Can E-Beam Technology Create Antimicrobial Fabrics?

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Research Article

Can E-beam technology create antimicrobial fabrics?

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Abstract. Silver has a long historical use as an antimicrobial metal; it exerts its antimicrobial effects oligodynamically. Current silver-fabric technologies are dependent upon either the use of silver thread in the weave or time release of silver salts from fabrics. Electron-beam technology uses a concentrated beam of electrons to induce crosslinking in irradiated materials. This research marks experimental work attempting to bond silver directly to cloth to create a composite. The study had four aims, to: 1) test treated synthetic fabrics for antimicrobial effectiveness; 2) distinguish differences in resistance between different bacterial genera; 3) test the longevity of treated fabrics through repeated washing; and 4) identify treatment conditions producing maximum effectiveness. Fabric was immersed in silver nitrate solution then irradiated to incite deposition. Effectiveness was measured using a Kirby-Bauer procedure to measure zones of inhibition around irradiated cloth circles. Following each round of assay, fabrics were laundered and the process repeated to gauge the effectiveness of the fabric in retaining antimicrobial activity. Data analysis demonstrated no significant differences in inhibition between gram positive and gram negative genera nor in irradiation dosages. Composites treated with silver nitrate but not irradiated lost antimicrobial properties after the initial washing, suggesting no fabric-metal bonding. However, the antimicrobial properties of the treated samples lessened after the initial washing but thereafter remained steady through the experimental period.

Introduction

Silver has a long, historical use as an antimicrobial metal; as early as 550BC, according to the writings of Herodotus (silverinstitute.org), silver was used for the storage of beverages during long voyages. Silver nitrate has been used medically in pediatry to treat neonatal conjunctivitis; legislation from 1909–1959 required this treatment in newborns, resulting in a decrease in infant blindness from 24% to 0.3% as measured by admissions to schools for the blind (Tortora et al., 2007). Other silver salts have been used as disinfectants and in the treatment of various infections such as moniliasis (candidiasis) and trichomoniasis. Silver sulfadiazine is used as both an antibiotic and anti-fungal topical, particularly in burn cases where nosocomial infections (*Staphylococcus* and *Pseudomonas*) are a high risk (Noronha and Almeida, 2000).

Silver exerts its antimicrobial effects oligodynamically, that is to say from the action of few

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molecules. It functions as many other heavy metals do against microbes, affecting membrane stability, inhibiting DNA replication, and interacting with thiol groups in proteins to denature them (Matsumura et al., 2003). However, the use of colloidal silver as a treatment is hotly debated between traditional and alternative medicines (van Hessalt et al., 2004; silvermedicine.org).

Silver is being tested in various industrial applications as a coating compound to retard or prevent bacterial growth and biofilm formation (Cowan et al., 2003). The application of silver to fabric to control the presence of odor-causing bacteria in socks and sports clothing has become commonplace. Silver-impregnated nylon (X-static) has been tested for its ability to retard and prevent bacterial growth in a large number of bacterial genera. MacKeen et al. (1987) incubated impregnated nylon fibers in bacterial cultures and evaluated for colony forming unit (CFU) reduction potential; these fibers were active because of the slow release of silver salts from the fibers.

The primary difficulty with methods depending on the slow release of silver is the loss of efficiency over time (e.g. continued laundering decreases the efficacy of the silver treatment as free silver is released) (Wright et al., 1998). A treatment that could withstand repeated washings without losing effectiveness would, therefore, be of value medicinally and economically.

This study marks research attempts to develop a method using Electron beam (e-beam) technology to directly deposit silver into fabric fibers for long term effectiveness in retarding bacterial growth. Other considerations addressed in experimental design were the choice of fabric (e.g. cotton, polyester, nylon) and whether or not deposition could be enhanced using a precipatory metal additive.

E-beam technology was chosen for this experiment as it has demonstrated the ability in other materials to induce increased tensile strength and multi-surface bonding as well as adding new physical properties. E-beam irradiation changes the physical properties of irradiated materials primarily through induced cross-linking (ebeamservices.com/ebeam_spe_antec.htm). CSU maintains collaborative research ties with Kent State University (KSU). KSU maintains a 150 kW, 5 MeV electron accelerator as part of their joint Program on Electron Beam Technology (PEBT); KSU supported this research through the use of their accelerator.

This project began with four hypotheses. First, it was expected from the literature (Yin et al., 1999) that gram positive bacteria would be more resistant than gram negative bacteria. As both groups contain pathogenic species, any discovery of susceptibility had future medicinal impact. Second, polyester was expected to form a less effective composite than polyethylene because the aromatic nature of polyesters is less reactive with e-beams (ebeamservices.com). Polyethylene was primarily evaluated because of its widespread usage in disposable hospital cloths. Polyester was evaluated for its potential usefulness longer term, such as in lab coats and military uniforms. In this study, polyester was maintained as a cotton: polyester blend to retain breathability in the fabric. Nothing was found in the literature on the testing of cotton fabrics using e-beams. Third, the efficacy of the composites was predicted to initially decrease with the first washing (marking the release of unbound silver) but to remain steady thereafter as remaining silver would be bound to the cloth. Lastly, it was thought that the addition of a metal catalyst might enhance silver deposition, so trials were conducted with and without metal grommets remaining in the cloth (Poondi et al., 1998). All of these hypotheses were examined in addition to examining the effects of varied irradiation levels on cloth to determine the best dosage for creating and maintaining antimicrobial activity.

Materials and Methods

Bacterial species and culture

Gram positive bacterial species used in this study were *Bacillus cereus, B. megaterium, B. subtilis, Corynebacterium hoffmanni, C. pseudodiptherium, Staphylococcus aureus, Streptococcus faecalis,* and *S. pyogenes.* Gram negative bacterial species used were *Escherichia coli, Klebsiella pneumoniae, Neisseria catarrhalis,*

N. perflava, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhimurium, and *Serratia marcescens*. These cultures were chosen as all were part of stock collections. All cultures were acquired from Presque Isle Cultures (Presque Isle, PA) and maintained under the organism's appropriate culture conditions.

Stock cultures were maintained on Mueller-Hinton (MH) Agar slants unless otherwise required. For inhibition assays, 100mL cultures of each bacterial type were grown up in MH broth, shaking overnight at 30°C. Fresh slants were restreaked every two weeks from stocks.

Differential media

Several different agar preparations were used in this study according to manufacturer (Difco) instructions. Assays were routinely conducted on Mueller-Hinton agar. Other media used to verify bacterial authenticity during the course of the study were King's B Agar (*Pseudomonas*), DNase agar (*Neisseria*), Tinsdale Agar (*Streptococcus* and *Corynebacterium*), SS agar (*Salmonella* and *Proteus*), MYP Agar (*Bacillus*), ME Agar (*Listeria*), Mannitol Salt agar (*Staphylococcus*), and EMB Agar (*Escherichia* and *Klebsiella*). *Serratia* was identified by the red pigment produced both on agar and in liquid media. All media were obtained through Fisher Scientific.

Cloth irradiation and washing

70:30 polyester: cotton lab coats and polyethylene disposable surgical hoods were purchased from Fisher Scientific and cut into 30cm squares. Each type of cloth was soaked in a 100mL solution of 50mM silver nitrate, placed in heat-sealed plastic bags and irradiated at the Kent State NEO Beam Facility at the following doses: 25 kGy, 50 kGy, 75 kGy, and 150 kGy. The fabrics were removed and air dried before being cut into 0.7cm circles for assay.

Following each round of assay, remaining cloth squares were laundered using Tide with color-safe bleach (Proctor & Gamble) in a mini-washer (Avanti Products, FL) to simulate laundering conditions. The washing conditions were cold water, approximately 10mL of detergent and the preprogrammed wash setting. The preprogrammed setting consisted of a wash cycle and three rinse cycles that lasted a total of thirty-five minutes. The capacity of the washer was 8L of water. Cloth squares were air dried before new circles were cut. It was decided to launder the squares and cut new discs each time since the discs were too small to retain for reuse.

When the potential benefit of a metal catalyst was being evaluated, two squares were cut per cloth (polyester-cotton and polyethylene). One included metal grommets attached to the cloth, and one did not. Irradiation doses and other procedures remained consistent in both studies. The grommets were only included during irradiation and were not included in the cut circles.

Assay procedures

Mueller-Hinton plates were inoculated from overnight cultures. A sterile cotton applicator was used to spread the culture on the plate at three different angles to ensure confluency; each plate was overlaid with triplicate samples of either irradiated polyester-cotton or polyethylene discs and incubated overnight at 30°C. Zones of inhibition (ZOIs) were measured the following day. The ZOIs were measured from the edge of the disc to the edge of the zone, as the discs were hand cut and not of uniform size. A sample result is included in Figure 1.

Controls

A pre-trial experiment was conducted to establish that the cloth had no innate antimicrobial properties. Polyester-cotton and polyethylene discs were dipped in silver nitrate solutions ranging from 1mM to 50mM concentrations, but not irradiated. Discs were plated using the previously stated method. Activity was visible on all discs tested. Following a round of laundering, the ZOI was reduced to zero. From this, it was verified that ZOIs could be produced and that washing completely removed any antimicrobial activity. Thus, there was no inherent binding of silver to the cloth prior to irradiation.

Control discs (polyester-cotton or polyethylene) were included in each round of assay that

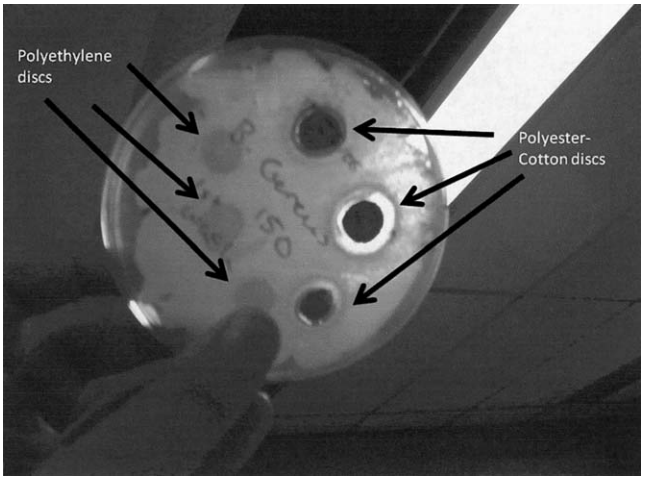

Figure 1. Zones of inhibition for *B. cereus*. This is an example of an assay plate with both polyester-cotton and polyethylene discs in triplicate.

were neither dipped in silver nitrate nor irradiated. These discs were plated using the same assay procedure. These cloth disks demonstrated no antimicrobial activity in all assays (data not shown).

Statistical analysis

Statistical analysis was performed using both Microsoft Excel 2003 and SSP software, version 2.75 (Smith, 2004). Two-sample t-test assuming unequal variances were performed on gram positive bacteria vs. gram negative bacteria and with metal vs. without metal data (the later data is not included as it was not significant). One-tailed tests were used in all analyses using a significance level of $p < 0.05$. An ANOVA single factor test was done on washings for each strength with $\alpha = 0.05$.

Results

Statistical tests were performed on the data to determine whether there were any significant differences between variables.

Susceptibility by gram designation

A t-test was run on gram positive bacteria versus gram negative bacteria, showing there was no significant difference between groups ($p = 0.0001$). The df, T stat and T critical values are shown in Table 2.

Differences in antimicrobial activity by cloth type

The results from irradiated polyethylene fabrics are not presented, as there was no measurable bonding between silver and cloth as evidenced by the loss of all growth retardation after one washing (data not shown).

Efficacy of e-beam dosage on antimicrobial activity

An ANOVA run on the different washes at each irradiation dose showed no significant loss of the cloth's ability to retard bacterial growth through three rounds of assay; silver-treated fabrics that were not irradiated lost all ability to retard growth after one washing. The p values for the ANOVA test are 0.246 for 25 kGy washings, 0.229 for 50 kGy washings, 0.194 for 75 kGy washings and 0.104 for 150 kGy washings. See Table 3 for df1, df2, F stat and F critical values.

Efficacy of a metal catalyst

Results of the fabric irradiated with grommets are, likewise, not presented, as there was no statistical difference between the fabric with grommets

Table 1. Zones of inhibition data summary.

		Min (cm)	Max (cm)	Mean (cm)	SEM (+/−)
Dosages	25 kGy	0.000	0.400	0.110	0.007
	50 kGy	0.000	0.400	0.120	0.006
	75 kGy	0.000	0.500	0.110	0.007
	150 kGy	0.000	0.400	0.110	0.006
Wash	1st	0.000	0.400	0.080	0.005
	2nd	0.000	0.500	0.110	0.006
	3rd	0.050	0.400	0.150	0.008
Gram	Positive	0.000	0.400	0.130	0.004
	Negative	0.000	0.500	0.100	0.004

Table 2. T-test values by gram designation.

		df	T	T critical	P two-tail
Gram	Positive Negative	778	3.872	1.963	0.0001

and fabric without. The minimum and maximum ZOIs are shown in Table 1.

Discussion

All ZOIs were measured from the edge of the disc to the edge of the zone since disc size varied slightly. In all cases except the controls, bacteria did not grow under or over the disc. This shows that bacteria were inhibited in all cases even if the ZOI was 0 cm. On control discs (ones without silver), bacteria grew under and on the cloth discs.

It was expected from the literature that gram positive bacteria would be more resistant to silver than gram negative bacteria; however, statistical analysis of data showed this was not the case. The null hypothesis for the t-test was that gram positive bacteria ZOIs would be different than gram negative ZOIs. With the p value less than 0.05 significance level, it was determined that the null hypothesis can be rejected. Therefore, there was no significant difference between groups.

Polyethylene lost all ability to retard growth after one washing, suggesting that there was no retention of silver in the cloth. For this reason, the polyethylene data is not included. This result disagreed with literature suggesting that polyethylene would be better able to crosslink than polyester (ebeamservices.com). The polyester: cotton blend proved superior to polyethylene in maintaining ZOIs through repeated washings. There was an initial loss of some antimicrobial activity following the first wash. This suggests that there was a combination of retained and free silver on the cloth that was removed by the first washing.

As neither samples of 100% polyester nor 100% cotton were tested in this experiment, it cannot be said with certainty that the observed antimicrobial activity was due to silver retention in the polyester alone. Activity could well be due to retention of the silver in the cotton or due to some interplay between cotton and polyester.

After the initial washing in which free silver was removed, there were no significant changes in ZOIs between washings. The null hypothesis was that there would be no difference between washings. Since the p value was greater than 0.05 for all the doses, the null hypothesis is accepted, showing that there is no significant difference. Through the three washings conducted in this study, the cloth's ability to maintain antimicrobial properties was retained. All four irradiation levels tested maintained bacteriostatic ability throughout the study. Statistical analysis did demonstrate a pattern of decreasing p values as irradiation dosage increased. See Table 3 for the p values. It may be that there is an upper limit to how much crosslinking is productive in composite bonding, and the highest dosage tested (150 kGy) is approaching that limit. Given the data, the best dosage choice for irradiation appears to be 25 kGy as it is a lower irradiation

Table 3. ANOVA values of irradiation level and washings.

		df1	df2	F	F critical	P (ANOVA)
25 kGy	Wash 1	2	51	1.440	3.179	0.246
	Wash 2					
	Wash 3					
50 kGy	Wash 1	2	51	1.518	3.179	0.229
	Wash 2					
	Wash 3					
75 kGy	Wash 1	2	51	1.691	3.179	0.194
	Wash 2					
	Wash 3					
150 kGy	Wash 1	2	51	2.366	3.179	0.104
	Wash 2					
	Wash 3					

dosage and the highest p value. The higher p value means it has the least significance between washes compared to the other dosages.

Metal grommets were included in some irradiation trials to test if the presence of a 'contaminating' metal would aid in ionic silver reduction and deposition. Statistical analysis showed no significant differences between trials with and without metal added. Thus, the conclusion was that grommets did not increase the efficacy of silver bonding.

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Literature Cited

Cowen, M.M., K.Z. Abshire, S.L. Houk, and S.N. Evans. 2003. Antimicrobial efficacy of a silver-zeolite matrix coating on stainless steel. Carrier Corporation, Syracuse, NY.

E-BEAM Services, Inc. Electron-Beam processing of plastics: an alternative to chemical additives. Visited 9/15/04. www.ebeamservices.com/ebeam_spe_antec.htm

MacKeen, P.C., S. Person, S.C. Warner, W. Snipes and S.E. Stevens, Jr. 1987. Silver-coated nylon fiber as an antibacterial agent. *Antimicrob Agents Chemother*: **31(1):**93–99.

Matsumura, Y., K. Yoshikata, S. Kunisaki, and T. Tsuchido. 2003. Mode of bactericidal action of silver zeolite and its comparison with that of silver nitrate. *Appl Environ Microbiol* **69(7):**4278–4281.

Noronha, C., and A. Almeida, 2000. Local burn treatment – topical antimicrobial agents. *Annals of Burns and Fire Disasters* **13(4):**216–219.

Poondi D., R. Subramanian, M. Otooni, and J. Singh. 1998. Synthesis of Silver Nanoparticles by a Laser-Liquid-Solid Interaction Technique. *J Mat Synth Proc* **6(2):**89–104.

Smith, Gary, 2004. Smith's Statistical Package. Visited 5/20/07. www.economics.pomona.edu/StatSite/SSP.html

The History of the Element Silver An Esoteric View – from Ancient to Modern Use. 2007. Visited 9/20/04. www.silvermedicine.org/history.html

The Indispensable Metal: Uses. 2003. Visited 9/23/04. www.silverinstitute.org/uses.php#medical

Tortora, G.J., B.R. Funke and C.L. Case. 2007: Microbiology: An Introduction, 9e. Person Education, Inc. /Benjamin Cummings, San Francisco, CA. pp. 187–213.

Wright J.B., D.L. Hansen, and R.E. Burrell, 1998. The comparative efficacy of two antimicrobial barrier dressings: in vitro examination of two controlled release of silver dressings. *Wounds* **10(6):**179–188.

van Hasselt, P., B.A. Gashe, J. Ahmad J. 2004. Colloidal silver as an antimicrobial agent: fact or fiction? *J Wound Care* **13(4):**154–55.

Yin, H.Q., R. Langford, and R.E. Burrell. 1999. Comparative evaluation of the antimicrobial activity of ACTICOAT* antimicrobial barrier dressing. *J Burn Care Rehab* **20:** 195–200.

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