Comparison of bacteria-retaining ability of absorbent wound dressings

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ABSTRACT

Fibrous materials in some modern absorbent wound dressings have the ability to sequester and retain bacteria; however, this ability varies according to the nature of the fibres. We studied the bacterial retention capacity of alginate and carboxymethylcellulose dressings, using an infected skin ulcer model on the backs of rats. Wound surfaces were inoculated with either *Staphylococcus aureus* or *Pseudomonas aeruginosa* at a concentration of $1{\cdot}5 \times 10^6$ colony-forming units per wound. AQUACEL® Hydrofiber®, Kaltostat® or Sorbsan® were applied to the contaminated wounds for 12 h. Each dressing was then divided into two pieces. Total viable bacterial count within the dressing was calculated using one piece, and bacterial count released from the dressing into physiological saline was determined using the other piece, enabling bacterial retention rate to be calculated. Bacterial counts in tissue were also determined. Each dressing was tested on each of 10 wounds contaminated with each bacterium. Statistical analyses were performed using one-way analysis of variance (ANOVA) for replicated measures combined with Duncan's multiple comparison test. AQUACEL® Hydrofiber® dressing was most effective in its ability to retain both *Staphylococcus aureus* and *Pseudomonas aeruginosa* ($p < 0{\cdot}05$). Bacterial counts in tissue showed no significant change with respect to pathogen or the type of dressing used. It can be concluded that the bacterial retaining ability of AQUACEL® Hydrofiber® dressing was found to be significantly higher than that of alginate dressings in an infected animal wound model.

Key words: Carboxymethylcellulose ● Chronic skin ulcer ● *Staphylococcus aureus* ● Wound dressing ● Wound preparation

INTRODUCTION

Patients suffering from chronic skin ulcers, represented by chronic leg ulcers and pressure ulcers, often require hospitalisation when infection occurs. Infection of diabetic ulcers can have serious consequences, posing challenges in terms of high morbidity and medical expenses and sometimes requiring leg amputation. Furthermore, postoperative infection of surgical wounds necessitates prolonged periods of hospitalisation (1).

Several decades have passed since the introduction of modern wound dressings for skin ulcers into the clinical setting. This treatment accelerates wound healing by keeping the wound surface in a moist environment and absorbing exudate (2). Because colonisation by bacteria accompanies almost all cases of chronic skin ulcers, use of modern wound dressings was initially associated with concern about the potential risk of infection caused by sealing the wound tightly and creating a moist environment. However,

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Key Points

- infection is a common problem in persons with chronic wounds
- moist wound dressings have become routine in the treatment of chronic wounds
- some have speculated that sealing a wound, to enable moist wound healing, may lead to an increased risk of infection
- recent studies have shown that no significant increase over traditional dressing is observed with the use of moist wound healing dressings

Key Points

- many modern dressings are designed to absorb large amounts of exudate
- bacteria are also absorbed by some dressings, for example alginates
- hydrofiber dressings have also been shown to absorb bacteria
- the purpose of this study was to determine the ability of fibrous dressings to retain bacteria

experimental studies have shown no increase in infection; indeed, they have clarified that the infection rate associated with modern wound dressings is somewhat lower than that observed with ointments and gauze (3,4). Additional factors such as thermal insulation, maintenance of circulation and activation of leucocytes, suppression of tissue necrosis and change of pH in the closed environment are all related to this lower rate of infection under moisture-retentive dressings (5).

Many of the modern wound dressings are designed to absorb large volumes of exudate and can absorb an amount of moisture of up to 15–20 times their own weight (6). It has also been clarified that bacteria on the wound surface move into the dressing as wound exudate is absorbed, and that these dressings with high levels of fluid retention function to absorb and retain bacteria, making them useful for wound bed preparation where there is severe colonisation (7). These dressings may help to reduce cross-infection by wound pathogens. However, the mechanism of this bacterial retention has not yet been clearly elucidated.

Alginate material is said to have high-level affinity for bacteria (8). Bowler *et al.* carried out *in vitro* experiments using *Pseudomonas aeruginosa* and *Staphylococcus aureus* and reported that AQUACEL® Hydrofiber® demonstrates superior bacterial retention when compared to alginate (9). They also confirmed by electron microscopy that carboxymethylcellulose (CMCH) confines bacteria inside the dressing due to the decreased gaps between fibres when the fibres absorb moisture and become swollen (10). The purpose of the current experiment was to study the ability of fibrous dressings to retain bacteria using an *in vivo* model of infected skin ulcers developed on the backs of rats.

MATERIALS AND METHODS

Materials

The following materials were used in the study: polyurethane film (3M Corporation, St Paul, MN, USA), Eakin seal®, Kaltostat®, AQUACEL® Hydrofiber® dressing (ConvaTec, Skillman, NJ, USA), Sorbsan® Flat dressing (Alcare, Tokyo, Japan), 100% woven cotton gauze (Osaki Medical Products Company, Nagoya, Japan), brain-heart infusion (BHI, Difco Laboratories, Detroit, MI, USA), pentobarbital sodium (Nembutal; Dainippon Pharmaceutical Co., Ltd, Osaka, Japan), *Pseudomonas aeruginosa* 5142rif (Serotype E, non mucoid), *Staphylococcus aureus* (clinical isolate MSSA no. 7743114), rats (Saitama Experimental Animals Supply Co., Ltd. Sugito, Saitama, Japan).

Methods

Non mucoid *P. aeruginosa* was kindly provided as a gift by Dr Ikeda, Department of Bacteriology at Teikyo University School of Medicine. Bacterial suspensions were prepared by the following method. Methicillin-susceptible *S. aureus* was a clinical isolate obtained from an infected surgical wound of an orthopaedic surgery patient. Bacteria were grown on blood agar overnight. One colony of each of these bacterial strains was incubated in BHI for 12 hours at 37°C. Cultured cells were centrifuged three times in physiological saline at 980 g and suspended in saline at a concentration of $0{\cdot}5\times10^8$ colony-forming units (CFU)/ml. After the suspension was vortexed, the colony count was determined by measuring absorbance at 600 A.

The method of creation of infected wound was described previously (11). Fifteen male Sprague–Dawley rats aged 12 weeks were used in this study. Rats were anaesthetised by intraperitoneal injection of pentobarbital (Nembutal, 30 mg/kg), the hair on the back of each animal was removed with clippers, and the skin was then sterilised with iodine. 15 mm × 15 mm full-thickness skin wounds that included the muscular membrane were prepared on the back of each rat. Four wounds were created in each rat and each wound was separated by 25 mm. Gauze that had been soaked in a 5000-fold dilution of adrenaline in physiological saline was placed on the wound surface for haemostasis. A 15 mm × 15 mm, 7·4 mg piece of fresh gauze was placed on the base of the ulcer, after which the gauze was soaked with bacterial suspension at a concentration of $1{\cdot}5\times10^6$ CFU/wound. After bacterial inoculation of the wound surface, the periphery of the wound was sealed with an Eakin® seal, and the entire area was then covered with polyurethane film to achieve a closed environment. We have previously shown that the wound in this condition produced exudate containing $1{\cdot}0\times10^8$ bacteria per wound (11). Wounds were assigned to the following six experimental groups, a *P. aeruginosa* and *S. aureus* group for

each of AQUACEL® Hydrofiber®, Kaltostat® and Sorbsan®. Each dressing was assigned to ten wounds inoculated with each bacterium. All animals were given drinking water containing acetaminophen at a concentration of 0·25 mg/ml.

The test dressings, 15 mm × 15 mm in size, were applied to the individual wounds on the second day after wound preparation, and the dressings were maintained in a closed environment. After 12 hours, the dressings were removed and the weight of each whole dressing was measured. Each dressing was then divided equally into two parts and the weight of each piece was determined. One half of each divided dressing was homogenised for 2 minutes at 37°C in 100 ml of physiological saline with 0·1% Tween 80, to enable determination of total viable bacterial count. The remaining portion of each dressing pieces was soaked in 100 ml of physiological saline with 0·1% Tween 80 at 37°C for 1 minute. After the dressings were removed from the solution, the bacterial count in the solution was measured as follows. One millilitre of the solution was sampled and diluted according to the tenfold dilution assay protocol. One millilitre of this diluted solution was transferred to an empty Petri dish (Funakoshi, Tokyo, Japan), to which 25 ml of Trypticase Soy Agar (TSA) at 50°C was added. The agar was allowed to set and then the plates were incubated for 18 hours, after which colonies were enumerated. The number of bacteria retained by the dressing was calculated by subtracting the count of bacteria leached into solution from the total bacterial count per piece of dressing obtained by homogenisation. Each dressing was changed after 12 h intervals. After three dressing changes, bacterial counts in tissue were measured. After removal of each dressing and removal of the gauze from the base of each wound, the tissue at the base of the wound was surgically removed and weighed. The tissue was placed in a stomacher bag containing 100 ml of physiological saline with 0·1% Tween 80 and washed for 2 minutes to sluice off surface bacteria, and the tissue was then homogenised for 15 seconds in a Polytron homogeniser, (Ishii Laboratory Works, Osaka, Japan) followed by 1 minute in a glass homogeniser. The tissue was then placed in a stomacher bag containing 100 ml of physiological saline with 0·1% Tween 80 and homogenised further for 2 minutes. Subsequently, 1 ml of this homogenate was sampled and diluted according to the tenfold dilution assay protocol. Two tryptone soy agar plates were inoculated with 0·1 ml of this diluted homogenate and incubated for 18 hours, after which the total viable count was determined.

The protocol for animal experimentation described herein was approved by the Animal Research Committee of Teikyo University School of Medicine.

Statistical analysis

The comparison of three dressings was performed using one-way analysis of variance (ANOVA) for replicated measures combined with Duncan's Multiple Comparison Test.

RESULTS

In both the *S. aureus* and *P. aeruginosa* test groups, the bacterial count for tissue to which AQUACEL® Hydrofiber® dressing was applied was lowest, followed in order by the counts in tissue to which Sorbsan® and Kaltostat® dressings were applied; however, these differences did not reach statistical significance (Figure 1 a,b). In each group, the average bacterial count value was high (approximately $2{\cdot}5 \times 10^8$ CFU/g tissue).

Observation of the amounts of *S. aureus* trapped in dressings revealed significant differences between groups; retention and immobilisation of *S. aureus* by AQUACEL® Hydrofiber®, Kaltostat® and Sorbsan® was 87%, 64% and 37%, respectively (Figure 2). Retention and immobilisation of *P. aeruginosa* by AQUACEL® Hydrofiber®, Kaltostat® and Sorbsan® were 81%, 59% and 29%, respectively (Figure 3).

DISCUSSION

Retention rates of both *S. aureus* and *P. aeruginosa* by AQUACEL® Hydrofiber® dressings were higher than those by alginates, agreeing with the results of the earlier *in vitro* load testing by Bowler *et al.* (9). However, while Bowler *et al*. reported that the rate of bacterial retention by alginates differed between *S. aureus* and *P. aeruginosa*, data from the current study showed very similar retention rates between the two pathogens. While protein and other blood cell components might contribute towards the difference between our results and those of Bowler *et al*., the cause remains unknown. The present study revealed significant

Key Points

- infected wound models were created as previously described in the *IWJ*
- each test dressing was assigned to ten wounds which were inoculated with bacteria
- dressings were then evaluated for their ability to absorb and retain bacteria
- statistical differences were observed between dressings when tested with *S aureus*

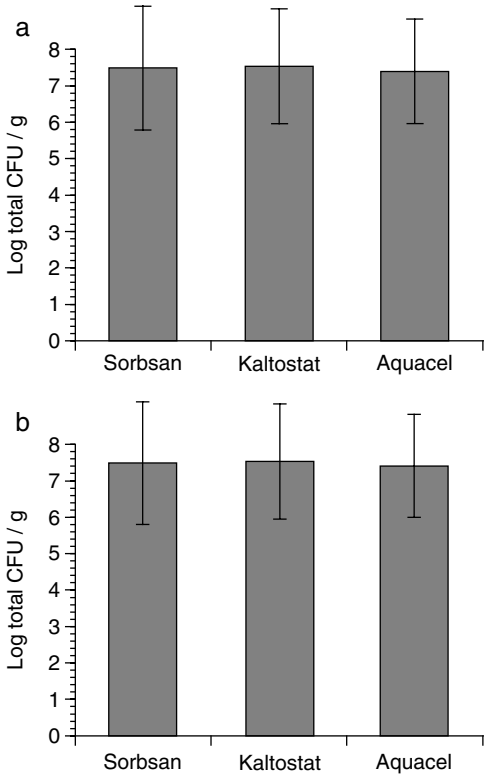

Figure 1. Effect of dressings on bacterial counts in tissue biopsies. Dressings were changed three times to new ones with 12 hours of interval. (a) *Staphylococcus aureus*; (b) *Pseudomonas aeruginosa*. Ten biopsies were taken per dressing and logarithmic transformation of the quantitative bacterial count per gram was calculated.

differences in the bacterial trapping rates of two alginate dressings; this was possibly due to differences of fibre thickness and chemical composition between individual alginate dressings.

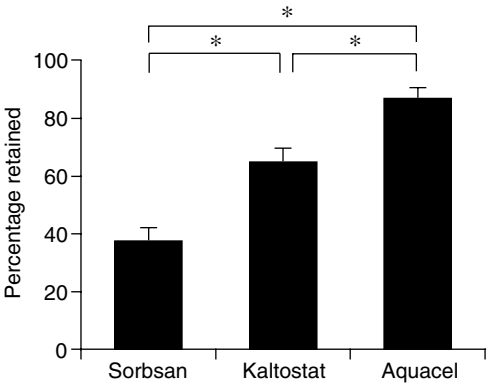

Figure 2. Mean percentage dressing retention using a *Staphylococcus aureus* challenge. Ventral bar represents the standard error of the mean (SEM). AQUACEL® Hydrofiber® dressing was most effective in its ability to retain *S. aureus* when comparing with Kaltostat® dressing and Sorbsan® dressing (*$p < 0{\cdot}05$).

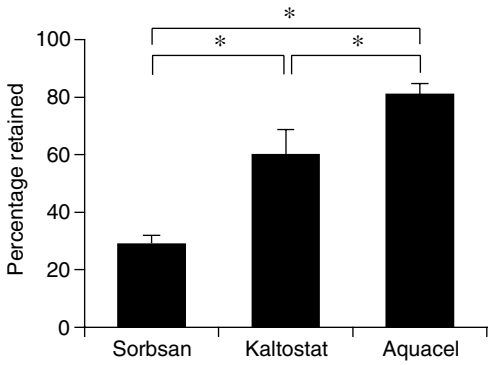

Figure 3. Mean percentage dressing retention using a *Pseudomonas aeruginosa* challenge. Ventral bar represents the standard error of the mean (SEM). AQUACEL® Hydrofiber® dressing was most effective in its ability to retain *Pseudomonas aeruginosa* when comparing with Kaltostat® dressing and Sorbsan® dressing (*$p < 0{\cdot}05$).

AQUACEL® Hydrofiber® dressing and alginates are both used widely to absorb exudate in chronic wounds, and these fibrous dressings have been reported to have the ability to retain and immobilise wound-derived microorganisms (12). Bowler reported marked variation in the ability of dressings to retain bacteria within their matrix and speculated the following mechanism to account for bacterial retention; following hydration, the interstitial space is reduced due to swelling of the fibrous component of hydrofibres, and a gel then forms that blocks further fluid flow along the fibres (9). Recently, Walker observed such gel formation by scanning electron microscopy. The AQUACEL® Hydrofiber® dressing formed a continuous coherent gel, caused by merging of fully hydrated fibres that were then indistinguishable from each other, and the bacteria appeared to be absorbed into this gel. Conversely, the alginate wound dressing did not form a coherent, single structure but instead displayed a patchwork of gelled regions with fibres still identifiable within the gel structure. Some bacterial populations, adherent to the surrounding non hydrated fibres, were visible (10).

Chronic ulcer surfaces are always colonised with mixed microorganisms; however, many factors determine the progression to infection (13,14). As bacterial burden increases, the colonised wound is transformed into a covert infection that may not involve extensive tissue invasion but is sufficient to inhibit wound healing. Cases in which colonisation proceeds to infection can be predicted in the aged and

Key Points

- retention rates for *S aureus* and *P aeruginosa* by hydrofiber dressings were higher than alginates
- bacterial retention has been shown to be a function of fibre thickness and gelling ability
- the ability of dressings to sequester and retain pathogens from a wound, a function that has been recognised in alginates and hydrofibers, should therefore decrease infection rates in chronic ulcers

in immuno-compromised patients. This concept of critical colonisation was demonstrated recently (15,16). In such cases, dressings with the ability to trap and retain bacteria and possibly prevent the formation of biofilms in wounds are clinically very advantageous and may also lead to a reduction in cross-infection by wound pathogens.

When used in the management of chronic ulcers, AQUACEL® Hydrofiber® dressings can play a compensatory role in infection control.

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