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Antimicrobial Efficacy of the Silver Wound Dressing Biatain Ag in a Disc Carrier Test Simulating Wound Secretion

M. Ebert[a] O. Assadian[c] N.-O. Hübner[a] T. Koburger[b] A. Kramer[a]
on behalf of the 'International Society of Chemotherapy Working Group Antiseptics' and the 'Working Section for Clinical Antiseptic of the German Society for Hospital Hygiene'

[a]Institute for Hygiene and Environmental Medicine, Ernst Moritz Arndt University, and [b]Hygiene Nord GmbH, Greifswald, Germany; [c]Clinical Institute for Hospital Hygiene, Medical University of Vienna, Vienna, Austria

Key Words
Silver wound dressing · Biatain Ag · Antimicrobial efficacy · Wound secretion

Abstract
Aim: The efficacy of antimicrobial compounds included in wound dressings has been determined using the quantitative suspension test according to EN 13727 before. However, as suspension tests are not an accurate reflection of the conditions under which wound antiseptics are used, it was investigated if a disc carrier test would yield results simulating practical conditions on wound surfaces. A silver-leaching foam wound dressing was used for evaluation of the disc carrier test method. ***Method:*** The disc carriers consisted of circular stainless-steel discs measuring 2 cm in diameter and 1.5 mm in thickness, complying with the requirements of EN 10088-2. Carriers were contaminated with *Staphylococcus aureus*, methicillin-resistant *S. aureus* or *Pseudomonas aeruginosa*, respectively, together with an artificial wound secretion and left to dry at room temperature for 30 min. The wound dressings being tested were placed on the discs for the length of the exposure time, and after neutralization by thioglycolate in phosphate-buffered saline the number of surviving test organisms was then counted. The logarithmic reduction factor was calculated from the difference between the initial inoculum and the number of recovered test organisms. ***Results:*** The disc carrier test allowed determination of an antimicrobial efficacy in a realistic setting. It also imposed more stringent requirements on efficacy over time than the quantitative suspension test. The silver foam wound dressing showed a time-dependent antimicrobial efficacy. After 24-hour application time, the reduction factors against *S. aureus*, *P. aeruginosa* and the methicillin-resistant *S. aureus* were 1.9 ± 0.15, 2.1 ± 0.14 and 3.1 ± 0.18, respectively. ***Conclusion:*** The disc carrier test was a useful method for testing the antimicrobial efficacy of a foam silver dressing. The antimicrobial dressing exhibited an antimicrobial effect after 3 h and achieved a reduction >2 log against the tested bacterial strains in the presence of a simulated wound secretion after 24 h.

Introduction

At present, there is no universally recognized test hierarchy for assessing the antimicrobial efficacy of wound antiseptics and antimicrobial wound dressings. The an-

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Univ.-Prof. Dr. Ojan Assadian, DTMH (Lond)
Clinical Institute for Hospital Hygiene, Medical University of Vienna
Vienna General Hospital, Waehringer Guertel 18–20
AT–1090 Vienna (Austria)
Tel. +43 1 40400 1904, E-Mail ojan.assadian@meduniwien.ac.at

timicrobial efficacy of wound antiseptics is generally investigated in a quantitative suspension test with and without biotope-adjusted challenge [1] according to EN 13727. In order to achieve plausible and correct results, it is paramount that a suitable neutralization medium is selected in order to avoid distortion of the results as a consequence of active substance traces entering the nutrient medium; if the neutralization process is not completely effective, the active substance traces in the test set-up will continue to be antimicrobially active resulting in a wrong impression that the active substance is more effective at a certain contact time than it really is. Currently, for wound antiseptics tested in a suspension test it is required to achieve a ≥ 5 log reduction with no organic challenge and a ≥ 3 log reduction in the presence of a biotope-typical organic challenge [1].

Because a quantitative suspension test cannot reproduce the conditions under which wound antisepsis is performed in clinical practice, we investigated first whether a carrier test was suitable for testing the antimicrobial efficacy as a phase 2, step 1 test under practice-relevant conditions. The model presented here is a closer reflection of the circumstances under which wound antiseptics are used because in contrast to a quantitative suspension test in which the antiseptic is mixed with the test micro-organisms placed in the suspension and the number of test organisms killed in samples of the suspension is compared with the number of test organisms in the control solution, in the carrier test the micro-organisms are placed on a disc, dried and only then covered with the wound antiseptic in the form of a liquid, ointment or wound dressing. The number of surviving test micro-organisms is counted once the exposure time has elapsed. This test model will also allow investigators to ascertain the impact on the efficacy of a challenge comparable to wound secretion.

As the efficacy of wound dressings containing silver is the subject of intense debate in the literature [2], and suspension tests of mild silver protein, silver sulphadiazine and silver nitrate have shown these substances to be practically ineffective when presented with a protein challenge in suspension [3], we aimed to compare the antimicrobial efficacy of a wound dressing containing silver with that of a wound dressing containing no silver in a carrier test.

Method

Study Substances

The study was conducted using Biatain Ag (Coloplast GmbH, Hamburg, Germany), a foam dressing containing homogeneously distributed silver, which continuously releases silver for up to 7 days when in contact with wound exudate. The identical foam dressing without silver (Biatain, Coloplast GmbH) served as control for the purpose of this study.

Pathogens

The test organisms used were *Staphylococcus aureus* ATCC 6538, methicillin-resistant *Staphylococcus aureus* North German epidemic strain and *Pseudomonas aeruginosa* ATCC 15442.

Challenge

The substance used as challenge, simulating wound fluid [3], was Eagle's minimal essential medium with Earle's salts and L-glutamine (PAA Laboratories, Germany), to which 10% fetal bovine serum (Gibco, Life Technologies GmbH, Darmstadt, Germany) was added.

Discs

The carrier discs used were circular stainless-steel discs, 2 cm in diameter and 1.5 mm thick (1.4301, surface grade 2B), complying with the requirements of EN 10088-2.

Neutralization

Before the carrier test was conducted, the appropriateness of possible neutralization media was evaluated in a pre-analytic test. The most suitable neutralized for the silver dressing was found to be 0.1% thioglycolate in phosphate-buffered saline. This compound was therefore used in the further carrier test.

Test Suspension

Test strains were cultured in an overnight culture on blood agar and incubated for 24 h at 36 ± 1°C. A colony was taken from each of the test strains, placed on blood agar once again and incubated again for 24 h at 36 ± 1°C. The resulting subcultures were prepared and used as working cultures. Prior to the start of the test, the suspensions were made by diluting 1 ml of the test strain solution in 9 ml of tryptone-NaCl.

Multiple dilution steps in tryptone-NaCl were performed in order to ensure that the tests could be conducted with sufficiently high test strain counts (approx. 10^8–10^9 CFU/ml). 0.1 ml of each of the 10^{-6}, 10^{-7} and 10^{-8} dilutions were transferred by a sterile spatula onto agar plates using the double method and incubated for 48 h at 36 ± 1°C. Colony counts were then performed to check the microbial density. The efficacy of the wound dressings was assessed by comparison with the final test strain counts of contaminated disc carriers which were left to dry but not covered with wound dressings serving as positive controls.

Performance and Assessment of the Test

The metal discs were placed in sterile Petri dishes. 0.05 ml of the test strain suspension in the challenge medium was placed on the surface, and a sterile glass spatula was used to gently spread the suspension in a circular direction. Care was taken to leave a 1- to 2-mm gap between the solution and the outer edge of the disc in order to ensure that the wound dressings completely covered the wetted surface (fig. 1).

The sterile samples cut to match the wound dressing were then placed in sterile Petri dishes into which challenge fluid had previously been transferred by pipette. They were left in place for 1 min to allow the samples to be completely wetted with the solution and to become saturated with it (the samples were turned in the solu-

tion after 30 s). The samples were then placed on the contaminated test carriers, with care being taken to ensure that the test carriers were completely covered by the wound dressings and that no gaps were left. In order to assure that contact with the contaminated disc test carrier surface was maintained, a second disc test carrier (mass 3.8 g) was placed on top of each sample for the duration of the exposure time (fig. 2).

After the exposure times had elapsed (5, 30 min, 3, 10 and 24 h), the disc test carriers were transferred to sterile examination tubes (Greiner Bio-One GmbH, Solingen, Germany) that had previously been filled with sterile glass beads and 10 ml of neutralizing agent. The metal disc test carriers were positioned with the contaminated surfaces facing downwards. They were able to move freely on the glass beads in the horizontal plane. The tubes were agitated on a vortexer for 2 min to dissolve the pathogens. After a neutralization time of 5–30 min, 1 ml of the test-neutralizer mixture was removed and placed in 9 ml of tryptone-NaCl. 0.1 ml of this mixture and subsequent dilutions were spread on sets of 2 Petri dishes. The colonies were counted after 48 h of incubation at 36 ± 1°C.

Only agar plates with colony counts ranging from 15 to 300 CFU were taken into account when evaluating the tests. The weighted averages of the consecutive dilutions were calculated as follows:

$$\hat{C} = \frac{c}{(n_1 + 0.1n_2)d}$$

where $\hat{C}$ = weighted average; c = sum of the colonies on all nutrient media assessed; n_1 = number of nutrient media assessed with the first dilution; n_2 = number of nutrient media assessed with the second dilution; d = dilution factor of the first dilution assessed.

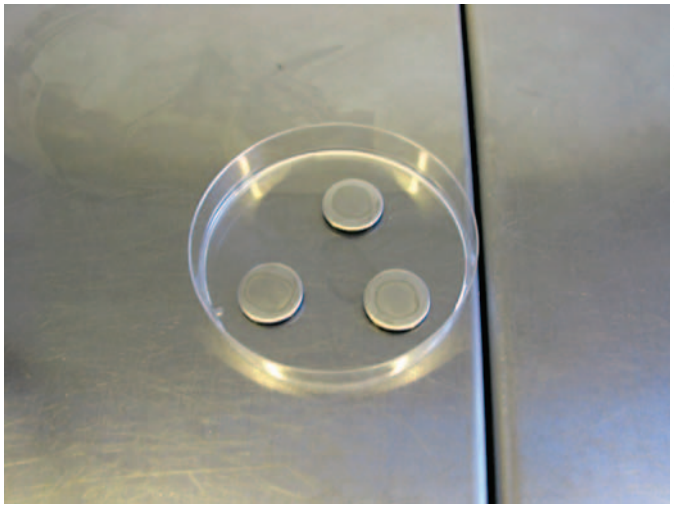

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Fig. 1. Dried pathogen suspension on a metal disc carrier.

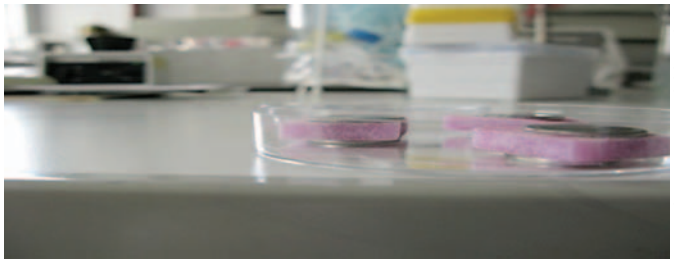

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Fig. 2. Test carrier with a wound dressing covered by an uncontaminated test carrier.

Results

The antimicrobial effect on both *Staphylococcus* strains started after 3 h at the earliest (significant log reduction compared to the positive control at the p = 0.05 significance level), while a similar effect on *P. aeruginosa* could be observed after already 30 min ($p < 0.05$, table 1). The antimicrobial effect increased with time, and the reduction factors after 24 h against *S. aureus*, *P. aeruginosa* and the methicillin-resistant *S. aureus* strain were 1.9 ± 0.15, 2.1 ± 0.14 and 3.1 ± 0.18, respectively (table 1).

Biatain without silver which served as negative control did not show any antimicrobial efficacy, as expected. In contrast, the non-antimicrobial wound dressing even showed an increase of >1 log in the number of test bacteria after 24 h (for *P. aeruginosa* almost even after 10 h). After 24 h, methicillin-resistant *S. aureus*, *S. aureus* and *P. aeruginosa* showed an increase of 1.1 ± 0.23, 1.7 ± 0.16 and 1.8 ± 0.17 log. This increase was most likely due to the moisture on the slide and almost reached 2 log in the case of *P. aeruginosa* (table 1).

Discussion

We found the disc carrier test to be a useful and easy-to-apply method to assess the antimicrobial efficacy of an antimicrobial wound dressing. Furthermore, the test method allows a more realistic assessment simulating conditions on the wound surface. It also imposes more stringent requirements on antimicrobial efficacy over time than a quantitative suspension test, since contact between the wound dressing and the test organism suspension to which the dressing is exposed is less intense than during a suspension test where the dressing is placed in a test solution containing test organisms at planktonic and dynamic conditions.

So far, it is accepted that if an antimicrobial compound fulfils the requirements for quantitative suspension tests, the next step will involve testing against an established

Table 1. Efficacy of the silver wound dressing Biatain Ag compared to an identical wound dressing without silver (mean of 9 test runs with each run assessed twice, giving a total of 18 results)

Exposure time	*S. aureus* (control 5.9 log)		MRSA (control 5.9 log)		*P. aeruginosa* (control 6.1 log)	
	Biatain Ag	Biatain	Biatain Ag	Biatain	Biatain Ag	Biatain
5 min	0.3 ± 0.14	0.1 ± 0.08	0.7 ± 0.25	0.5 ± 0.18	0.2 ± 0.12	0.1 ± 0.12
30 min	0.3 ± 0.1	0.1 ± 0.1	0.7 ± 0.19	0.3 ± 0.27	1.1 ± 0.15*	0.3 ± 0.16
3 h	1.3 ± 0.1*	0.0 ± 0.11	2.0 ± 0.15*	0.6 ± 0.26	1.2 ± 0.17*	0.1 ± 0.15
10 h	1.6 ± 0.34*	0.1 ± 0.09	2.2 ± 0.23*	0.4 ± 0.15	1.3 ± 0.13*	–0.9 ± 0.13
24 h	1.9 ± 0.15*	–1.7 ± 0.16	3.1 ± 0.18*	1.1 ± 0.23	2.1 ± 0.14*	–1.8 ± 0.17

MRSA = Methicillin-resistant *S. aureus.* * $p < 0.05$: significant difference to control, assessed by t test.

reference standard in healthy volunteers [4]; yet, this is not possible for wound antiseptics, as no models for an infected wound in healthy subjects exist [5]. Therefore, the only possibility is to test the antimicrobial efficacy on mesh graft donor sites in patients, when such a procedure is clinically indicated [6]. However, this test will allow only conclusions on antimicrobial efficacy against physiological colonization flora on the wound surface, since controlled contamination with pathogens is unethical. The only true possibility of testing antimicrobial efficacy and the clinical benefit is therefore to conduct a clinical randomized controlled trial (RCT) on wounds that can be compared with each other, such as chronic infected crural ulcers. Because of the prohibitive costs of RCTs and the difficulties associated with coordination of multi-centre trials, before even considering RCTs, as much information as possible should be available on the antimicrobial efficacy of the compounds to be tested. The information needed is more than what can be provided by suspension tests and must be derived from other tests, such as examination of efficacy in disc carrier tests (initially with no biofilm, and once the substance has been shown to be effective with a biofilm placed on the disc carrier prior to the test) as well as comprehensive in vitro and semi in vivo tolerance testing.

Quantitative suspension tests have found silver compounds confronted with a protein challenge to be ineffective [3], but the silver wound dressing Biatain Ag tested here was found to have a significant bactericidal effect after 10–24 h and against *P. aeruginosa* even after a 30-min exposure time.

However, the test results do not allow the conclusion that wound dressings containing silver also lead to the desired clinical effect of improved chronic wound healing because the in vitro nature of the disc carrier test will not examine the possible cytotoxic effect on wounds. It is always important to rule out the risk of wound healing being inhibited by silver ions [7] in silver ion-leaching wound dressings releasing silver into the wound. In vitro tests have shown that the addition of ionic silver to alginate improves antimicrobial efficacy and the ability to bond to elastase, matrix metalloproteinase-2 and pro-inflammatory cytokine, and increases anti-oxidative capacity; however, this is achieved on the backside of a significant keratinocyte inhibition [8].

A Cochrane analysis assessing the clinical efficacy of silver wound dressings found only 3 RCTs with a short follow-up. All tested silver wound dressings did not achieve any significant improvement in ulcer healing, although the ulcers treated with a silver foam dressing did tend to decrease in size. The authors conclude that there is insufficient evidence for the use of wound dressings or topical antiseptics containing silver in the treatment of infected or contaminated wounds [9]. In another meta-analysis, 8 studies from 1,957 relevant sources were assessed. The conclusion was that wound dressings containing silver significantly improved the healing process of chronic, poorly healing wounds, with reduced wound odour, exudation and pain [10]. However, a further meta-analysis published in the same year found no clear evidence in favour of recommending foam dressings or alginate wound dressings containing silver for the treatment of infected chronic wounds for a period of 4 weeks [11]. Nevertheless, foam dressings containing silver did achieve a trend-wise greater reduction in wound area, exudation and odour compared to wound dressings not containing silver. Because the silver dressing was antiseptically effective in our disc carrier model, we conclude

that RCTs with a longer follow-up period are necessary in order to identify the most suitable wound dressing for the treatment of contaminated and infected acute and chronic wounds [11].

Disclosure Statement

The study received financial support from Coloplast GmbH, Hamburg. The sponsor had no influence on the study design or on the interpretation of the results. None of the authors have any financial or other conflict of interest to report.

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