Evaluation of Cytotoxicity and Antimicrobial Activity of Acticoat® Burn Dressing for Management of Microbial Contamination in Cultured Skin Substitutes Grafted to Athymic Mice

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Cultured skin substitutes (CSS) have become a useful adjunctive treatment for closure of burn wounds, but CSS are avascular and remain susceptible to microbial destruction longer than split-thickness skin grafts. Irrigation of CSS grafted to burn wounds with a topical antimicrobial solution (TAS) has been shown to promote engraftment of CSS, but TAS usage has potential limitations. Acticoat® Burn Dressing (Acticoat®; Westaim Biomedical, Exeter, NH) is a silver-coated barrier dressing reported to exhibit antimicrobial activity and to reduce infection in partial-thickness and full-thickness wounds. This study evaluated the cytotoxicity of Acticoat® with CSS and the efficacy of Acticoat® for the management of microbial contamination in CSS grafted to full-thickness wounds in athymic mice. The cytotoxicity of Acticoat® was assessed in preliminary studies after 1 week of exposure to CSS during in vitro maturation or healing on wounds in athymic mice. Histologies were analyzed and cellular viability in the CSS was determined by MTT conversion on days 0, 1, and 7 of Acticoat® exposure. At 1, 2, 3, and 4 weeks after grafting, wounds were traced, and areas of healing CSS were calculated by image analysis. At 4 weeks, wound biopsies were evaluated and scored for engraftment of human cells. In a subsequent study, wounds were inoculated with strain SBI-N of *Pseudomonas aeruginosa* at 1×10^5 cfu/wound before the application of CSS or inoculated onto the surface of Acticoat®. At 4 weeks, swab cultures were collected from the surface of CSS and scored for the presence of SBI-N. Statistical significance was accepted at the 95% confidence level ($P < .05$). The data show that exposure in vitro of CSS to Acticoat® was cytotoxic within 1 day, but 1 week of exposure in vivo did not injure CSS or inhibit wound healing. Contaminated wounds treated with Acticoat® healed similarly to control treatments, with comparable rates of engraftment, and detection of SBI-N on the surface of only one graft. No SBI-N was detected on CSS after inoculation onto the surface of Acticoat®. These results suggest that Acticoat® may be suitable as a protective dressing to reduce environmental contamination of CSS, if used in conjunction with additional antimicrobials to control organisms present in the wound. (J Burn Care Rehabil 2005;26:238–246)

Cultured skin substitutes (CSS) provide therapeutic alternatives for the closure of massive burn wounds.[1–3] However, current models of CSS have anatomical and physiologic deficiencies compared with the grafts of native skin. These limitations include lack of a vascular plexus and incomplete barrier function at the time of grafting.[3,4] As a result, CSS have increased susceptibility to destruction from microorganisms present in the wound and/or the environment.[5] Irrigation of CSS grafted to burn wounds with topical antimicrobial solutions containing antibacterial and antifungal compounds has been demonstrated to reduce microbial destruction of avascular CSS during vascularization.[6–9] These solutions contain a redundancy of antimicrobials to reduce the chance of resistance development by organisms during clinical treatment.[7,10] However, the possible development of drug resistance, the off-label

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use of some antimicrobial components, and the time and labor required for preparation motivate the investigation of alternative methods for management of microbial contamination of CSS.

The antimicrobial activity of silver-containing dressing materials has been evaluated widely in both in vitro[11–14] and in vivo[15–17] studies. These products consist of ionic silver (Ag^+) either coated onto or contained within synthetic materials. Silver dressings reportedly exhibit antimicrobial activity that is comparable with or superior to traditional silver-based topical compounds (solutions, creams, or ointments) used on burn wounds.[13] A previous study from this laboratory described in vitro studies that evaluated the antimicrobial activity of a silver-coated dressing, Acticoat® (Westaim Biomedical, Exeter, NH) and suggested effective barrier protection from environmental microorganisms.[11] The current studies were conducted to evaluate the cytotoxicity of Acticoat® with CSS and to determine whether Acticoat® is effective for the management of microbial contamination in full-thickness wounds treated with CSS.

MATERIALS AND METHODS

Experimental Design

For all experiments reported here, CSS consisted of collagen-based substrates populated with human fibroblasts and keratinocytes to generate a composite skin substitute. A preliminary study was conducted to evaluate the cytotoxicity of Acticoat® with CSS after 1 week of exposure in vitro or in vivo after grafting to athymic mice. After the determination that in vivo contact between Acticoat® and CSS was nondestructive, a second experiment was performed to identify a concentration of *Pseudomonas aeruginosa* (Psa) inoculated onto the wound bed that resulted in complete loss of CSS grafted to athymic mice in the absence of drug treatment (negative control) but without lethal risks to the animals. After identification of 1×10^5 cfu/wound as a suitable inoculum for significant graft loss, a third experiment was conducted to compare the efficacy of Acticoat® with a topical antimicrobial solution (TAS)[6] for the control of microbial contamination in CSS grafted to athymic mice. Full-thickness skin wounds on athymic mice were inoculated with 1×10^5 cfu/wound of Psa (+Psa) before the application of CSS and treated with Acticoat® or TAS for 1 week after surgery. Controls for the contaminated wound study included sterile water (H_2O) irrigation of +Psa wounds (vehicle control), and treatment of CSS grafts with Acticoat®, TAS, or H_2O in the absence of Psa contamination (positive controls). An additional condition was included in which 1×10^5 cfu Psa were inoculated onto the surface of Acticoat®, without wound bed contamination, to evaluate the barrier protection from environmental microorganisms provided by the dressing.

Preparation of Cultured Skin Substitutes

CSS were prepared from human dermal fibroblasts and epidermal keratinocytes sequentially inoculated onto collagen-glycosaminoglycan substrates.[18,19] There was a 1-day interval between fibroblast and keratinocyte inoculation, with the latter identified as culture day 0. CSS were incubated (5% CO_2, 37°C, saturated humidity) at the air–liquid interface of the culture medium[20] beginning on culture day 1 for the duration of the in vitro maturation and evaluation period. Nutrient media[21] were changed daily throughout in vitro culture. All CSS were meshed (Model 078, Concept Inc., St. Petersburg, FL), but not expanded, 1 day before contact with Acticoat® for in vitro studies or grafting to athymic mice for in vivo studies. This was performed to be analogous with procedures used for the clinical application of CSS.[1] Subsequent to meshing, CSS grafted to athymic mice were cut into 2-cm × 2-cm grafts the day before surgery.

Animal Surgery

All animals were acquired, housed and studied under a protocol approved by the University of Cincinnati Institutional Animal Care and Use Committee. Grafting of CSS to athymic mice was performed under aseptic conditions using procedures previously described.[18,20,22] CSS were grafted orthotopically to full-thickness skin wounds (2 cm × 2 cm) surgically created to the depth of the panniculus carnosus on the flanks of athymic mice. Experimental wounds (4 cm^2) were inoculated with 0, 1×10^4, 1×10^5, or 1×10^6 cfu Psa (strain SBI-N) before the application of CSS onto the wound. CSS in H_2O and TAS groups were covered with N-Terface® (Winfield Laboratories, Richardson TX), and CSS in the Acticoat® groups were covered with Acticoat® prewetted with sterile water and oriented so the blue-mesh surface was in contact with the CSS. Grafts were attached with stent-sutures through the CSS and N-Terface® or Acticoat® at the corners and midpoints of the wound margin. All wound perimeters were traced before final dressing to facilitate subsequent evaluation of wound area during healing.[20] In the final study, one group received an inoculum of 1×10^5 cfu Psa onto the surface of the Acticoat® (surface) in the absence of wound bed contamination. Cotton gauze was applied on top of the N-terface® or Acticoat® and opposing sutures were tied over to secure the gauze.

The dressings were covered with OpSite® (Smith and Nephew Medical Ltd., Hull, UK), and sealed to the surrounding murine skin with benzoin tincture around the perimeter. Complete dressings were wrapped with Coban® (3M, Minneapolis, MN) with a small aperture left in the Coban® above the gauze. All grafts were irrigated with 1.5 ml of either sterile water (Acticoat® and H_2O groups) or TAS immediately after surgery and then daily with TAS or at ≤48-h intervals with H_2O for 1 week. Irrigation solutions were administered through the OpSite®, via the aperture in the Coban®, to saturate the cotton gauze and keep it moist between administrations.[6] Daily observations confirmed the gauze remained fully wetted during the entire treatment period, serving as a moisture reservoir for the Acticoat®. At 1 week after surgery, all treatments were discontinued, dressings and sutures were removed (Acticoat® and gauze were wet to the touch), data were collected, and the healing wounds were redressed with N-terface®, gauze, and Coban®. Dressings were changed, and data were collected at weekly intervals for the remainder of the in vivo study periods.

Assessment of Acticoat® Cytotoxicity

The initial evaluation of Acticoat® with CSS was performed to assess the potential cytotoxicity of the silver ions to the cellular components of the skin substitutes. Cytotoxicity was first evaluated by exposure of Acticoat® to CSS during in vitro incubation. Subsequently, parallel CSS were assessed in vivo by the application of Acticoat® on CSS grafted to athymic mice. For in vitro experiments, CSS (n = 4/group) were incubated with (Acticoat®) or without (control) an overlay of prewetted Acticoat®, oriented with the blue-mesh surface contacting the CSS, for 7 days starting on culture day 13. Punch biopsies (9 × 3mm) were collected from each CSS on days 0, 1, and 7 of Acticoat® exposure and used to measure cellular viability as determined by an MTT (Sigma Chemical Co., St. Louis, MO) conversion assay.[19,21] The assay reflects mitochondrial enzyme activity in viable cells as indicated by the conversion of the tetrazolium salt MTT to formazan, with a corresponding color change of the reaction solution.[23] Data, mean ± SEM, are expressed as the percentage of pretreatment (day 0) MTT values (OD_{590}) calculated independently for each sample in the control and Acticoat® groups. Biopsies were collected from CSS at the same time points for histologic evaluation by light microscopy. Parallel CSS, without previous Acticoat® exposure, were grafted to noncontaminated wounds on athymic mice (n = 7–8/group) on culture day 16 and dressed with Acticoat® as described previously. Dressings for the control groups (H_2O and ointment) consisted of N-Terface® and gauze irrigated with sterile water (vehicle control) or N-Terface® and gauze coated with antibiotic ointment (positive control), respectively. The ointment (1:1:1, Bactroban®: Neosporin®: Nystatin) is the standard dressing used for CSS studies on athymic mice[22] and also is used in clinical application of CSS after the discontinuation of dressing irrigation.[1] Bactroban® contains 20 mg/g mupirocin, and Neosporin® contains 400 units/g bacitracin, 3.5 mg/g neomycin, and 5,000 units/g polymyxin B. Wound areas were measured at weekly intervals[20] through 4 weeks, and mice were euthanized, wound biopsies (two/animal) were collected, and were processed for light microscopy or immunostained for HLA-ABC antigens to verify engraftment of human cells[20,24,25] at 4 weeks after grafting. Data (mean ± SEM) are expressed as percentage of original wound area (%OWA) and percentage of HLA-ABC positive/group (%HLA+).

Titration of *Pseudomonas aeruginosa* Inoculum

CSS were prepared and grafted to athymic mice on culture day 16 as described previously. Before CSS placement, wounds (n = 3/group) were contaminated with 1×10^4, 1×10^5, or 1×10^6 cfu Psa (strain SBI-N) in 0.1 ml of saline. Dressings and irrigation schedules were identical to those previously described for vehicle controls (H_2O). Irrigation was discontinued at 1 week after surgery, dressings and sutures were removed, and wounds were redressed with N-Terface®, gauze and Coban®. Two weeks after grafting, surface cultures were collected from the CSS with a BBL CultureSwab® (BD Biosciences, Sparks, MD), mice were euthanized, and wound biopsies collected and stained for HLA-ABC antigens. Data (mean ± SEM) are presented as percentage of Psa positive/group (%Psa+) and %HLA+.

Antimicrobial Activity of Acticoat® on Psa-Contaminated Wounds

CSS were prepared as described previously and grafted to athymic mice (n = 4–6/group) on culture day 16. Three groups received inoculation of 1×10^5 cfu Psa (strain SBI-N), and four groups received no Psa inoculation before placement of CSS on wounds in all seven groups. One group each ± Psa inoculation was treated with Acticoat®, TAS, or H_2O dressings as previously described. One group without Psa inoculation was dressed with Acticoat® and inoculated with 1×10^5 cfu Psa (strain SBI-N) on the external surface of the Acticoat® (surface) before placement of the cotton gauze. Irrigation, dressing

change, and data collection schedules were identical to those described in the animal surgery section. Wound areas were measured at surgery and weekly through postoperative week 4. At 4 weeks after grafting, surface cultures were collected from the CSS, mice were euthanized, and wound biopsies collected and stained for HLA-ABC antigens. Data (mean ± SEM) are expressed as %OWA, %Psa+ and %HLA+.

Microbial Culture and Serotyping

All contaminated wounds were inoculated with Psa strain SBI-N, which is a clinically isolated strain previously shown to be an O serotype G strain[26] susceptible to TAS[6] or Acticoat®[11] treatment. The use of a strain with known serotype facilitated confirmation that Psa recovered from experimental wounds were the same serotype as the organisms inoculated onto the wounds at the time of CSS grafting. Swab cultures collected from the surface of grafted CSS were used to inoculate tryptic soy with 5% blood agar plates. The plates were incubated overnight at 35°C and subsequently examined for microbial growth. If cultures were identified as positive for Psa, then one sample from each treatment group was serotyped for cross-reactivity to SBI-N.[6] All tested organisms were identified to be consistent with the serotype of SBI-N.

Data Collection and Analysis

Data from the in vitro analysis of Acticoat® cytotoxicity were collected on days 0, 1, and 7 of Acticoat® exposure, and data from all in vivo studies were collected at weekly intervals (unless previously stated) through postoperative week 2 or 4. MTT values were determined independently for each CSS by averaging values from three wells containing 3 × 3-mm punch biopsies per well. MTT data are expressed as percentages of the pretreatment (day 0) value (OD_{590}). Samples were measured in a 24-well plate using a SpectraCount® microplate photometer (Packard Instrument Co., Meriden, CT). Samples for light microscopy were fixed, processed, embedded in glycol-methacrylate, sectioned, and stained with toluidine blue using standard techniques. Wound area (%OWA) data were calculated from direct tracings of wound perimeters followed by computerized image analysis and are expressed as percentages of the wound area at the time of surgery.[20] Immunostaining for HLA-ABC antigens (%HLA+) was performed on wound biopsies embedded in M1 matrix (Lipshaw, Pittsburgh, PA), cryo-sectioned and labeled with a fluorescein-conjugated antibody to HLA-ABC (#CLHLA01F, Accurate Chemical and Scientific Corp., Westbury, NY). All histologic evaluation was performed with a Nikon Microphot-FXA microscope (Nikon, Melville, NY), and images were captured using a Spot Camera 1.5.0 (Diagnostic Instruments, Inc., Sterling Heights, MI). Quantitative data (mean ± SEM) were analyzed by χ^2 test, analysis of variance, related measures analysis of variance, and/or Tukey's test to determine significant differences, $p \leq .05$ (unless indicated otherwise), between or among experimental groups.

RESULTS

Acticoat® Cytotoxicity. Reduced mitochondrial metabolism in CSS caused by exposure to Acticoat® during in vitro maturation is shown in Figure 1. Cellular viability in CSS decreased significantly from pretreatment (day 0) values (100 ± 0%) after exposure to Acticoat® for 1 day (53.6 ± 2.1%), or 1 week (44.4 ± 2.3%). There were no significant differences among control CSS after 1 day (105.7 ± 6.6%), or 1 week (89.5 ± 7.5%) of incubation. Acticoat®-treated CSS samples at 1 day or 1 week of incubation also were significantly different from untreated samples at the same time points.

Representative images from the histologic evaluation of CSS during 1 week of Acticoat® exposure in vitro are presented in Figure 2 (controls: A, C, and E; + Acticoat®: B, D, and F) and correlate well to the cellular viability data. The photomicrographs in the top row were collected on culture day 13 and show CSS (Figure 2A) and CSS + Acticoat® (Figure 2B) before the appli-

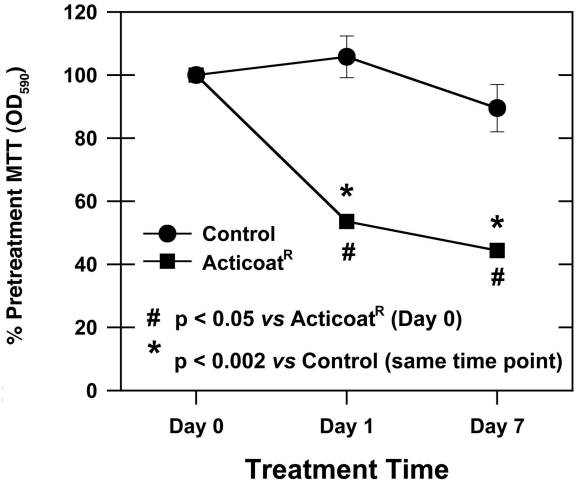

Figure 1. Cellular viability as measured by MTT conversion in control cultured skin substitutes and cultured skin substitutes + Acticoat® during 1 week of in vitro exposure. Data (mean ± SEM) are expressed as percentages of the pretreatment (day 0) MTT values (OD_{590}) calculated for each sample in the control and Acticoat® groups. Significant reductions (*, #) in viability caused by Acticoat® exposure are indicated.

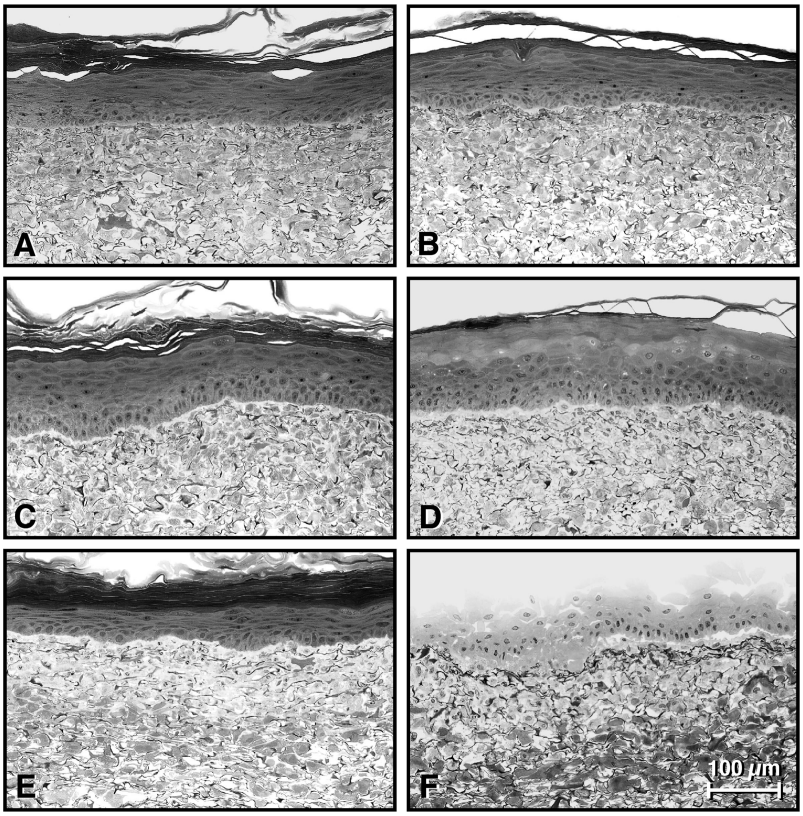

Figure 2. Time–course histologies collected from control cultured skin substitutes (CSS; A, C, and E) and CSS + Acticoat® (B, D, and F) at day 0 (A and B) day 1 (C and D), and day 7 (E and F) of in vitro evaluation of the cytotoxicity of Acticoat® to CSS. Scale bar = 100 μm for all six panels.

cation of the Acticoat® (day 0). Both CSS have histiotypic morphologies with a stratified, well-differentiated epidermal component covered by multiple cornified layers and a dermal component heavily populated with fibroblasts. In the middle row, the control CSS (Figure 2C) is unchanged after 1 additional day in culture, but the CSS + Acticoat® (Figure 2D) has enlarged and vacuolated cells throughout the epidermis and generally poor morphology after 1 day of Acticoat® exposure. After 7 days of exposure the CSS + Acticoat® (Figure 2F) is obviously injured, with a detaching and disorganized epidermis, large vacuolated cells in both the epidermis and dermis, reduced numbers of nucleated cells, and no cornified layers. The control CSS (Figure 2E) has an increased number of flattened, cornified layers but is otherwise very similar to the sample collected 1 week earlier (Figure 2A).

CSS were grafted to noncontaminated wounds on athymic mice and treated with Acticoat®, antibiotic ointment, or vehicle controls (H_2O) for 1 week after surgery. Wound area data are shown in Figure 3A and indicate no significant difference in %OWA among the treatment groups at any time point. Wounds from all three conditions stabilized at ~40 %OWA by 4 weeks after grafting. Therefore, Acticoat® exposure to CSS during the first postoperative week is noncytotoxic, noninjurious, and does not inhibit wound healing. One week of Acticoat® exposure did not result in a significant change in HLA-ABC expression by epidermal keratinocytes at 4 weeks after surgery, as shown in Figure 3B. Human cells were detected in six of seven wounds (85.7%) treated with Acticoat® compared with seven of eight wounds (87.5%) treated with ointment or H_2O as controls. This result demonstrates that Acticoat® is noncytotoxic to CSS after treatment of full-thickness wounds on athymic mice.

Photomicrographs of representative biopsies from the Ointment (Figure 4A), Acticoat® (Figure 4B), and H_2O (Figure 4C) treatment groups, collected from healed CSS at 4 weeks after surgery, are presented in Figure 4. The tissues exhibit a cornified and fully differentiated epidermis, putative rete-ridges, and a thick dermis heavily populated with fibroblasts. This morphology is consistent throughout all three samples and confirms that in vivo treatment with Acticoat® on CSS is nondamaging.

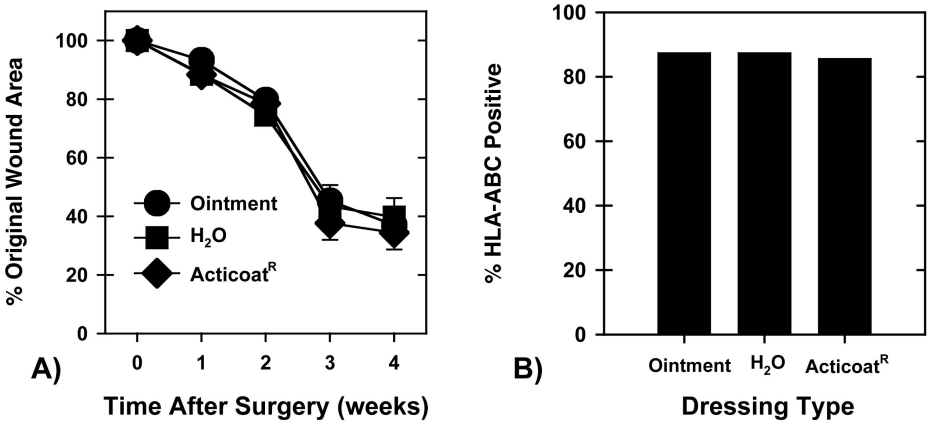

Figure 3. Cytotoxicity of Acticoat® to cultured skin substitutes (CSS) after 1 week of in vivo exposure on full-thickness wounds in athymic mice. The area of healing wounds through postoperative week 4 and the engraftment (at 4 weeks) of human cells from CSS were evaluated in CSS + Acticoat® and control groups. Data (mean ± SEM) are presented as the percentage of the original wound area (A) and the percentage of HLA–ABC-positive wounds/group (B), respectively.

The data presented suggest that Acticoat® is cytotoxic and unsuitable for use with CSS during in vitro maturation before surgical application. However, 1 week of Acticoat® exposure after surgery is noninjurious and does not inhibit wound healing of CSS grafted to athymic mice.

***Pseudomonas aeruginosa* Titration.** Surgical wounds on athymic mice were contaminated with 1×10^4, 1×10^5, or 1×10^6 cfu SBI-N before the application of CSS. Figure 5 presents results of assays for detection of Psa from swab cultures of the wound surfaces (%Psa+) and for detection of HLA-ABC expression by human keratinocytes (%HLA+) at 2 weeks after grafting of CSS. All wounds inoculated with 1×10^4 SBI-N were positive for HLA-ABC, and two of three (66.7%) were positive for Psa. A significant reduction in HLA-ABC expression was observed in wounds inoculated with 1×10^5 SBI-N (33.3%) and 1×10^6 SBI-N (0%). Psa was detected in 100% of the wounds from the two higher SBI-N inocula. On the basis of these results, 1×10^5 SBI-N was chosen as the inoculation density for subsequent contaminated wound studies presented in this report.

Acticoat® Efficacy on Contaminated Wounds. Wound area data (%OWA), collected at weekly intervals through postoperative week 4, are shown in Figure 6. All wounds were healed by 4 weeks after grafting of CSS. Grafted wounds without Psa inoculation had areas of approximately 60 %OWA in all three treatment groups. Grafted wounds with 1×10^5 SBI-N on the wound beds and Acticoat® (wound bed) or TAS treatment had areas of approximately 40 %OWA. Similarly, grafted wounds inoculated with Psa on the surface of the Acticoat® (surface) were approximately 40 %OWA at 4 weeks. Treatments of Acticoat® (wound bed or surface) or TAS after Psa contamination were statistically different from controls but not each other. Grafted wounds inoculated with 1×10^5 SBI-N and treated with H_2O had areas of approximately 10 %OWA and were significantly smaller than all other groups.

Data presented in Figure 7 confirm the engraftment of human keratinocytes (%HLA+) and the detection of Psa from wound surface cultures (%Psa+) at 4 weeks after grafting of CSS. All healed wounds without Psa inoculation (controls) were 100 %HLA+ independent of treatment. Wounds inoculated with Psa and treated with Acticoat® (wound bed) or TAS exhibited 80% (4/5) or 82% (5/6) HLA+ wounds, respectively. Grafted wounds treated with Acticoat® followed by Psa inoculation on the surface of the Acticoat® (surface) were 82 %HLA+ (5/6). No wounds inoculated with Psa and treated with H_2O were positive (0 %HLA+) for engraftment of human cells. Psa inoculated on the wound bed was not detected with TAS (0 %Psa+) treatment but was detected in one of five grafted wounds treated with Acticoat® (20 %Psa+) and all wounds treated with H_2O (100 %Psa+) only. Psa was not detected after SBI-N inoculation onto the surface of Acticoat® (0 %Psa+). The Psa inoculation group treated with H_2O only was statistically different from all other groups for both %HLA+ and %Psa+.

These results suggest that Acticoat® may be effective as a protective dressing to reduce CSS contamination from environmental microorganisms. However, additional antimicrobials may be required for increased antimicrobial activity on burns to protect CSS from wound contamination.

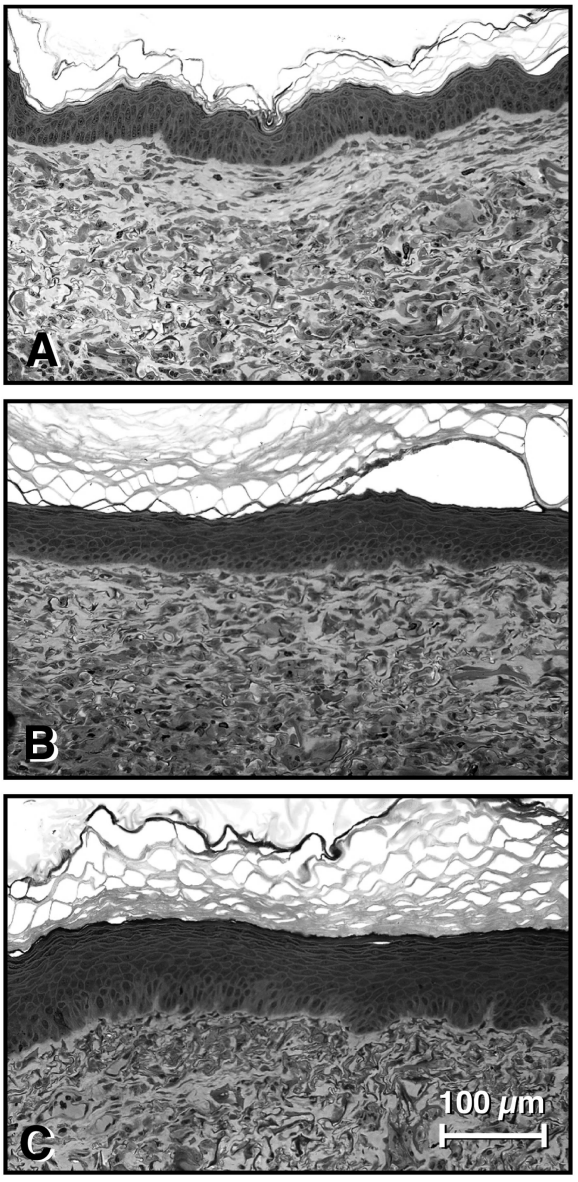

Figure 4. Light micrographs of representative histologies collected at 4 weeks after surgery from full-thickness wounds in athymic mice grafted with cultured skin substitutes. Healing wounds were treated during the first postoperative week with antibiotic ointment (A), Acticoat® (B), or sterile H_2O (C). Scale bar = 100 μm for all three panels.

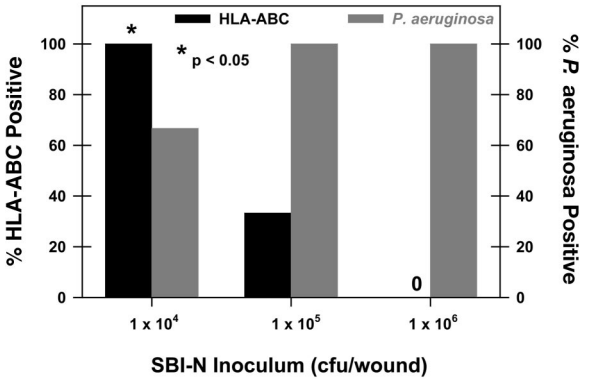

Figure 5. Titration of *Pseudomonas aeruginosa* (strain SBI-N) inoculum on full-thickness skin wounds in athymic mice grafted with cultured skin substitutes in the absence of antimicrobial treatment. At 2 weeks after surgery, wounds were scored for the engraftment of human cells and the presence of SBI-N in swab cultures. Data (mean ± SEM) are presented as the percentage of HLA-ABC positive and *P. aeruginosa*-positive wounds/group. Significantly greater (*) engraftment of human cells as compared with other inoculation densities is indicated.

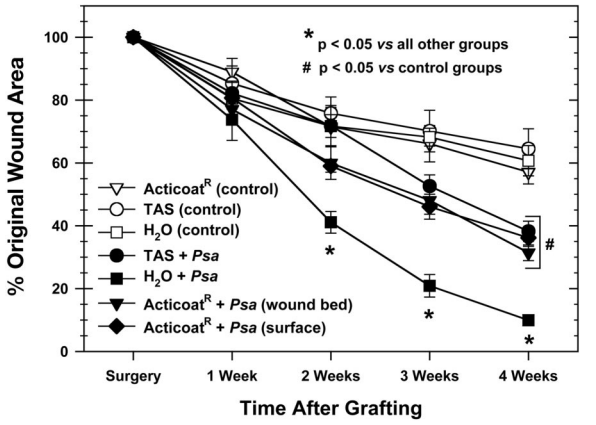

Figure 6. Kinetic assessment of the percent of original wound area during healing of full-thickness wounds in athymic mice after inoculation of SBI-N (1×10^5 cfu/wound) and grafting of cultured skin substitutes. Data (mean ± SEM) were collected from the various treatments that were maintained for 1 week after surgery. Significant reductions (*, #) in wound area among treatment groups are indicated.

DISCUSSION

The preparation of the multicomponent TAS used for the clinical treatment of burn wounds with CSS is very labor intensive. Additionally, the possible development of drug resistance by microorganisms exposed to TAS and the requirement for an investigational protocol for the off-label use of some components of the topical solution[8] supports the investigation of alternative methods to control microbial contamination in wounds treated with CSS. Any alternative must be noncytotoxic to permit engraftment of human cells from CSS, have effective antimicrobial activity against clinical isolates of microorganisms, and provide protection for CSS from organisms present in both the wound and the environment.

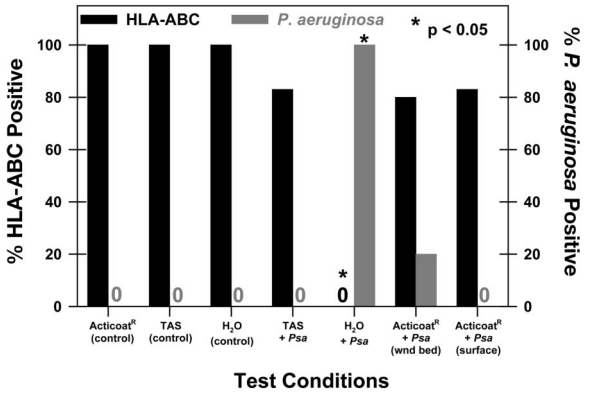

Figure 7. Evaluation of the antimicrobial efficacy of Acticoat® to control *P. aeruginosa* (strain SBI-N) contamination in full-thickness wounds grafted with cultured skin substitutes in athymic mice. The engraftment of human cells from cultured skin substitutes and the presence of SBI-N in swab cultures were evaluated at 4 weeks after surgery. Data (mean ± SEM) are presented as the percentage of HLA–ABC-positive and *P. aeruginosa*-positive wounds/group. Significantly reduced engraftment of human cells and increased persistence of SBI-N in the absence of antimicrobial treatment are indicated (*).

The results from the in vitro assessment of Acticoat® combined with CSS (Figures 1, 2) suggest that the ionic silver (Ag^+)-coated material is cytotoxic and injurious within 1 day of exposure during CSS incubation in vitro. This apparent toxicity may have resulted from 1) complexation/precipitation of vital nutrients from the culture media, 2) significant changes in media osmolarity associated with release of Ag^+ from the Acticoat®, 3) interaction of Ag^+ with the cellular components of the CSS, and/or 4) elevated rates (cytotoxic) of Ag^+ release attributable to the culture conditions. By whichever mechanism, the Acticoat® produced significant reduction in cellular viability and obvious cellular injury within 24 hours of contact with CSS.

The in vitro assays suggest that a preliminary in vivo assessment of Acticoat® cytotoxicity on a clean wound grafted with CSS was necessary before testing the antimicrobial efficacy on contaminated wounds. The data presented (Figure 3) indicate that 7 days of Acticoat® exposure to healing CSS on athymic mice did not produce statistical differences in wound area or engraftment of human cells as compared with control groups. The morphology of healed wounds (Figure 4) treated with Acticoat® during the first postoperative week was comparable with healed wounds from the control conditions. In vivo, free Ag^+ may complex with proteins or other macromolecules in the wound that are not present in vitro, resulting in relatively lower concentrations of free Ag^+ during the in vivo assay period. These results suggest that Acticoat® is not toxic in grafting of full-thickness wounds and may be suitable as an antimicrobial dressing for use in combination with CSS to treat burn wounds.

The density of Psa (SBI-N) required for uniform detection (100 %Psa+) and significant reduction in the engraftment of human cells (%HLA+) was determined to be 1×10^5 SBI-N (Figure 5). This density is consistent with previous studies from this laboratory[6] that were conducted to characterize the efficacy of the TAS routinely used in combination with CSS for the clinical treatment of burn wounds[7–10] and used as a control treatment in the current study.

The antimicrobial efficacy of Acticoat® was evaluated on full-thickness wounds in athymic mice that were contaminated intentionally with 1×10^5 cfu of a clinical Psa isolate (SBI-N) before grafting of CSS or onto the surface of the Acticoat® after CSS treatment (Figures 6 and 7). The former inoculation condition was a test for activity against wound bed contamination and required penetration of active antimicrobial agents (Ag^+) through the CSS to prevent graft destruction and subsequent positive surface cultures. The latter condition tested the ability of Acticoat® to protect CSS from environmental microorganisms. The data indicate that wound bed contamination was eliminated completely by treatment with TAS (0/6), whereas Psa was detected after Acticoat® treatment (1/5). However, if Psa is inoculated on the external surface of Acticoat®, no organisms were detected (0/6). These results suggest that Acticoat® in combination with CSS provided complete protection from environmental contamination but that Acticoat® is not uniformly effective against wound bed contamination. Therefore, it may be advisable to administer additional active compounds during Acticoat® treatment to reduce the numbers of microorganisms present on burn wounds to undetectable levels, as with TAS. The apparent discrepancy between in vitro and in vivo cytoxicity studies and the prospective benefits of Acticoat® on contaminated wounds emphasizes the need for complementary analyses to fully evaluate the cytoxicity and efficacy of wound treatments. This experimental design demonstrated that Acticoat® did not inhibit engraftment of human cells or wound healing by CSS and may be effective as a protective dressing to reduce graft contamination from the environment. If combined with suitable agents to control wound bed microorganisms, Acticoat® may reduce the pharmacologic complexity of topical antimicrobials to manage contamination of CSS applied to burn wounds.

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