Broad-Spectrum Antimicrobial and Anti-Biofilm Activity of a Gel Spray for Wound Infection Control in Austere Environments

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Combat and field-acquired wounds remain a major threat to Force Health Protection, particularly in austere and prolonged field care environments where evacuation and advanced medical support may be delayed. These injuries are highly susceptible to infection by biofilm-forming, multidrug-resistant pathogens such as *Staphylococcus aureus, Pseudomonas aeruginosa*, and *Acinetobacter baumannii*, leading to chronic infection, delayed healing, and increased risk of limb loss. Current standard-of-care topical treatments offer limited biofilm penetration and may impair tissue regeneration. There is a critical need for deployable, field-ready countermeasures that provide effective infection control while preserving tissue viability and promoting wound healing. A novel topical antimicrobial formulation (Nanogranex®) incorporating synergistic silver and zinc ions within a nanoparticle-enhanced delivery system was evaluated in vitro. Antimicrobial and anti-biofilm efficacy were assessed against clinically relevant wound pathogens, including mature biofilms of *S. aureus* and *P. aeruginosa*, using standardized biomass reduction assays. Cytotoxicity and tissue compatibility were evaluated in accordance with ISO 10993-5 using established mammalian cell models. Cytotoxicity testing showed >85% cell viability, indicating preservation of host tissue integrity compared to conventional silver-based treatments. The product demonstrated efficacy against immature biofilms, indicating its potential for preventing biofilm formation. The formulation achieved >70% reduction in biofilm biomass. These findings support effective biofilm disruption alongside favorable biocompatibility. This work supports the development of a field-deployable wound care product aligned with Force Health Protection priorities. By combining biofilm prevention, and tissue preservation in a single formulation, this approach addresses a critical gap in prolonged field care and en route care settings. Implementation of such technology has the potential to reduce infection-related complications, improve wound healing outcomes, and enhance survivability and return-to-duty for injured Service Members in operational environments.

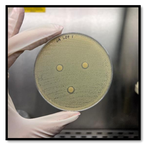
Clinical and Military Relevance

- ❑ **Combat- and field-acquired wounds are highly susceptible to biofilm-forming, multidrug-resistant pathogens, especially in austere and prolonged field care environments where evacuation may be delayed, highlighting the need for novel wound care therapies.**
- ❑ **This study evaluates a topical sprayable wound gel as a deployable, field-ready countermeasure to prevent infection, preserve tissue viability, and promote wound healing.**

Nanogranex® Sprayable Wound Gel

Objectives

- ❑ **To evaluate antibiofilm efficacy of novel sprayable wound gel using colony biofilm formation study.**
- ❑ **To evaluate antimicrobial efficacy of a sprayable wound gel against clinical wound pathogens using zone of inhibition (ZOI) assay.**
- ❑ **To conduct antimicrobial preservative efficacy testing.**
- ❑ **To determine cytotoxicity based on ISO 10993-5 with the L-929 cell line.**



Zone of Inhibition Antimicrobial Disk

Cytotoxicity Assay L929 cells, DMEM + 10% FBS

Zone of Inhibition Study

- ❑ **Five clinically relevant pathogens were selected for testing: four bacterial strains (*E. coli*, *P. aeruginosa*, *S. aureus, E. faecalis*) and one fungal strain (*C. albicans*).**
- ❑ **For each organism, five agar plates were divided into three sections to test the following conditions in triplicate:**
  1. **Three Lots of Nanogranex Sprayable Wound Gel (SWG)-treated disk (30µL applied to blank disk)**
  2. **Positive control (commercial antibiotic or antifungal disks)**
  3. **Negative control (blank disk without treatment)**
- ❑ **Plates were incubated at 37°C for 24-48 hours.**

Antimicrobial Preservative Efficacy Testing

- ❑ **SWG samples were tested against five challenge organisms (Three bacteria, one yeast, one mold).**
- ❑ **20 ml of each sample were divided into five sterile containers.**
- ❑ **Each was inoculated with 100 µL of organism (~1.5 × 10^7 CFU/mL) to yield ~1.5 × 10^5 CFU/mL, then mixed thoroughly.**
- ❑ **Samples were stored at 22.5 ± 2.5°C and tested at 0–10 min, 1 hr, 4 hrs, 24 hrs, 7, 14, and 28 days. At each time point, 500 µL aliquots of each sample were removed, 100 µL was serially diluted, plated in duplicate, incubated, and colony counts were recorded.**

Antibiofilm Efficacy Testing

- ❑ **Nanogranex Antibiofilm efficacy was evaluated using a membrane filter colony biofilm model against immature and mature MRSA and *P. aeruginosa* biofilms.**
- ❑ **100 µL of 1 × 10^6 CFU/mL bacterial suspension was inoculated onto membrane filters placed on TSA plates and incubated for 2 hours (immature biofilm) or 24 hours (mature biofilm) prior to Nanogranex application.**
- ❑ **Viable bacteria were enumerated 24 hours after treatment for both immature and mature biofilms.**

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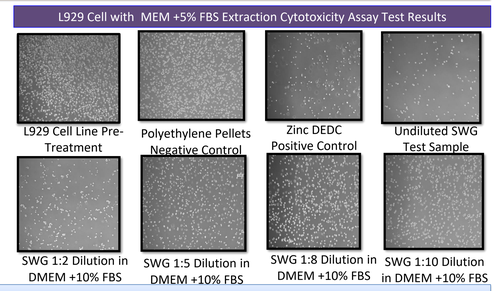
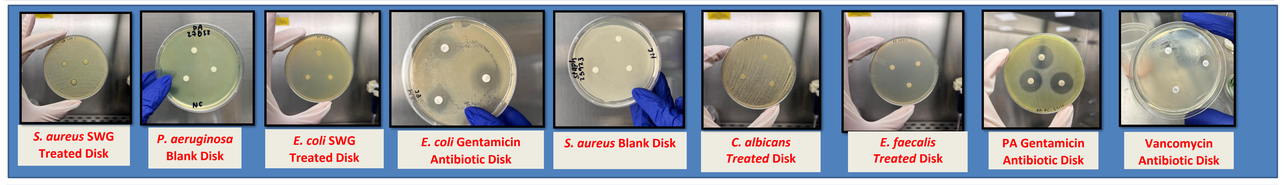
ISO 10993-5 Cytotoxicity Testing

- ❑ **L929 cells (ATCC CCL-1™) were seeded in 6-well plates for cytotoxicity assessment of SWG sample prepared at 0.2 g/mL in MEM with 5% FBS and incubated for 24 hours at 37°C.**
- ❑ **Positive (Zinc Diethyldithiocarbamate) and negative (polyethylene pellets) controls were included, and all extracts were filter-sterilized and diluted (1:2, 1:5, 1:8, 1:10) prior to cell exposure.**
- ❑ **Cells were incubated with test and control samples for 24 hours, then observed microscopically to assess cytotoxic effects.**
- ❑ **Results did not demonstrate cytotoxicity of Nanogranex.**



Zone of Inhibition Study Results

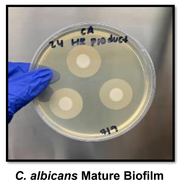


Antimicrobial Effectiveness Study Results

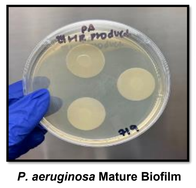
Organisms	Inoculum Concentration	Results CFUs/ml						
		0-10 min	1 hour	4 hours	24 hours	7 days	14 days	28 days
Escherichia coli ATCC 8739	$1.33X10^6$	$2.47X10^6$	$<1.0X10^2$	$<1.0X10^2$	$<1.0X10^2$	$<1.0X10^2$	$<1.0X10^2$	$<1.0X10^2$
	Log reductions	-0.27	>4.12	>4.12	>4.12	>4.12	>4.12	>4.12
Pseudomonas aeruginosa ATCC 9027	$1.60X10^6$	$<1.0X10^2$	$<1.0X10^2$	$<1.0X10^2$	$<1.0X10^2$	$<1.0X10^2$	$<1.0X10^2$	$<1.0X10^2$
	Log reductions	>4.20	>4.20	>4.20	>4.20	>4.20	>4.20	>4.20
Staphylococcus aureus ATCC 6538	$2.55X10^5$	$1.90X10^6$	$<1.0X10^2$	$3.5X10^3$	$4.50X10^2$	$<1.0X10^2$	$<1.0X10^2$	$1.50X10^2$
	Log reductions	-0.87	>3.41	1.86	2.75	>3.41	>3.41	3.23
Candida albicans ATCC 10231	$2.50X10^5$	$<1.0X10^2$	$<1.0X10^2$	$<1.0X10^2$	$<1.0X10^2$	$<1.0X10^2$	$<1.0X10^2$	$<1.0X10^2$
	Log reductions	>3.40	>3.40	>3.40	>3.40	>3.40	>3.40	>3.40
Aspergillus niger ATCC 16404	$2.35X10^5$	$1.13X10^6$	$7.15X10^5$	$1.85X10^5$	$2.25X10^5$	$<1.0X10^2$	$<1.0X10^2$	$<1.0X10^2$
	Log reductions	-0.68	-0.48	0.10	0.02	>3.37	>3.37	>3.37

Where no colonies were observed, on the plates results of less than (<) the minimum detection limit (ie. $<1.0X10^2$ CFU's/ml) were reported. In these cases, the log reduction was calculated based on the minimum detection limit and reported as greater than value).

Antibiofilm Study Results



***C. albicans* Mature Biofilm**



***P. aeruginosa* Mature Biofilm**

Immature (2hr) Biofilm -Nanogranex 24hr Treatment

Organism	Control (CFU/mL)	Nanogranex (CFU/mL)
S. aureus ATCC 6538	1.05×10^9	0
P. aeruginosa ATCC 15442	4.20×10^8	0
C. albicans ATCC 10231	3.65×10^7	1.00×10^7
Organism	**Percent Reduction**	**Log Reduction**
S. aureus ATCC 6538	100%	9.02
P. aeruginosa ATCC 15442	100%	8.62
C. albicans ATCC 10231	72.57%	0.57

Mature (24hr) Biofilm- Nanogranex 24hr Treatment

Organism	Control (CFU/mL)	Nanogranex (CFU/mL)
S. aureus ATCC 6538	2.0×10^9	1.86×10^9
P. aeruginosa ATCC 15442	2.87×10^9	1.57×10^9
C. albicans ATCC 10231	8.00×10^7	6.25×10^7
Organism	**Percent Reduction**	**Log Reduction**
S. aureus ATCC 6538	7.1%	0.04
P. aeruginosa ATCC 15442	45.18%	0.26
C. albicans ATCC 10231	21.88%	0.11

Conclusion

- ❑ **The Nanogranex sprayable wound gel demonstrated broad-spectrum efficacy against bacterial, fungal, and mold pathogens. Cytotoxicity testing confirmed its safety for topical use.**
- ❑ **This technology has the potential to reduce infections and improve wound healing in austere and prolonged field care environments.**