

Assessment of a silver-coated barrier dressing for potential use with skin grafts on excised burns

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Abstract

Acticoat[®] burn dressing is a silver-coated dressing with antimicrobial activity purported to reduce infection from environmental organisms in partial and full-thickness wounds. Acticoat[®] was tested for activity as an antimicrobial treatment and as an antimicrobial barrier dressing in three in vitro assays. It was found that a modified disc assay method gave false negative results but in an assay in which bacteria were inoculated on top of samples of Acticoat[®], bacterial numbers were reduced, over time, with all microorganisms tested. Acticoat[®] served as a barrier for bacteria, inoculated onto it, from contaminating the surface of an agar plate under the Acticoat[®]. The data show that Acticoat[®] has: antimicrobial capabilities, but to be effective hours of contact between Acticoat[®] and the microorganisms are required; and the capacity to serve as an antimicrobial barrier dressing. These findings support the conclusion that Acticoat[®] has activity to reduce microbial contamination of wounds from environmental sources.

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1. Introduction

Cultured skin substitutes have provided a new approach for the closure of wounds [1,2]. However, these avascular and only partially keratinized grafts are fragile and subject to microbial destruction [3,4]. Because of this, topical antimicrobial mixtures containing both antibacterial and antifungal agents, have been formulated to protect these grafts from the destructive aspects of microbial growth and have been shown to be effective in vitro [5–7]. Some have also been shown to be effective in vivo [8]. However, while data have been presented that the redundancy of antimicrobials contained in these mixtures reduces the likelihood of resistance development occurring with their clinical use [9], that possibility still exists. Further, these preparations require off-label use of some antimicrobials and are time consuming to prepare. For these reasons, alternative approaches, to provide protection from microbial destruction of cultured skin substitutes are being investigated constantly.

Recently, a silver-coated dressing, has become available for use in burn patients. This dressing, known as Acticoat[®] Antimicrobial Burn Dressing has been shown to have antimicrobial activity equal to or better than some silver con-

taining topical antimicrobial substances commonly used in burn patients [10], and has been used as an effective antimicrobial dressing in patients [11].

In this report, we present results of a study which examined, the in vitro antimicrobial activity associated with the Acticoat[®] dressing, as well as its function as an antimicrobial barrier, to determine whether the dressing could provide an alternative to current antimicrobial mixtures for use with cultured skin grafts.

2. Materials and methods

2.1. Microorganisms

Strains of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans* and miscellaneous gram negative members of the Enterobacteriaceae were isolated from the wounds of burn patients.

2.2. Test materials

Acticoat[®] Antimicrobial Barrier Dressing (Westaim Biochemical Corp., Fort Saskatchewan, Alta., Canada); N-Terface[®] (Winfield Laboratories, Inc., Richardson, TX) and Op-Site[®] (Smith and Nephew, Inc., Largo, FL) were

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purchased from the respective companies. Sterile filter paper was used as a negative control.

2.3. In vitro test methods

2.3.1. Disc testing

Acticoat[®] discs were prepared by punching out circles from Acticoat[®] sheets using a 6 mm biopsy punch. Microorganisms, grown overnight and diluted to the density of a 0.5 McFarland standard, were poured over the surface of Mueller–Hinton agar plates, the excess decanted, and the surface of the plates allowed to dry. Discs of Acticoat[®], both dry and wetted with sterile water, were placed on the surface of the agar with the blue-mesh side in contact with the plate. The plates were incubated overnight, at 35 °C, and clear zones around the disc were measured. Any clear zone of ≥ 1 mm in radius was interpreted to indicate that (1) silver ions had released from the Acticoat[®] and migrated through the agar matrix, and (2) silver was in concentrations high enough to inhibit the growth of the test microorganism in the area of the clear zone.

2.3.2. Antimicrobial timed assay

Inocula were prepared as described above. Five 0.5 in. squares of Acticoat[®] were placed on the surface of Mueller–Hinton plates and 10 μ l of the test microorganism was placed on the center of each square. At serial intervals (immediately, 1, 2, 4 and 8 h) one square was aseptically removed from the agar surface, placed in a tube containing 10 ml of sterile saline, mixed for 15 s on the #8 setting of a Vortex Genie (Scientific Industries, Springfield, MA). After vortexing 1.0 μ l of the sample was streaked, uniformly, over the surface of Tryptocase Soy Agar (TSA) plates using a sterile plastic loop. Plates were incubated overnight, at 35 °C, and colony counts performed. Two strains each, *S. aureus*, *P. aeruginosa*, *C. albicans* and, one each, *Escherichia coli* and *Enterobacter cloacae* were tested in this manner. The *E. coli* and *E. cloacae* were isolates that were designated Acticoat[®] resistant based on results from the disc assay. One strain each, *P. aeruginosa* and *E. coli* were treated in the same way and placed on filter paper squares, rather than Acticoat[®] squares. These served as negative controls.

2.3.3. Barrier function testing

Inocula and Acticoat[®] squares were prepared and placed on the surface of Mueller–Hinton plates and inoculated as described above. In this case, however, one square was aseptically removed from the surface of the agar at the prescribed interval (same as above) and discarded. Plates were incubated, at 35 °C for 24 h, only being removed from incubation for sample retrieval at 1, 2, 4 and 8 h. After 24 h of incubation, the plates were observed for evidence of microbial growth in each area from where a square had been removed previously. Acticoat[®] squares were compared, on the same plate with filter paper squares, serving as a negative control,

or with squares of two additional dressing materials commonly used in burn patients, Op-Site[®] and N-Terface[®]. Two strains each of *E. coli*, and one strain each, *S. aureus*, *P. aeruginosa*, *K. pneumoniae*, were used in these assays. The *K. pneumoniae* and *E. coli* isolates were ones designated as Acticoat[®] resistant using the disc assay.

3. Results

3.1. Disc testing

Based on the criterion that we established for susceptibility of a test microorganism to Acticoat[®] using our disc assay (≥ 1 mm clear zone radius around the 6 mm Acticoat[®] disc), all strains of *P. aeruginosa* were susceptible as were 12 of 15 *S. aureus*. Only 2 of 14 miscellaneous gram negative bacteria (one each, *E. coli* and *A. lwoffii*) were judged susceptible using this criterion (Table 1). While clear zone sizes suggesting susceptibility were present when all isolates of *C. albicans* were tested, there were numerous pin-point colonies visible in all of the clear zones. There was no difference observed whether or not the discs were wetted prior to use.

3.2. Antimicrobial timed assay

When test organisms were inoculated on the tops of squares of Acticoat[®] or filter paper and the numbers of CFUs remaining on the squares were estimated after various contact times (Table 2) the following results were obtained. (1) Numbers of *P. aeruginosa* and *E. coli*, on filter paper squares, while somewhat variable at the different time points, remained essentially unchanged from initial inoculation throughout the 8 h test period, indicating no antimicrobial activity. (2) All other test microorganisms, again with some variation at some time points, showed reduced numbers as a function of time in contact with the Acticoat[®] square. (3) After 8 h of contact with Acticoat[®],

Table 1
Acticoat[®] susceptibility of selected microorganisms tested in a topical antimicrobial test assay system

Organism	N	No. of susceptible	Average zone diameter \pm S.E.M. (range/mm)
<i>S. aureus</i>	15	12	7.40 \pm 0.16 (7–8)
<i>P. aeruginosa</i>	14	14	9.85 \pm 0.32 (8–12)
Miscellaneous gram negative bacteria ^a	14	2 ^b	8.50 \pm 1.50 (7–10)
<i>C. albicans</i>	13	13 ^c	8.85 \pm 0.34 (7–11)

^a Five *Escherichia coli*, three *Enterobacter cloacae*, two *Citrobacter koseri*, one each: *Klebsiella pneumoniae*, *Morganella morganii*, *Proteus mirabilis*, and *Acinetobacter lwoffii*.

^b One each *E. coli* and *A. lwoffii*.

^c Numerous pin-point colonies visible in all of the clear zones.

Table 2
Reduction in microbial load relative to time in contact with Acticoat®

Organism	Initial	1 h	2 h	4 h	8 h
<i>S. aureus</i>	73 ^a	>100	65	22	18
<i>S. aureus</i>	30	75	50	34	3
<i>P. aeruginosa</i>	>100	>100	80	35	0
<i>P. aeruginosa</i>	>100	>100	>100	55	0
<i>E. coli</i>	80	>100	2	50	1
<i>E. cloacae</i>	100	>100	29	0	0
<i>C. albicans</i>	1	0	0	0	0
<i>C. albicans</i>	35	1	0	0	0
Control: <i>P. aeruginosa</i>	>100	>100	>100	95	>100
Control: <i>E. coli</i>	50	37	43	45	45

^a CFUs contained in 1.0 µl sample.

no microorganisms could be recovered in five of eight different isolates tested and only small numbers of CFUs were isolated from the other three test organisms. (4) The *E. coli* isolate in which only one CFU was found after 8 h of contact with Acticoat® and the *E. cloacae* isolate, where

no CFUs were recovered from the 4 or 8 h samples, were isolates previously judged to be resistant to Acticoat® based on disc test results.

3.3. Barrier function testing

Test microorganisms were inoculated on the top of squares of Acticoat® in contact with the surface of a nutrient containing agar plate and removed after varying periods of time, the following results were obtained. (1) In no instance was there growth of the test organisms, two *S. aureus*; two *P. aeruginosa* and one each *E. coli*, *E. cloacae* and *K. pneumoniae* (the latter three bacteria had been judged to be resistant to Acticoat® based on disc test results), on the surface of the plate under where the Acticoat® square had been located. (2) Growth was found to be present, in all cases, under filter paper squares, used as a negative control. (3) Similarly, growth was found under all squares of N-Terface®. (4) Growth was found under squares of Op-Site® as well, but with no consistency. In some cases, for example, growth was apparent only under the squares removed at 4 and 24 h (*P. aeruginosa*) and in another at 2 and 24 h (*S. aureus*). See illustrations in Fig. 1.

4. Discussion

The experiments performed in this study were designed to determine whether: (1) the silver ions contained in Acticoat® demonstrate antimicrobial activity on various microorganisms, (2) the time of contact with Acticoat® affects its efficacy and (3) Acticoat® acts as an antimicrobial barrier, not allowing live microorganisms which are on the surface to penetrate through the Acticoat® layers. Multiple in vitro experiments were performed to address these questions.

Results presented on disc testing (Table 1) suggested that this assay was able to determine whether organisms were susceptible or resistant to the silver ions leached from discs of Acticoat® placed on the surface of agar plates previously inoculated with test organisms. Using the criterion of clear zone diameters of ≥ 1 mm around the disc as indicators of susceptibility of the test organisms to the action of the silver ions, all *P. aeruginosa*, *C. albicans* and 12/15 *S. aureus* tested were judged to be susceptible. In contrast, using a variety of non-Pseudomonas gram negative rods as the test microorganisms, only 2/14 were judged to be susceptible. From these results, we assumed that the disc assay was able to discriminate between organisms susceptible or resistant to silver ions.

However, this conclusion was contradicted by results obtained in the antimicrobial timed assay experiment (Table 2). In this case, with some minor variations, there were time-dependent reductions in quantitative counts of all microorganisms inoculated on the tops of samples of Acticoat® placed on the surface of nutrient agar plates. In all cases reductions from the initial inoculum number were approximately 25–100% after 8 h in contact with Acticoat®.

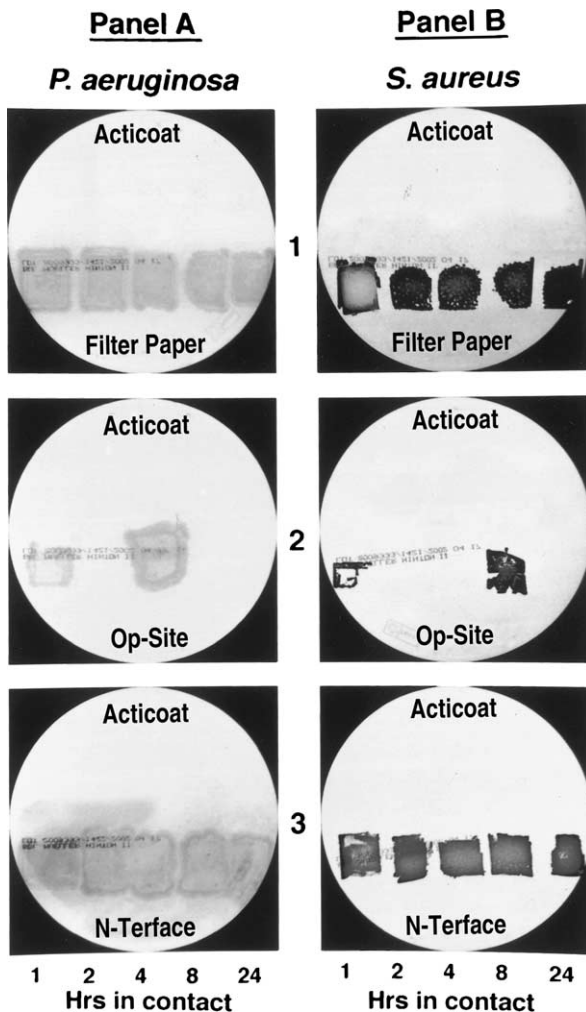


Fig. 1. Barrier function testing of Acticoat®. In vitro barrier function of Acticoat® compared with (1) filter paper (negative control), (2) Op-Site® and (3) N-Terface® when tested against *P. aeruginosa* (panel A) and *S. aureus* (panel B).

Conversely, no consistent reduction in initial numbers was observed with the two control assays. The reduction in numbers as a function of time of contact with Acticoat[®] was observed both in bacteria that were judged to be susceptible to silver ions based on disc assay testing and also in organisms (*E. coli* and *E. cloacae*) which were judged to be silver ion resistant in that assay. These results led us to conclude that time of contact of microorganisms with the silver containing Acticoat[®] is a consideration in their susceptibility to the antimicrobial action of silver ion. Further, we concluded that the disc assay was not suitable to distinguish between silver ion susceptible or resistant microorganisms. Falsely resistant organisms could be reported because the silver ions continuously diffusing into the agar from the Acticoat[®] disc created a decreasing concentration gradient, which did not allow enough time of contact for some organisms to concentrations of silver which would kill them.

Results from our in vitro barrier function testing of Acticoat[®] demonstrated that Acticoat[®] served as an impenetrable barrier for all organisms tested. No growth of any test microorganism, those judged susceptible or resistant by the disc assay, was found on the agar surface under inoculated samples of Acticoat[®] at any time of testing. In contrast to this, organisms placed on the surfaces of filter paper (negative control) or another commonly used wound dressing material, N-Terface[®], were found to grow on the agar surface under the test square at all test times. Data from evaluation of another wound dressing material, Op-Site[®], were inconsistent with growth on agar surfaces under the test squares at some testing times and not at others. In any case, the collective results of these in vitro experiments suggest that the silver-ion containing Acticoat[®] has both inherent antimicrobial properties and the capacity to serve as an antimicrobial barrier dressing. These results suggest that Acticoat[®] may be suitable for protection against environmental microorganisms in use with cultured skin substitutes

to treat full-thickness wounds, if combined with appropriate antimicrobials for management of organisms present in the wound.

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