Journal of Surgical Research **176,** 701–707 (2012)
doi:10.1016/j.jss.2011.11.1040

# Effective Method to Remove Wound Bacteria: Comparison of Various Debridement Modalities in an *In Vivo* Porcine Model

Aron G. Nusbaum, B.S.,* Joel Gil, B.S.,* Marian K. Rippy, D.V.M., Ph.D.,† Brian Warne, D.P.M.,‡ Jose Valdes,* Abel Claro, B.S.,* and Stephen C. Davis, B.S.*,[1]

*Department of Dermatology and Cutaneous Surgery, University of Miami Miller School of Medicine, Miami, Florida; †Rippy Pathology Solutions, Woodbury, Minnesota; and ‡Good Samaritan Hospital, San Jose, California

Originally submitted September 16, 2011; accepted for publication November 30, 2011

***Background.*** **Debridement is one of the crucial steps for successful wound care. In addition to removing necrotic tissue, debridement has been shown to reduce wound-associated bacteria that delay healing. Using an *in vivo* porcine model, we compared the effects of various methods of debridement, including hydrosurgery and plasma-mediated bipolar radiofrequency ablation (PBRA), on bacterial removal and wound healing.**

***Methods.*** **One hundred thirty-five deep dermal wounds were inoculated with methicillin resistant *Staphylococcus aureus* (MRSA) and covered with a polyurethane dressing for 48 h to allow for biofilm formation. Wounds were then treated with either PBRA (at two settings), hydrosurgery, sharp debridement, or no debridement. Biopsies were collected for microbiology and histologic assessment on d 0, 2, 9, and 21 post-treatment.**

***Results.*** **All treatment groups showed a statistically significant reduction in MRSA counts relative to no debridement at all times points ($P < 0.05$). PBRA at a maximum setting had the lowest MRSA counts at all recovery times and, compared with all other treatment groups, a statistically significant difference was observed on d 21 ($P < 0.05$). No detrimental effects on the healing process were noted with any of the debridement methods.**

***Conclusion.*** **While sharp debridement has been established as the traditional gold standard for rapid removal of necrotic, infected tissue, our results suggest that novel debridement modalities show clinical promise for the treatment of chronic ulcers and burn wounds, especially when bacteria are present.** 

***Key Words:*** **debridement; wound healing; wound bacteria; plasma mediated bipolar radiofrequency ablation; hydrosurgery.**

## INTRODUCTION

The treatment of chronic wounds significantly impacts the health care system with $25 billion spent annually [1, 2]. It has been estimated that 2.5 million Americans are affected by venous ulcers, 1.3 to 3 million suffer from pressure ulcers, and 1 million diabetics are at risk for developing neuropathic ulcers over any 3-y period [3]. The inability of these wounds to heal, along with their associated complications such as osteomyelitis and sepsis create an ongoing challenge for clinicians. Incidence of wound closure in chronic wounds is low, with estimates ranging from 25%–50% in venous and diabetic ulcers after 20 wk of treatment [3].

One of the most important concepts in current practice is that of wound bed preparation, which encompasses debridement, management of exudate, and control of bacterial burden [4]. This concept is based on restoring the altered chronic wound environment to that of an acute wound that progresses naturally towards complete healing. Failure of chronic wounds to re-epithelialize has been associated with multiple factors such as epidermal hyperproliferation at the nonhealing wound edge, high proinflammatory cytokine levels, elevated matrix metalloprotease levels, low growth factor activities, and senescent wound cells [5]. Devitalized tissue has been shown to not only delay the healing process but also to provide a fertile environment for bacterial proliferation [4, 6, 7]. It has been suggested that colonization greater than

[1] To whom correspondence and reprint requests should be addressed at Department of Dermatology and Cutaneous Surgery, University of Miami Miller School of Medicine, 1600 N.W. 10th Avenue, Room 2089, Miami, FL 33136. E-mail: sdavis@med.miami.edu.



0022-4804/$36.00

$10^5$ CFU/g of tissue impairs wound healing [8], even without signs of overt infection [9]. Elements which may contribute to this microbial effect on chronic wounds include virulence factors [9], polymicrobial synergy [8], and biofilm formation [9–11]. Biofilms are diverse communities of microorganisms embedded in a self-produced matrix of extracellular polymeric substance (EPS), which are firmly attached to a biologic or nonbiologic surface [10, 11]. They are found in approximately 60% to 80% of chronic wounds [12] and present a unique challenge to clinicians due to their persistent nature [13, 14], elusiveness to standard culture techniques [12, 13], and resistance to conventional antimicrobial therapy [11, 15, 16 ]. We have previously demonstrated the presence of biofilms in porcine wounds and found that once formed, the bacteria are much more difficult to eradicate [10]. Debridement is a critical step in the management of chronic wounds. By removing molecular [2], physical [7], and microbiologic [3] barriers to healing, debridement not only facilitates endogenous healing but also renders the wound amenable to a variety of novel therapeutic modalities.

While various debridement methods are available, sharp debridement (SD) has been considered the gold standard for rapid removal of necrotic, infected tissue [7]. Recently, hydrosurgery has been introduced as a safe and effective method to selectively remove necrotic tissue while accurately preserving viable structures [17–20] These features, along with its reported speed [21], ease of operation [18], and ability to reduce bacterial load [20], have led to its increasing use in the clinical setting for debridement of a wide range of wound types [17, 22]. The hydrosurgery system (HS) projects a high velocity water jet across the operating window that is subsequently collected into a vacuum container. Fluid projected parallel to the wound surface creates a venturi effect, which suctions targeted tissue into the path of the cutting stream [23] while concomitantly aspirating debris [17].

The ongoing search for novel debridement methods has led to the study of plasma-mediated bipolar radiofrequency ablation (PBRA), which uses radiofrequency energy to excite electrolytes in a saline medium, creating a focused plasma capable of ablating or coagulating tissue depending on the voltage applied [24]. PBRA has been shown to effectively remove surface debris without causing thermal injury to surrounding tissues [25, 26] and an earlier pilot study performed by the authors showed the capability of PBRA to debride wounds and reduce bacterial load [27].

The present study was conducted to determine the effects of PBRA, the HS, and SD on bacterial counts and wound healing in an *in vivo* deep dermal porcine wound model inoculated with methicillin resistant *Staphylococcus aureus* (MRSA).

## MATERIALS AND METHODS

The experimental animal protocols used for this study were approved by the University of Miami Institutional Animal Care and Use Committee, and all the procedures followed the federal guidelines for the care and use of laboratory animals (U.S. Department of Health and Human Services, U.S. Department of Agriculture). The studies were conducted in compliance with the University of Miami's Department of Dermatology and Cutaneous Surgery Standard Operating Procedure (SOPs). Animals were monitored daily for any observable signs of pain or discomfort. To help minimize possible discomfort, an analgesic buprenorphine 0.03 mg/kg (Buprenex injectable; Reckitt Benckiser, Hull, England) was given intramuscularly to each animal on the first day, and every third day thereafter, in addition to the use of a fentanyl transdermal system: 1 $\mu$g/h/Kg (Duragesic; Alza Corp, Mountain View, CA) during the entire experiment.

### Experimental Animals

A porcine model was used for our experimental research due to the morphologic similarities between swine skin and human skin. Wound healing data from porcine models have also been shown to correlate more closely to humans than rodents [28, 29]. Nine (9) young female specific pathogen free pigs weighing 35–40 kg were kept in house for at least 1 wk prior to initiating the experiment. The animals were fed a basal diet *ad libitum* and housed individually in our animal facilities (American Association for Accreditation of Laboratory Animal Care accredited) with controlled temperature (19–21°C) and lighting (12h/12h light/dark cycles).

### Animal Preparation

The animals were anesthetized, and the hair on the back was clipped with standard animal clippers. Skin on the back and sides surrounding and including the wound area of the animals was prepared by washing with a nonantibiotic soap and sterile water. The animals were blotted dry with sterile gauze. Each animal was anesthetized intramuscularly with tiletamine HCl plus zolazepam (5.0 mg/kg) (Telazol; Laderle Parenterals Inc., Carolina, Puerto Rico), xylazine (0.2 mg/kg) (X-jet; Phoenix Scientific Inc., St. Joseph, MO), and atropine (0.04 mg/kg) (Atrojet SA; Phoenix Scientific Inc.) followed by mask inhalation of an isoflurane (Isothesia; Abbott Laboratories, Chicago, IL) and oxygen combination.

### Experimental Design-MRSA Infected Deep Dermal Wound Model

Fifteen (15) deep reticular dermal wounds (22 mm × 22 mm × 3 mm deep) were made in the paravertebral and thoracic area of each of nine (9) animals with a specialized electrokeratome fitted with a 22 mm blade. The wounds were separated from one another by 15 mm of unwounded skin. All 135 wounds were inoculated with a pathogenic strain of methicillin resistant *Staphylococcus aureus* (MRSA USA300) as described under "Wound Inoculation", and covered with a polyurethane film dressing for 48 h to allow biofilm formation [10] prior to treatment.

### Wound Inoculation

A bacterial inoculum suspension was made by swabbing a 3 cm diameter area of the overnight growth from a culture plate into 4.5 mL of sterile water. This resulted in a suspension consisting of approximately $10^{10}$ colony forming units/mL (CFU/mL). Serial dilutions were made until a concentration of $10^6$ CFU/mL was achieved. A sample of this suspension was plated onto culture media to quantify the exact concentration of viable organisms before the experiment. The inoculum suspension was used directly to inoculate each wound by pipeting a 25 $\mu$L aliquot into a sterile glass cylinder (22 mm diameter) in