IMPACT OF UNIQUE PROCESS ON CHEMISTRY AND ANTIMICROBIAL ACTIVITY OF NANOSTRUCTURED SILVER

Dr. Patricia Nadworny[1,2]; Dr. Robert Burrell[1,2]

[1]FeynMed Solutions (2725119 Alberta Ltd.), 4722 76 Avenue NW, Edmonton, Alberta, T6B 0A5, patricia@feynmed.com
[2]13-263, Donadeo Innovation Centre for Engineering, Department of Biomedical Engineering, Faculties of Engineering and Medicine & Dentistry
University of Alberta, 9211 116 St. NW, Edmonton, AB, T6G 1H9, rburrell@ualberta.ca

ABSTRACT

Background: Nanocrystalline silver wound products have been used for ~30 years. Although they have demonstrated strong biological activity, they are slow and costly to manufacture, both in capital and operating costs, resulting in limited availability. A next-generation process for synthesis of nanostructured silver thin films was developed to address these issues. The study's purpose was to examine the impact of modulating variables in this unique process on the resultant chemistry and antimicrobial activity.

Methods: Nanostructured silver was deposited on high density polyethylene (HDPE) by DC magnetron sputtering (99.99% Ag, 12.7x51.4cm target at 15°C, 10 cm working distance). Variables including:

- Ar/O_2 ratio (0%-4.5% O_2, remainder Ar)
- Water injection rate (0-51.6 µL/min)
- Water injection method (see Results section for details)
- Sputtering power (0.5-1.8A at 380V)
- Run length (15-30min)
- Static or moving HDPE (0-2.0 cm/min), and
- Working gas pressure (13-60 mTorr)

were systematically altered, and the impact on total silver deposited, percent ammonium hydroxide-soluble (active/AHS) silver, and antimicrobial activity was tested. Total silver per unit surface area of dressing was determined by digesting 1"x1" pieces in 20 mL of 50% HNO_3 in distilled H_2O for 20min. AHS silver was determined by digesting 1"x1" pieces in 20 mL of NH_4OH for 10min. Both digestions were diluted and analysed by atomic absorption spectrometry (AAS) or inductively coupled plasma mass spectrometry (ICP-MS). Antimicrobial activity was tested via log reductions: A 16h *P. aeruginosa* culture was inoculated into 50 mL calf serum (a wound fluid model) and incubated for 16h to produce a $1x10^9$ CFU (colony forming unit) inoculum. Dressings prepared from two silver-coated pieces of HDPE with a piece of gauze in between (2.5x2.5cm) received 200 µL inoculum and were incubated for 1h at 37°C. They were then placed in a sodium thioglycolate/saline/Tween-80 (STS) solution (to inactivate the silver and recover microorganisms), vortexed, serially diluted, and spot plated. CFUs were counted after ~24h incubation at 37°C.

Results: Direct injection of H_2O into the sputtering chamber, and injection of H_2O into Ar in the absence of O_2, did not result in sufficient AHS silver or bactericidal activity (log reductions ≤2). However, addition of H_2O, via approaches listed in the Results section, to appropriate $Ar:O_2$ blends synergistically increased %AHS silver and bactericidal activity, in some cases generating log reductions >8.3 (total kill). This allows for production at higher power levels (increasing production rate), with lower vacuum requirements, decreasing both capital and operating expenses.

Conclusion: Under appropriate conditions, dressings with strong consistent antimicrobial activity can be manufactured at much lower costs, and significantly increased speed, relative to currently available nanocrystalline silver dressings.

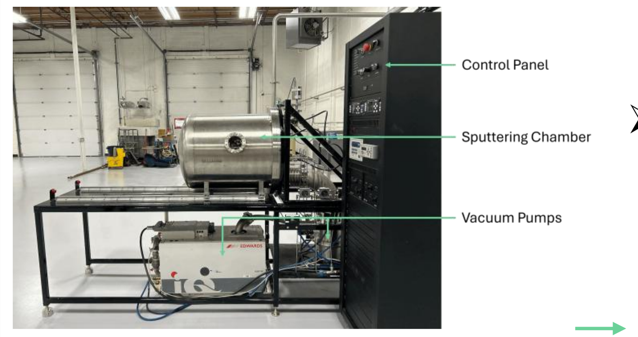
BACKGROUND

- >10M patients globally have chronic wounds, with <50% of wounds healing by 12 weeks
- Chronic wounds are on the rise due to aging populations and comorbidities (e.g., diabetes)
- The cost to heal a chronic wound is ~10K-$34K USD/wound
- Diabetic foot ulcers have a 5y mortality rate of ~30%, rising to ~50% in the event of amputation
- The wound care market is projected at $30B USD/y by 2030
  - ~$16B USD/y attributed to advanced wound healing products

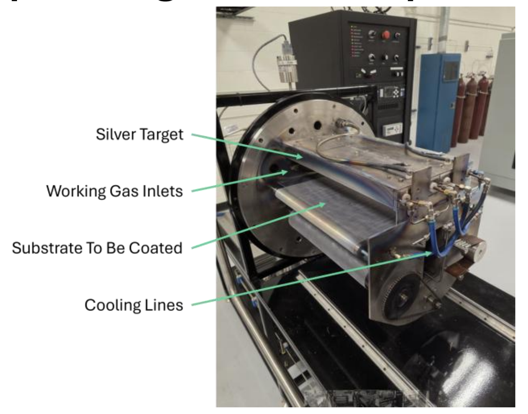
References:
Jarbrink, Syst Rev 2016;5:152. Denny, Healthcare Quarterly 2014;17:7-10.
https://www.marketsandmarkets.com/Market-Reports/wound-care-market-371.html
Fife *et al.* Adv Wound Care 2017;7:77-94. Sen, Adv Wound Care 2019;8:39-48.

MATERIALS AND METHODS - MANUFACTURING

Sputtering Machine – Closed

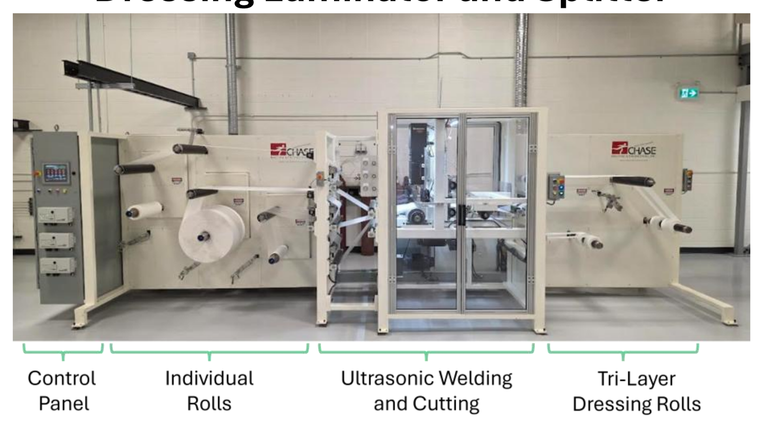


Sputtering Machine – Open



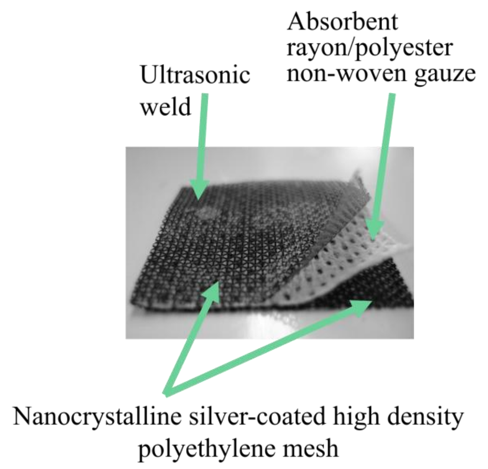
- Silver thin films deposited on HDPE in extreme sputtering machine with various modifications to support water injection
- Variables systematically altered

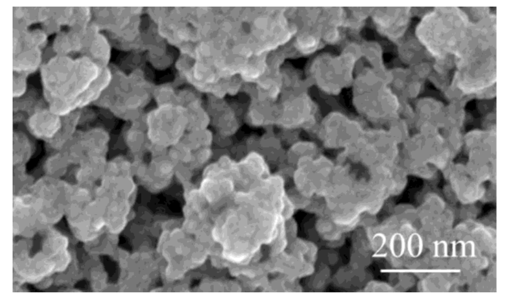
Dressing Laminator and Splitter



- 2 layers of thin film are assembled with absorbent gauze layer in between and cut to size to create final product

Final Product

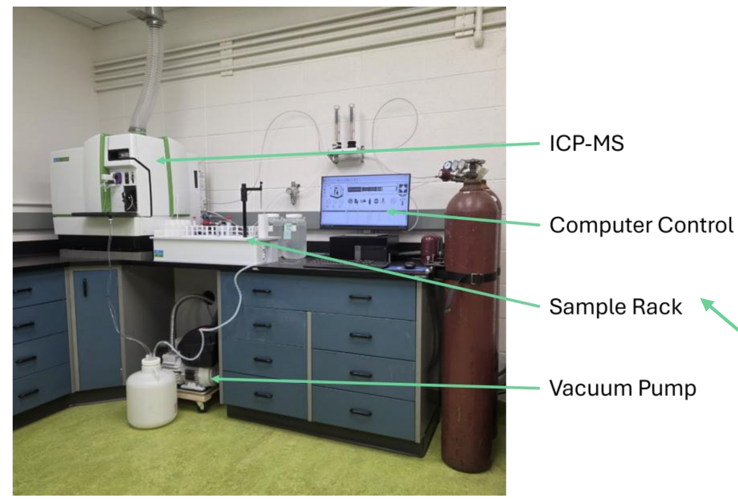




MATERIALS AND METHODS - TESTING

Total Silver Analysis

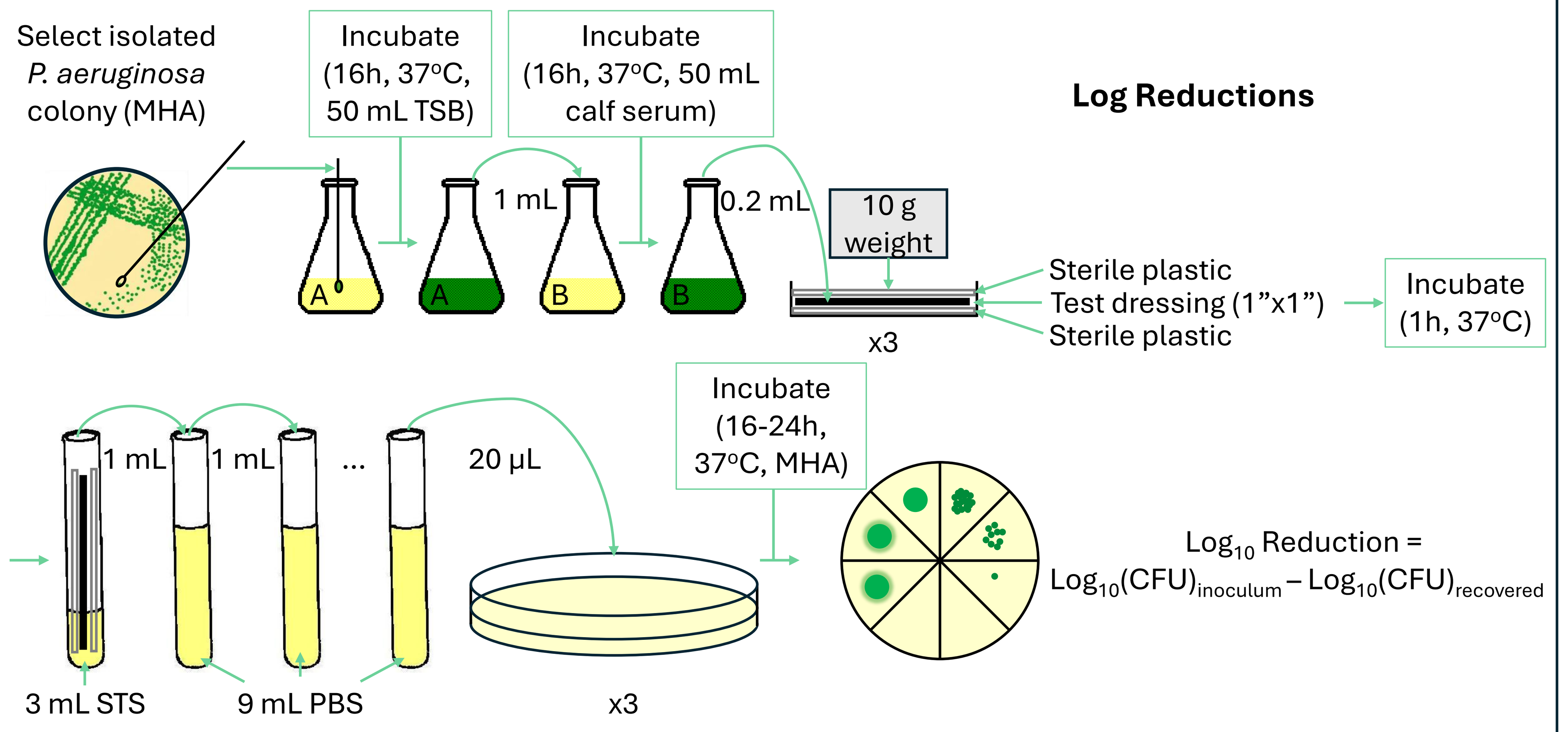
1"x1" piece of thin film
↓
20 mL nitric acid (65% solution diluted 1:2 in dH_2O)
↓
20 min at 100°C; remove dressing
↓
Cool; 1:2 dilution in dH_2O



AHS Silver Analysis

1"x1" piece of thin film
↓
20 mL of 20 g/L ammonium hydroxide (30%)
↓
5 min at RT; remove dressing
↓
1:5 dilution in dH_2O

Log Reductions



RESULTS AND DISCUSSION

Results/Discussion

- Sputtering using H_2O with various addition methods in absence of O_2 did not result in antimicrobial activity
- Sputtering in increasing O_2 in absence of H_2O improved antimicrobial activity over H_2O alone (not total kill)
- Synergistic effect seen when oxygen and H_2O combined during sputtering process
  - Allows for sputtering at higher power and rate, lower O_2 concentration
- Adding H_2O upstream of the MFC with various injection rates resulted in total kill at 2% O_2
- Bubbling Ar through heated water prior to entering the sputtering machine, and adding water via microvalve downstream of the MFC prior to the sputtering chamber, also resulting in highly microbicidal dressings

Conclusions

- Dressings with strong consistent antimicrobial activity can be manufactured at much lower costs and significantly increased speed, relative to currently available nanocrystalline silver dressings

Future Work

- Continued examination of effect of variables on activity, to fine tune process
- Expansion of *in vitro* testing to demonstrate broad antimicrobial activity and longevity
- *In vitro* and *in vivo* biocompatibility and efficacy testing
- Establish quality systems for regulatory compliance
- Sterilization and stability testing

RESULTS AND DISCUSSION, CONTINUED

Run Length (min)	H_2O Injection Method*	% O_2**	H_2O Injection Rate (µL/min)	Web Speed (cm/min)	Current (A)	Working Gas Pressure (mTorr)	Total Ag (mg/in²)	AHS Ag (%)	Log Reduction
15	1	N/A	35	0	1.5	N/A	1.01	21.7	<2
30	None	0	0	1.5	1.5	40	2.52	3.4	2.1
30	None	0	0	1.5	1.8	40	5.04	3.3	2.8
30	None	1	0	1.5	1.5	40	2.83	9.4	4.9
30	None	2	0	1.5	1.5	40	2.32	21.3	4.9
30	None	3	0	1.5	1.5	40	2.93	22.3	5.0
30	None	4	0	1.5	1.5	40	2.64	26.4	5.0
30	2	0	2	1.5	1.8	40	4.96	2.1	2.7
30	2	0	4	1.5	1.8	40	4.80	2.1	2.1
30	2	0	8	1.5	1.8	40	4.32	3.5	2.1
30	2	0	9	1.5	1.5	40	4.32	3.1	<2
30	2	0	9	1.5	1.5	40	3.21	1.7	2.4
30	2	0	20	1.5	1.5	40	4.16	3.6	<2
30	2	0	33	1.5	1.5	40	4.80	2.1	<2
30	2	1	9	1.5	1.5	40	2.47	32.8	6.2
30	2	2	1	0	1.5	40	4.70	24.2	>8.3
30	2	2	1	1.5	1.5	40	4.70	24.2	>7
30	2	2	3	0	1.5	40	6.86	23.6	>8.3
30	2	2	3	1.5	1.5	40	6.86	23.6	>7
30	2	2	5	0	1.5	40	5.66	22.4	>8.3
30	2	2	5	1.5	1.5	40	5.66	22.4	>7
30	2	2	7	0	1.5	40	4.03	26.0	>8.3
30	2	2	7	1.5	1.5	40	4.03	26.0	>7
30	2	2	9	0	1.5	40	6.67	15.7	>8.3
30	2	2	9	1.5	1.5	40	6.67	19.3	>7
30	2	2	9	1.5	1.5	40	3.29	39.2	6.0
30	2	3	9	1.5	1.5	40	2.74	63.2	6.3
30	2	4	9	1.5	1.5	40	2.99	74.0	6.6
30	2	4.25	6	0	0.5	13	NR	35	3.2
30	2	4.25	6	0	0.9	13	NR	35	4.1
30	2	4.25	6	0	1.8	40	NR	35	5.5
30	2	4.25	6	0	1.8	50	NR	48	4.2
30	2	4.25	9	0	1.8	40	3.46	48.2	7
30	3	1	N/A	0	1.5	40	5.14	41.0	6.9
30	3	1	N/A	0	1.5	40	5.32	45.7	>6
30	4	2	9.1	0	1.5	40	3.20	26.0	7.5
30	4	2	51.6	0	1.5	40	4.11	17.3	7.5

* 1 = Water added directly to chamber via microvalve (no Ar or O_2 gas)
2 = Water added to Ar upstream of mass flow controller (MFC) via syringe pump with piping heated to 50°C
3 = Ar bubbled through heated stainless steel column containing 1400 (1st run) or 1500 (2nd run) mL H_2O at 88°C
4 = Water added via microvalve downstream of MFC, prior to sputtering chamber, at 21°C
** Remainder Ar.
N/A = Not applicable.
NR = Not recorded.
Green highlighted data = Log reduction >4 (acceptable by FDA standards). Values with ">" indicates total kill.
Blue highlighted data = Log reduction between 3-4 (>3 indicates biocidal)
White highlighted data = Log reduction between 2-3 (not biocidal, some measured activity)
Grey highlighted data = Log reduction <2 (no activity measured)

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