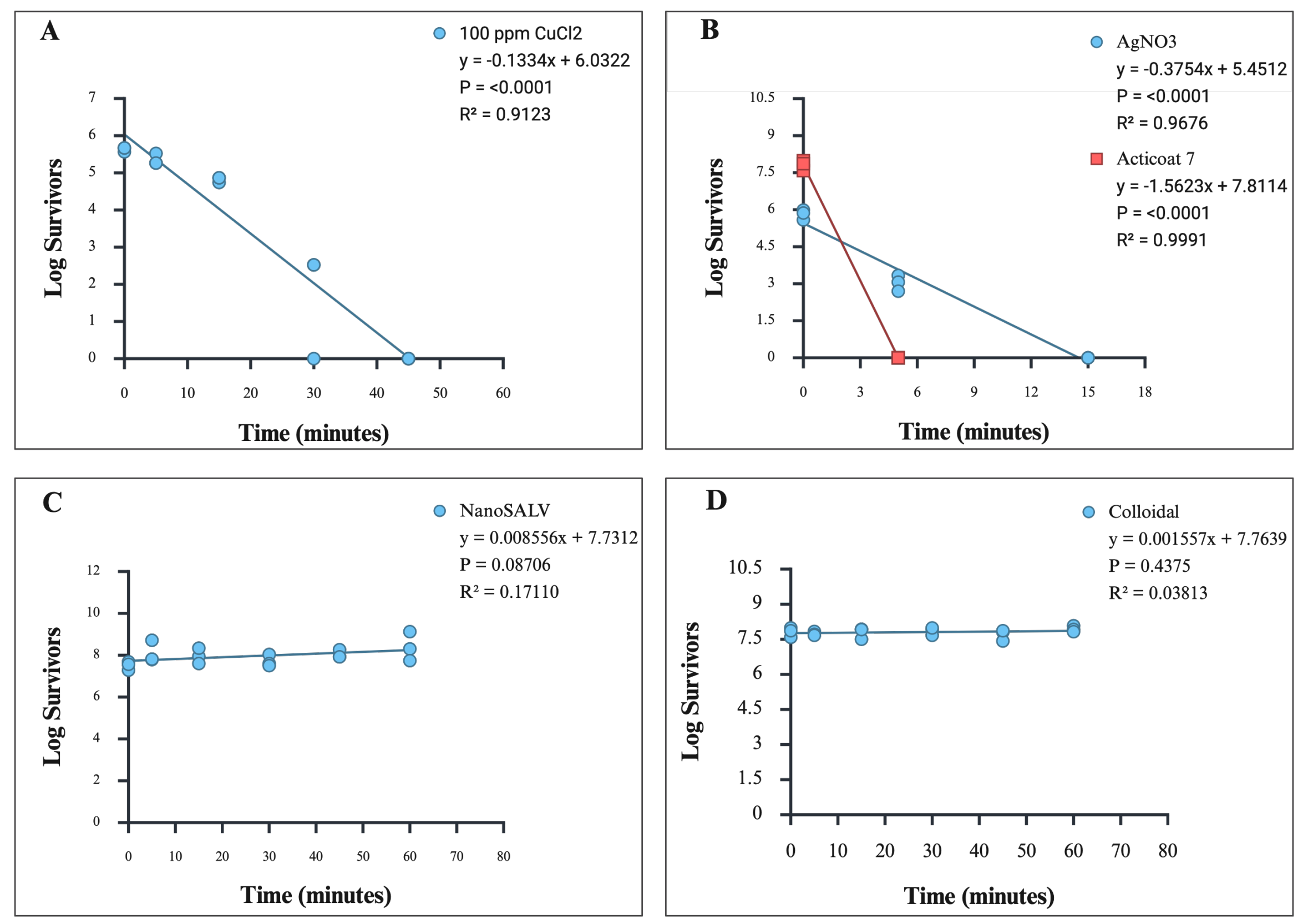


Figure 2. Log survivor results after *S.aureus* incubated for either 5, 15, 30, 45, or 60 minutes with (A) 100 ppb $CuCl_2$, (B) 40 ppm $AgNO_3$ and Acticoat 7™, (C) 1:1 ratio of bacteria to microtitania/silica gel, and (D) 40 ppm colloidal silver.

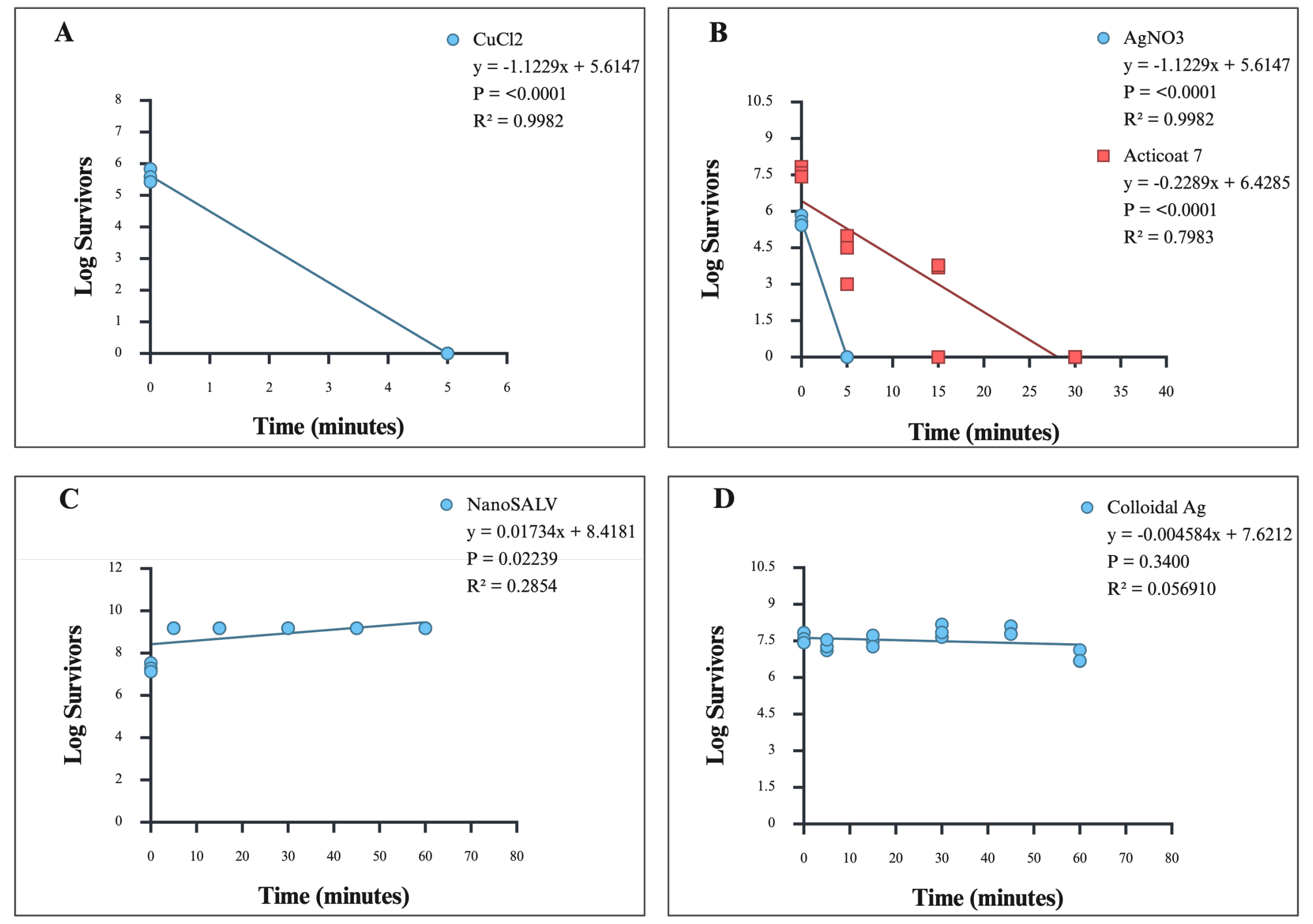


Figure 3. Log survivor results after *P. aeruginosa* incubated for either 5, 15, 30, 45, or 60 minutes with (A) 0.1 ppb $CuCl_2$, (B) 40 ppm $AgNO_3$ and Acticoat 7™, (C) 1:1 ratio of bacteria to microtitania/silica gel, and (D) 40 ppm colloidal silver. All the data points for microtitania/silica gel were the greater than maximum countable number.

RESULTS

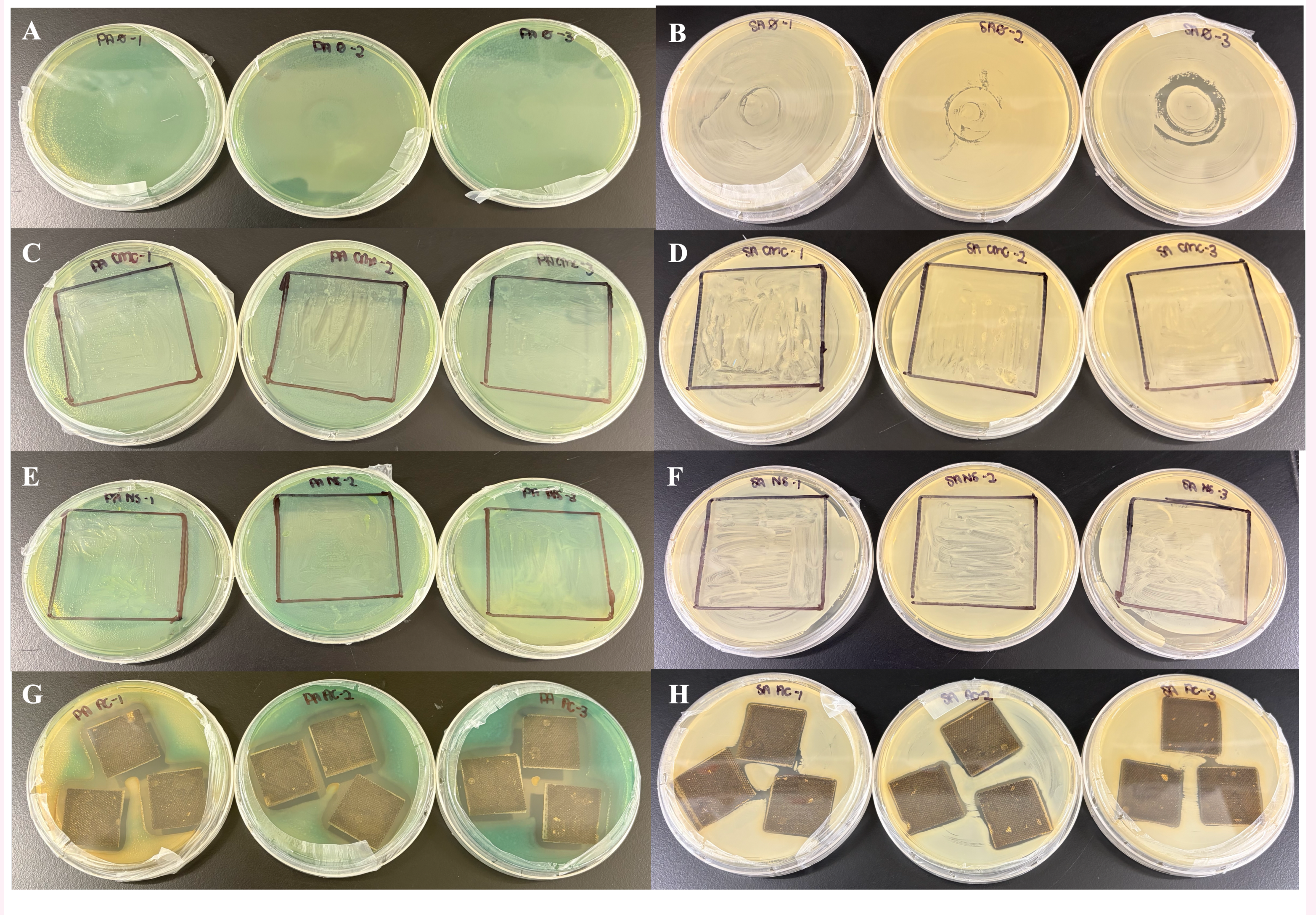


Figure 4. Bacterial lawns grown for the determination of efficacy of microtitania/silica after treatment and incubation for 18 hours. A,C,E,G= *P. aeruginosa*, B,D,F,H= *S. aureus*. A and B are after spread plating and no treatment, C and D are after 0.06 g of CMC was spread on a 2" x 2" square of the plate. E and F are after 0.06 g of microtitania/silica gel was spread on a 2" x 2" square of the plate. Finally, F and H are after 3 1" x 1" sheets of Acticoat 7™ were moistened with distilled water and placed onto the agar surface.

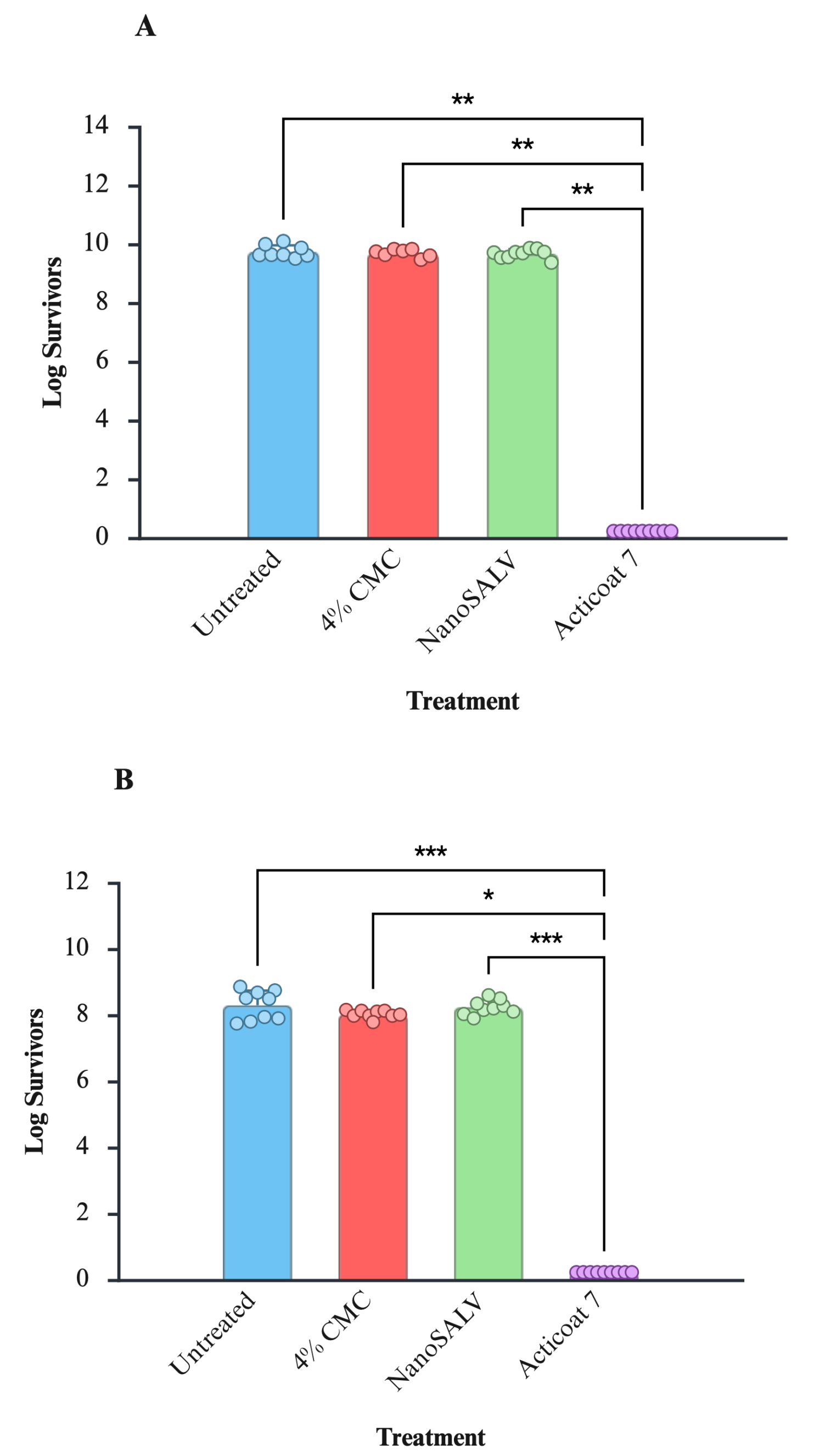


Figure 5. Log survivors after trephination and enumeration of an overnight agar plate treated with either CMC, microtitania/silica, or Acticoat 7™, or untreated control plates. (A) *S. aureus*, and (B) *P. aeruginosa*. The log survivors for Acticoat 7™ were below the detection limit for both of the bacteria tested. Standard deviation error bars are shown.

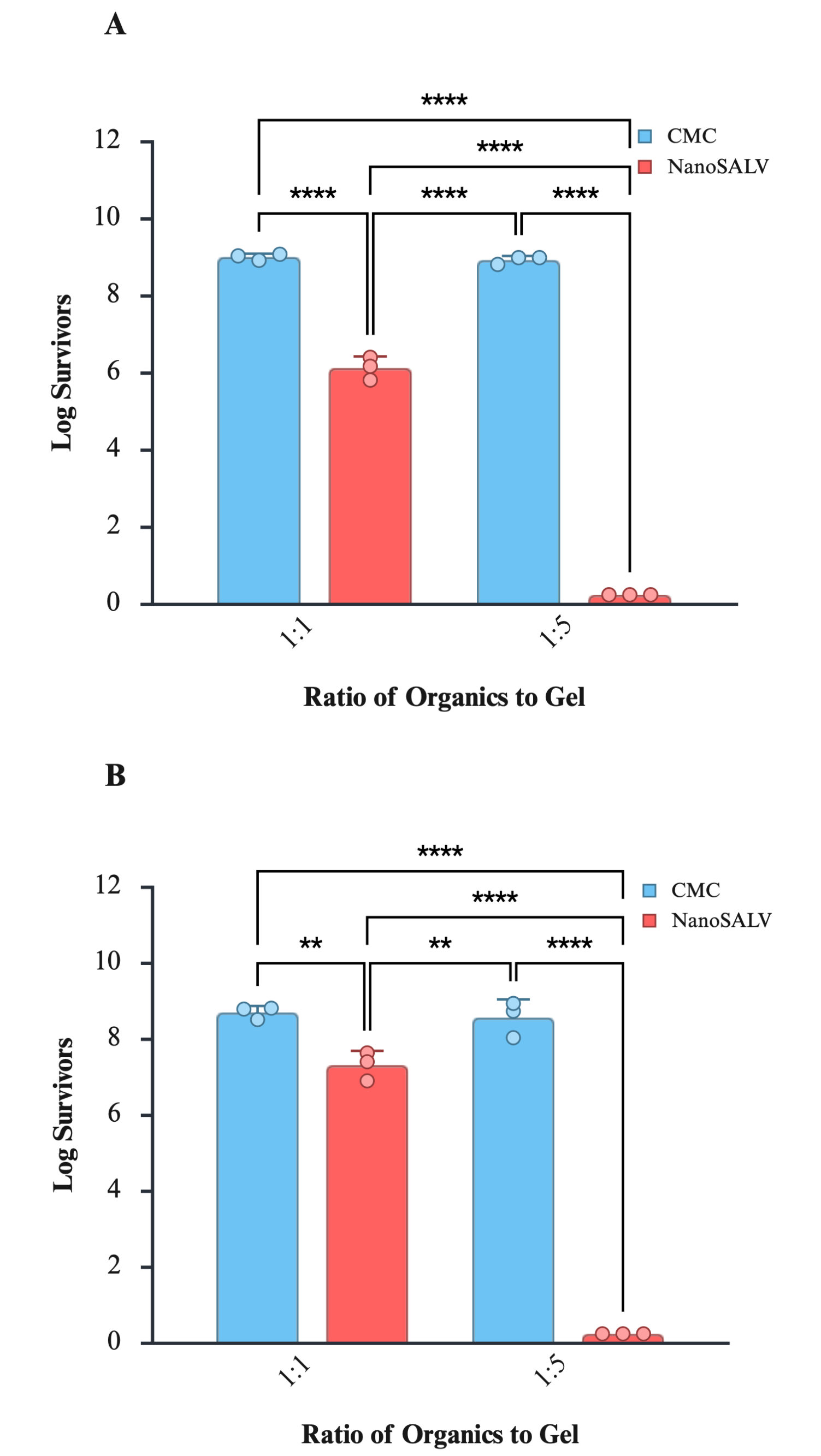


Figure 6. Log survivor results for (A) *S. aureus* and (B) *P. aeruginosa* after 1 hour incubation with various ratios of organic matter in gel-based products. Log survivor value for 1:5 NanoSALV was below the detection limit for both of the bacteria tested. Standard deviation error bars are shown.

RESULTS/DISCUSSION

S. aureus was sensitive to 100 ppm $CuCl_2$, showing a 6-log reduction after 45 minutes (Figure 2). Acticoat 7™ reduced the *S. aureus* population by 8 log in 5 minutes, and 40 ppm $AgNO_3$ reduced the *S. aureus* population by 6 logs in 15 minutes. The microtitania/silica gel, when mixed 1:1 with the inoculum, had no effect, and neither did 40 ppm colloidal silver on the *S. aureus* population. The data show that nanocrystalline silver is far more effective against *S. aureus* than $AgNO_3$ or $CuCl_2$. The microtitania/silica system and colloidal silver had no effect after an hour. Similar results were seen with *P. aeruginosa* (Figure 3). The nanocrystalline silver is the most effective, followed closely by $CuCl_2$ and $AgNO_3$, while the microtitania/silica system and colloidal silver had no effect.

In a Petri plate-based simulation of wound treatment using microtitania/silica, 4% CMC, and Acticoat 7™ (Figure 4), it was shown that the catalytic treatment was no different from a control CMC or a control untreated TSA media. In the case of Acticoat 7™, clear zones of inhibition indicated at least bacteriostatic activity. The log survivors from each Petri plate are shown in Figure 5 for *S. aureus* and *P. aeruginosa*. No organisms were recovered from under the Acticoat, indicating that it exhibited a bactericidal effect.

The microtitania/silica data is shown in Figure 6, where it can be seen that the 1:1 mixture of microtitania/silica with inoculum resulted in ~2 log reductions for *S. aureus*, and ~1 log reduction for *P. aeruginosa*. The 1:5 inoculum to microtitania/silica ratio resulted in an 8-log reduction for both *P. aeruginosa* and *S. aureus*.

The primary mechanism of action for microtitania/silica is the reactive oxygen species generated by the titania in both the light and dark reactions. These reactive oxygen species attack organic matter generally and derive their antimicrobial activity from the reaction between the ROS and the organisms. In these experiments, the inocula were grown in calf serum containing ~60 g/L of organic matter. At a 1:1 ratio of microtitania/silica to the inoculum, the level of organic matter is sufficient to remove most of the antimicrobial activity. At a 1:5 ratio, there is 80% less organic matter, and thus, more reactive species are available to attack the microorganisms. In the Petri plate experiment, the organic matter in the TSA is ~20 g/L. Each plate contained ~15 mL of agar, corresponding to 0.3 g of organic matter. This was sufficient to tie up the ROS from 0.06 g /25 cm² of micro titania/silica, the recommended dose per treatment, based on the assumption that a 15 g tube of the product contains ~60 applications (value obtained from NanoSALV product information). An exudative wound would release 5.25 g/25 cm² of serous exudate per day, providing more organic matter than the Petri plates (10). It is unlikely that microtitania/silica would provide any anti-inflammatory activity to an exudative wound, given that it produces ROS, a key driver of inflammation. The data presented here clearly show that small size alone is insufficient to generate antimicrobial activity. Colloidal silver had the smallest particle size tested and, while composed of silver, has no antimicrobial activity. This same phenomenon has been described elsewhere (5). The activity of the microtitania/silica is controlled by the presence of additional organic matter. When the organic matter level is high, efficacy is limited, while in situations with low organic matter, which would be impossible in a wound environment, there is strong efficacy observed. The nanocrystalline silver is effective regardless of the concentration of organic matter. This activity is driven by the nanostructure, which releases multiple silver species that are integral to the product's antimicrobial activity.

FUTURE RESEARCH

Future research in this field could include further investigation of nanocrystalline silver wound dressings. As shown in Figure 7, as the crystallite size decreases, the volume of intergrain atoms (see Figure 8) increases. As discussed in the paper by Nadworny et al., (5) the intergrain atoms are both the higher-oxidation-state atoms that confer the antimicrobial activity of these dressings and the neutral clusters which are believed to be anti-inflammatory.

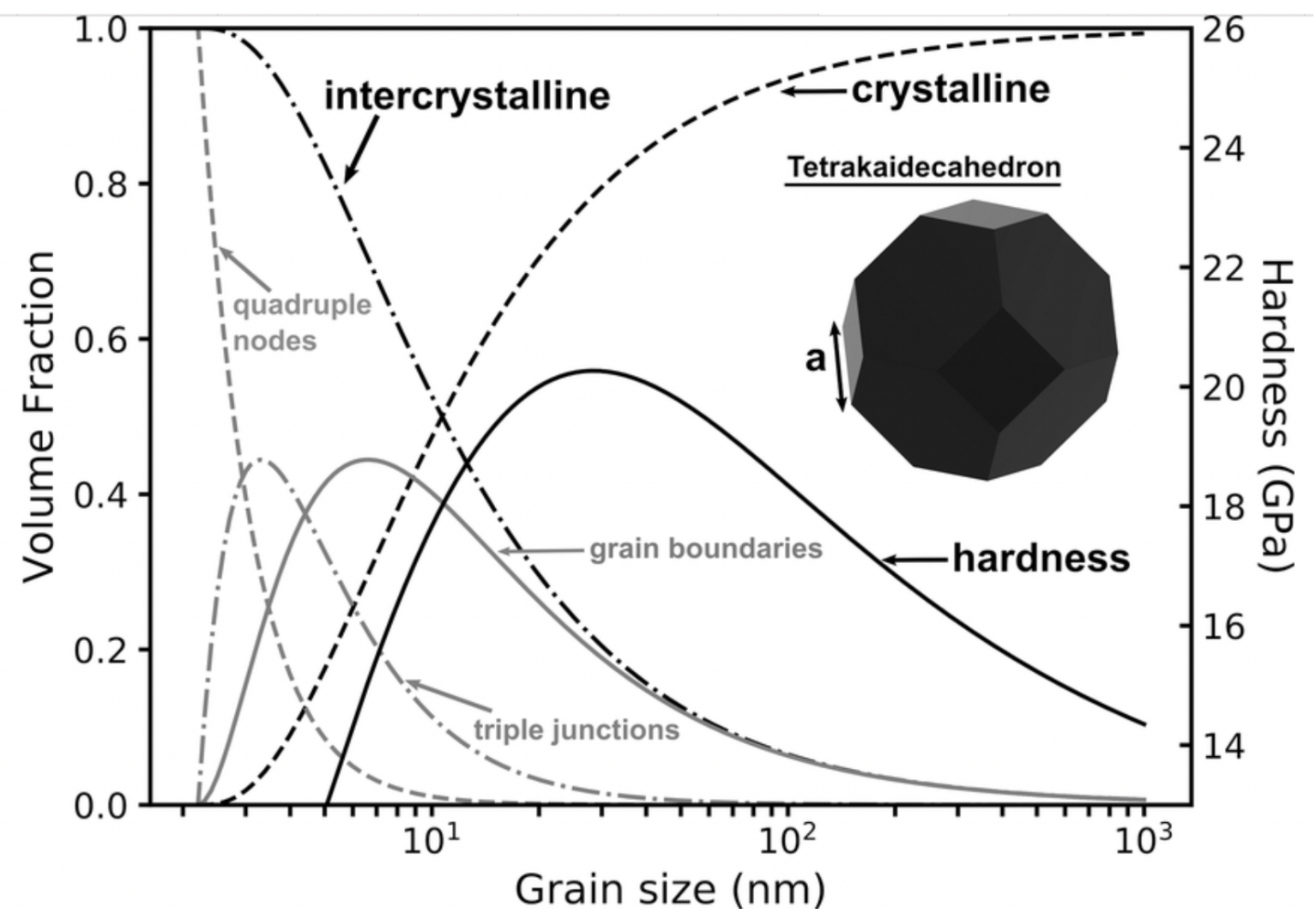


Figure 7. The effect of grain size on intercrystalline volume. As the grain size (nm) decreases, the volume of intercrystalline atoms increases. This figure was obtained from Microstructural effects on hardness and optical transparency of birefringent aluminosilicate nanoceramics - Scientific Figure on ResearchGate. Available from: https://www.researchgate.net/figure/Volume-fractions-of-the-intercrystalline-and-crystalline-components-with-respect-to_fig3_338231601 [accessed 25 Mar 2026].

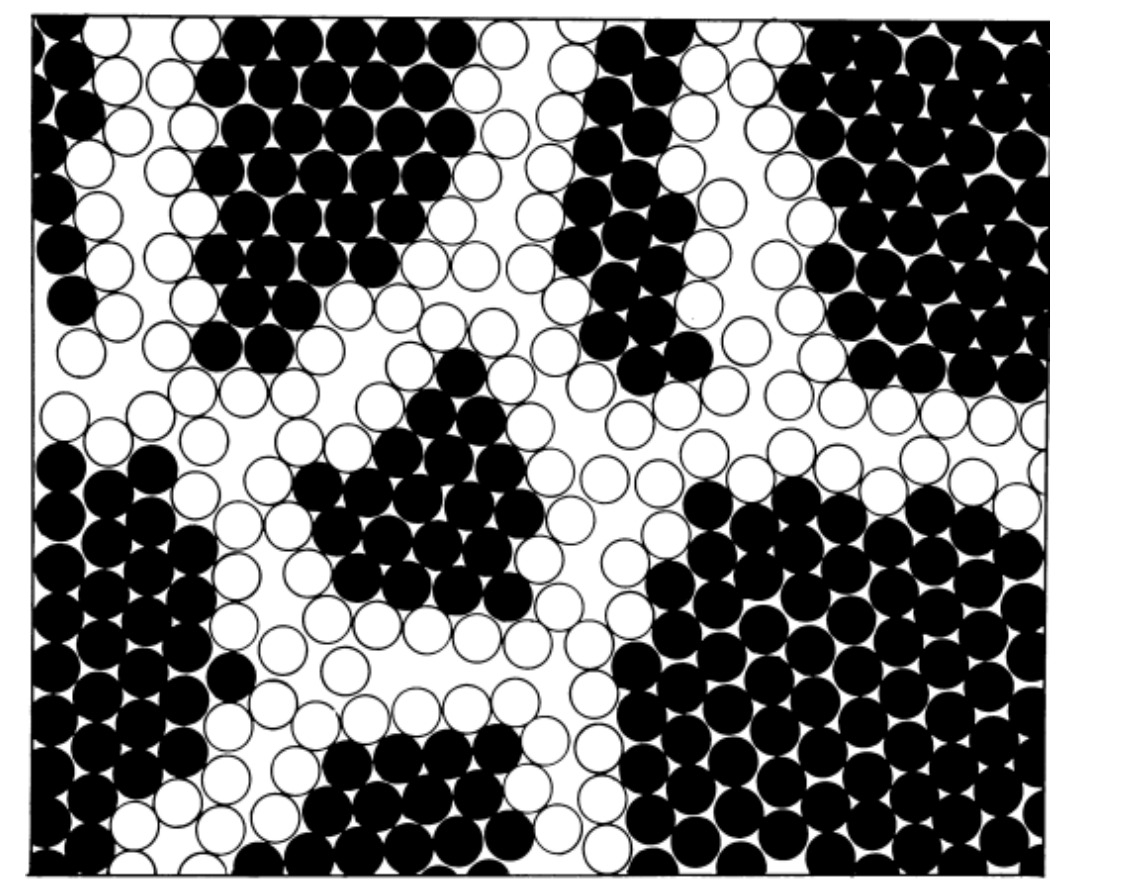


Figure 8. Two-dimensional model of a nanostructured material. The atoms in the centers of the crystals are indicated in black. The ones in the boundary core regions are represented as open circles. This figure was obtained from Gleiter, H (2000). Nanostructured materials: Basic concepts and microstructure. Acta Materialia, 48(1). https://doi.org/10.1016/S1359-6454(99)00285-2

CONCLUSION

1. Being small, or nano-sized does not guarantee efficacy.
2. Colloidal silver behaves like bulk silver, with limited efficacy.
3. Nanocrystalline silver, with its large grain boundary volume is an excellent antimicrobial.
4. Catalytic materials that produce ROS only work in the absence of organic matter, limiting their use in wound care.

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