Animal models of external traumatic wound infections

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Background: Despite advances in traumatic wound care and management, infections remain a leading cause of mortality, morbidity and economic disruption in millions of wound patients around the world. Animal models have become standard tools for studying a wide array of external traumatic wound infections and testing new antimicrobial strategies.

Results: Animal models of external traumatic wound infections reported by different investigators vary in animal species used, microorganism strains, the number of microorganisms applied, the size of the wounds and for burn infections, the length of time the heated object or liquid is in contact with the skin.

Methods: This review covers experimental infections in animal models of surgical wounds, skin abrasions, burns, lacerations, excisional wounds and open fractures.

Conclusions: As antibiotic resistance continues to increase, more new antimicrobial approaches are urgently needed. These should be tested using standard protocols for infections in external traumatic wounds in animal models.

Introduction

Despite advances in trauma care and management, infections remain a leading cause of mortality, morbidity and economic disruption in millions of wound patients around the world. Challenges remain in countering microorganisms even where antimicrobials are available. Much remains to be learned about basic aspects of the host-pathogen relationship and the complexity of the immune response to infection. In order to develop new therapies, we need to have a detailed understanding of the events that are triggered in a host after infection.

Animal models have become standard tools for the study of a wide array of external traumatic wound infections. However, a comprehensive review of the animal models of external traumatic wound infections has never been conducted. In this review, we will discuss the animal models of infected external traumatic wounds including surgical wounds, skin abrasions, burns, lacerations, excisional wounds and open fractures.

Animal Models of Surgical Site Infections

Surgical site infections (SSIs) are considered to be “low-inoculum” infections. These infections develop in apparently sterile operative environments when low numbers of contaminating microorganisms interact with devitalized tissues and foreign material (e.g., hematomas, sutures, prosthetic devices) in such a way as to remain viable in the face of antimicrobials and host defenses. SSIs account for 22% of healthcare-acquired infections (HAIs) and are a leading cause of patient mortality and morbidity as well as increased health cost. The ideal animal model of a SSI would include the following features: low inocula of pathogenic microorganisms that predictably induce infection, the infecting process is amenable to modification by use of prophylactic antimicrobials, and the end point meets the clinical definition of infection (i.e., the presence of purulent material yielding viable bacteria).

Superficial incision—foreign body model. McRipley and Whitney developed an SSI model by creating incisions on the backs of mice followed by the inoculation of microorganisms to the incisions. Female CF-1 mice weighing 18–20 g were used. On the day of infection superficial surgical wounds were produced on the shaved backs of anesthetized mice by making a longitudinal midline incision, 2.3 ± 0.2 cm in length and extending down to the panniculus carnosus. The skin on either side of the incision was retracted, and the wound was infected by the insertion of a contaminated segment of cotton thread (suture) through the skin with a suturing needle in such a way that the suture lay diagonally across the panniculus, with the ends extending slightly from the skin. The wound eventually covered approximately 6% of the total body surface area (TBSA) of a mouse. Results indicated that 10 Staphylococcus aureus cells/suture were insufficient to induce an infection, whereas an inoculum of 10^3 or 10^5 cells could elicit an infection. When the wound had been infected with S. aureus or Pseudomonas aeruginosa, clinical signs of infection were present but were not striking. Suppuration was noted occasionally beneath the retracted skin. No deaths occurred in mice infected with S. aureus and infection persisted for 1 to 2 weeks; only a few deaths were recorded in mice infected with P. aeruginosa.
*P. aeruginosa* was isolated from all internal organs cultured of mice that died, indicating generalized infection.

The model was used to study the efficacy of antimicrobials, virulence of bacteria in SSI and real-time monitoring of bacterial infections in vivo using bioluminescent bacteria.

Esperen et al. reported a mouse model of SSI using a catheter segment as the foreign body. Ten- to 12-week-old CF-1 female mice weighing approximately 25 g were operated under ether anesthesia. A 1 cm incision was made in the lateral abdominal wall by an aseptic technique. A catheter segment was placed in the peritoneal cavity, and the wound was closed with one suture. The sutures were removed from all mice before 18 h after the operation. The model was investigated with a range of different inocula from 2.1 x 10^3 to 2.5 x 10^7 colony-forming units (CFU).

Approximately 1 h after operation, experimental infection was produced by intraperitoneal (I.P.) challenge in the lateral abdominal wall opposite to the operation wound with 1 mL of staphylococci suspension. It was reported that a challenge dose of greater than 1 x 10^7 CFU gave nearly 100% infection rates, while an inoculum of greater than 5 x 10^6 CFU resulted in mortality. The infection persisted as an intra-abdominal abscess surrounding the catheter for at least 30 d.

Another foreign-body mouse model (using male albino Webster-derived CD-1 mice) of SSI was introduced by Actor and Grappel by surgical implantation of a 6.35 mm filter paper disc saturated with 10% sterile carrageenan solution. The skin of the ventral abdomen was incised in the midline with the treated discs being implanted in the underlying subcutaneous tissue. *Bacteroides fragilis* and *Escherichia coli* were used to establish wound infections. The implantation of a carrageenan-saturated disc along with an appropriate inoculum of microorganism resulted in uniform infections that could be quantified. In some cases the organisms spread from the local site, and the infections progressed to lethality, especially in neutropenic mice treated with cyclophosphamide (CY).

**Foreign body subcutaneous injection model.** Ford et al. reported the use of microcarriers as abscess promoters in the formation of *S. aureus* subcutaneous abscesses in mice. *S. aureus* mixed with dextran or gelatin microcarrier beads injected subcutaneously into CF-1 mice (20 g, male or female) resulted in the formation of reproducible, sustained abscesses with as few as 2 x 10^7 CFU. Without microcarrier beads, 4 x 10^7 CFU were required to produce an abscess. The abscesses that developed with microcarriers attained a diameter of up to 1.5 cm and persisted for several days before discharging through the skin. A similar mouse model was also reported by Bunce et al.

Kaiser et al. developed a model of SSI by inoculating suspensions of *S. aureus* and dextran microbeads into intramuscular sites on the dorsum of Hartley guinea pigs. Hartley guinea pigs of either sex weighing 450–550 g were used. Three strains of *S. aureus* were evaluated with the inoculum ranging from 1 to 160 CFU. To minimize the overgrowth of pathogenic microorganisms within the gastrointestinal tract of the guinea pigs, gentamicin and polymyxin B were added to the drinking water of the guinea pigs 24 h before the day of bacterial inoculation. It was observed that the infection rate rose with increasing inoculum size. As inocula rose from 0.625 to 160 organisms, 0–100% of lesions yielded staphylococcal growth on subculture. Inspection and biopsy of the lesions 3–4 d after intramuscular inoculation of viable bacteria typically revealed an indurated, well-circumscribed mass of microbeads and inflammatory tissue -1 cm in diameter. These lesions usually adhered tightly to adjacent tissues and could not be removed without dissection.

**Pocket wound model.** Yarboro et al. reported a rat model of SSI to mimic the situation encountered in orthopedic surgery. Female Sprague-Dawley retired breeder rats with weights ranging from 200 to 400 g were used. After isoflurane anesthesia, the lateral aspect of the right thigh was then shaved with electric clippers. The skin was opened with a 10 mm longitudinal incision in the lateral aspect of the thigh with use of scissors. A 2 x 2 cm pocket was formed, with blunt dissection, in the quadriceps muscle down to the femur. A 32-gauge stainless-steel suture was placed around the femur inside the wound to act as a surgical implant or foreign body to promote infection. Approximately 8.0 x 10^6 CFUs of gentamicin-sensitive *S. aureus* was pipetted into the pocket. High mortality was observed in the animals with six of nine rats dying. The model was used to evaluate the effect of prophylaxis against SSI with local or systemic antibiotics.

Using a similar procedure, Bergamini et al. introduced a SSI model in male guinea pigs weighing approximately 750 g. Three incisions, 2 cm in length, were made on each side of the midline within the shaved area of each animal’s back. The incisions were made down to but not through the deep fascia and extended laterally to create pouches approximately 2 x 2 cm square. No foreign body was applied in this model. The wounds were inoculated with 1.5 x 10^6 or 1 x 10^7 CFU of *S. aureus*. The model was used to investigate the efficacy of combined topical and systemic antibiotic prophylaxis in experimental wound infection.

**Deep incision—foreign body model.** A deep SSI model using rats (male Sprague-Dawley, 450 to 550 g) was documented by Fallon et al. Skin preparation consisted of clipping the hair from the back followed by skin cleansing. A standard incision measuring 4 cm in length and 5 mm lateral and parallel to the vertebral column was carried through the skin. The incision was then continued to a depth of approximately 1 cm into the underlying paraspinal muscles. Sterile sand (100 mg) was introduced into each wound as an infection-potentiating foreign body and the wounds were inoculated with 100 µL of a methicillin-resistant *S. aureus* (MRSA) suspension containing approximately 5.0 x 10^7 CFU. The skin incisions were closed with surgical staples. Clinical evidence of wound infection, defined as the presence of an abscess or frank pus within the wound, was observed in 8 of 13 (62%) animals. The authors used this model to evaluate and compare the efficacy of topical versus systemic therapy with cefazolin for the treatment of *S. aureus* infections.

In a guinea pig model developed by Moesgaard et al. a 2 cm skin incision was made 1 cm to the right of the midline in the shoulder region, extending down to, but not through, the deep fascia. Two single sutures of 4/0 atraumatic dexon (polyglycolic acid) were placed in the muscular fascia. Contamination with *E. coli* plus *B. fragilis* was carried out with 0.1 mL of each of the bacterial suspensions spread evenly over the sides and the base...
Table 1. Representative animal models of surgical site infections (SSIs)

| Animal species          | Microorganism and inoculum | Foreign body | Methods used to produce wounds and infections | End point | Refs |
|-------------------------|----------------------------|--------------|-----------------------------------------------|-----------|-----|
| Female CF-1 mouse       | S. aureus; P. aeruginosa 10^3 or 10^5 CFU | Segment of cotton thread (Suture) | A longitudinal midline incision, 2.3 ± 0.2 cm in length and extending down to the panniculus carnosus was made on the back. Each wound was infected by the insertion of a contaminated suture through the skin. | No deaths occurred in mice infected with S. aureus, infection persisted for 1 to 2 weeks; a few deaths were recorded in mice infected with P. aeruginosa. | 10 |
| Female CF-1 mouse       | S. aureus >1 x 10^6 CFU | Catheter segment | A 1 cm incision was made in the lateral abdominal wall. Infection was produced by I.P. challenge in the lateral abdominal wall opposite to the operation wound with S. aureus suspension. | A challenge dose >1 x 10^7 CFU gave nearly 100% infection rates, while an inoculum >5 x 10^7 CFU resulted in mortality. The infection persisted as an intra-abdominal abscess surrounding the catheter for at least 30 days. | 15 |
| Male albino Webster-derived CD-1 mouse | B. fragilis (10^5–10^8 CFU); E. coli (10^5–10^8 CFU) | A 6.35 mm filter paper disc | Mice were made neutropenic by the I.P. challenge of cyclophosphamide. The skin of the ventral abdomen was incised in the midline. The infection was induced by the implantation of a carrageenan-saturated disc along with microorganisms in the underlying subcutaneous tissue. | In some cases the organisms spread from the local site, and the infections progressed to lethality, especially in neutropenic mice treated with cyclophosphamide. | 16 |
| Male or female CF-1 mouse | S. aureus 2 x 10^7 CFU | Dextran or gelatin beads | S. aureus mixed with dextran or gelatin beads were injected subcutaneously into mice. | The abscesses that developed with microcarriers attained a diameter of up to 1.5 cm and persisted for several days. | 17 |
| Guinea pig              | S. aureus 160 CFU | Dextran microbeads | Inoculating suspensions of S. aureus and dextran microbeads into intramuscular sites on the dorsum of guinea pigs. | As inocula rose from 0.625 to 160 organisms, 0–100% of lesions yielded staphylococcal growth on subculture. | 9 |
| Female Sprague-Dawley rat | S. aureus 8 x 10^7 CFU | 32 gauge stainless steel (suture) | A 1 cm longitudinal incision was made in the lateral aspect of the thigh. A 2 x 2 cm pocket was formed in the quadriceps muscle down to the femur. S. aureus was pipetted into the pocket. | High mortality was observed in the animals with six of nine rats dying. | 19 |
| Female Sprague-Dawley rat | S. aureus 5 x 10^7 CFU | Sterile sand (100 mg) | A standard incision measuring 4 cm in length and 5 mm lateral and parallel to the vertebral column was carried through the skin. The incision was then continued to a depth of approximately 1 cm into the underlying paraspinous muscles. Sterile sand was introduced into each wound and the wound was inoculated with 100 µL of MRSA suspension. | Clinical evidence of wound infection was observed in 8 of 13 (62%) animals. | 23 |

of the wound with micro-syringes. Wound infection, defined as accumulation of pus draining spontaneously or after opening of the wound, developed in 19 out of 22 guinea pigs (86%) after intra-incisional contamination with 10^7 E. coli plus 10^8 B. fragilis before wound closure. A similar guinea pig model (with a 4 cm vertical incision injected with a 0.4 mL inoculum of S. aureus at a concentration of 10^8, 10^6 or 10^5 CFU/mL) was also described by Stratford et al.25 to investigate the effect of lidocaine and epinephrine on S. aureus infection.

Summary. There are numerous studies on the use of animal models of SSIs. Species of animals used include mouse, rat and guinea pig. The approaches to inflict surgical wounds and induce infections include incisional wounds with foreign bodies, subcutaneous injection of foreign bodies and microorganisms into pocket wounds with or without foreign bodies. Foreign bodies used include sutures, paper discs, dextran microbeads, sand and catheter segments. Depth of wound ranges from superficial sites down to the panniculus carnosus (in mice) to deep sites (1–2 cm) into the muscles (in rats or guinea pigs). Surgical wounds were made on the back, abdominal, thigh or, less frequently, the shoulder region of the animals. S. aureus is the most commonly studied bacterial species in the animal models. Table 1 shows a summary of representative animal models of SSIs.

In accordance with the depth of the infection, SSIs are clinically classified into superficial incisional SSI, deep incisional SSI and organ/space SSI26 (Fig. 1). Accordingly, animal models of SSIs can also be classified into the models to mimic superficial incisional SSI,10,15–17 deep incisional SSI,9,23–25 and organ/space SSI,19,22 respectively. For superficial SSI, mouse is the commonly used species. For deep incisional SSI and organ/space SSI, larger animals (e.g., rats and guinea pigs) are usually used because the size of mice is insufficient for mimicking these lesions. According
Skin abrasions can occur on any part of the body but usually affect bony areas, such as the hands, forearms, elbows, knees or shins. Most skin abrasions are shallow and do not extend far into the skin. An abrasion is often contaminated with dirt, gravel and other foreign material. Infection is a risk, especially with crushed or dirty abrasions. Due to the feature of skin abrasion wound, skin abrasion infections are usually superficial infections localized in the epidermis and upper layer of dermis.

**Needle scratch model.** Dai et al.\(^27\) developed a mouse model of skin abrasion infected with genetically engineered bioluminescent MRSA. Female BALB/c mice, 6–8-week-old and weighing 17–21 g, were used. At days 4 and 1 before the infection, mice were administered two doses of CY. The first dose, 150 mg CY per kg mouse body weight (150 mg/kg) was injected I.P. followed by the second dose of 100 mg/kg. This treatment reduced peripheral blood neutrophils to <100/mL blood, fostering a more vulnerable environment in the mice to infection. Skin abrasion wounds were made on the dorsal surfaces of mice using 28-gauge needles by creating 6 x 6 crossed scratch lines within a defined 1 x 1 cm\(^2\) area. The scratches were made in such a manner that they only damaged the stratum corneum and upper-layer of the epidermis but not the dermis. Five minutes after wounding, an aliquot of 50 µL suspension containing 10\(^{8}\) CFU of bioluminescent MRSA was inoculated over each defined area containing the crossed scratches with a pipette tip. A stable infection was developed in the mouse wounds as characterized by bioluminescence imaging. In the non-treated wounds, the scratches were expanded during the following few days (Fig. 2A) and infections were spread over the area surrounding the scratches as indicated by bioluminescence imaging. Gram staining of the histological section of a representative skin abrasion specimen (harvested at day 3 post-infection) (Fig. 2B) showed that MRSA was localized superficially in the epidermis. The model was used to demonstrate the effectiveness of photodynamic therapy; the topical application of photosensitizers targeted to bacteria followed by illumination.

A similar model was presented by Zolfaghari et al.\(^28\) In this model using female C57 black mice of 8-week-old, 25 mm\(^2\) square-shaped wounds were created in the skin of the back by scarification using a 27-gauge needle, run ten times parallel in one direction and another ten times perpendicular to the original tracks. Ten microliters of the MRSA suspension was placed on each wound (4 x 10\(^7\) CFU), and incubated for 1 h prior to treatment. This method also resulted in a reproducible MRSA wound colonization model, which persisted for up to 5 d post-inoculation.

**Blade scrape model.** Kraft et al.\(^29\) reported a mouse model of superficial cutaneous bacterial infections resulting after a minor wounding process. Male CF-1 mice (24 to 28 g) were treated with 0 to 150 mg of CY per kg 4 days before infection. Mice were anesthetized before superficial wounding. One drop (0.02 mL) of the prepared culture suspension containing 0 to 10\(^6\) CFU S. aureus or S. epidermidis was applied to the shaved skin, which was scraped with a no. 24 scalpel blade until a reddened area appeared (just short of drawing blood). The wound site (~10 mm\(^2\)) was occluded with a 15 mm\(^2\) piece of sterile plastic film secured with a 25 mm-wide girdle of surgical tape. At specified

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**Figure 1.** Surgical site infections (SSIs) are classified as superficial incisional SSI, deep incisional SSI and organ/space SSIs.\(^29\)

**Figure 2.** (A) Wound morphology at day 4 post-infection of a representative mouse wound. (B) A Gram-stained section of a mouse skin abrasion specimen showing the biofilms formed by Gram-positive MRSA near the skin surface. Dark blue area: biofilms of MRSA. The mouse skin abrasion specimen was harvested at day 3 post-infection.
intervals after challenge, occlusive dressings were removed and wound sites were examined for white-gray abscesses. At a CY dose of 50 mg/kg and an inoculum of 10^7 CFU, 89% of the mice (96 of 108) developed large abscesses (15 mm diameter). The model was used to by the authors to compare cutaneous infection in normal and immunocompromised mice,29 and by Hahn et al.30 to investigate the effects of superficial exudates of neutrophils on prevention of Bacillus anthracis infections.

**Tape stripping model.** Kugelberg et al.31 presented an animal model of superficial skin infections using a tape stripping method. Six- to 8-week-old female BALB/c mice were used for all experiments. The mice were anesthetized and the fur was stripped from the mice with an elastic adhesive bandage. An area stripped from the mice with an elastic adhesive bandage. An area

**Mal model of superficial skin infections using a tape stripping Bacillus anthracis** to evaluate a topical antibiotic for model was used with slight modifications by other investigators establishment of a staphylococcal infection in this model. The fine-grade, sterile sandpaper until the skin was glistening but not was removed. The exposed skin was then gently abraded with paper. A 2 x 2 cm area of abdominal skin was shaved until all fur was removed. The exposed skin was then gently abraded with fine-grade, sterile sandpaper until the skin was glistening but not bleeding. Next, 10^6 S. aureus cells concentrated from an over-night bacterial culture in stationary phase. The number of CFU recoverable from the wound 4 h after application of 10^7 CFU of S. aureus was 7.03 ± 0.37 log10. This is evidence of the successful establishment of a staphylococcal infection in this model. The model was used with slight modifications by other investigators to evaluate a topical antibiotic for S. aureus infections32 and elucidate the mechanisms by which the organisms are cleared from the skin.33

**Sand paper model.** Gaspari et al.34 developed a skin abrasion model by abrading the skin in Transgenic (Tg) mice with sand paper. A 2 x 2 cm area of abdominal skin was shaved until all fur was removed. The exposed skin was then gently abraded with fine-grade, sterile sandpaper until the skin was glistening but not bleeding. Next, 10^6 Candida albicans blast conidia were pipetted onto the surface of the skin in a 20 μL volume of sterile PBS. The area was occluded with a 0.5 x 0.5 cm piece of sterile gauze that was moistened with sterile PBS. This layer was further occluded with a 1 x 1 cm piece of plastic sheeting. Histological analysis showed that the infection remained localized to the stratum corneum of the epidermis, rarely invaded the viable epidermis and never invaded the dermis. The infection persisted over 5 to 7 d in the mice. The model was used by the authors to determine whether enhanced cutaneous delayed type hypersensitivity (DTH) is seen in these Tg mice in response to cutaneous fungal infections.

**Dermatome model.** A very early dermatome skin abrasion model was reported by Munster et al.35 Partial thickness skin corresponding to 20% TBSA was removed from the anesthetized Sprague Dawley rats (180–200 g) by an electro-dermatome, with an aperture of 20/1,000 inches. Rats were inoculated on the wound surface with 10^8 CFU of P. aeruginosa. All animals died within four days, with histological evidence of invasion and widespread visceral seeding. The model was reported to be reproducible, measurable and controllable. In addition, it was simple and rapid.

In another study, a superficial abrasion model in rabbits was created by Jeray et al.36 also using an electric dermatome. New Zealand White rabbits (either females or males) of approximately the same age and size (2.8 to 3.9 kg) were used. The hair on the dorsum of the anesthetized rabbits was removed using clippers. A degrading agent was used to remove the skin oils. Two 2 x 2 cm sites were marked with a skin marker, one caudal and one cranial. One site served as a control, with the skin intact, while the other served as the abrasion site. An electric dermatome set to a thickness of 0.0015 in created a superficial abrasion (roughly 2 x 2 cm) on the otherwise unprepared sites. Both sites on each rabbit were inoculated with 50 μL of encapsulated S. aureus. Results showed that the amount of bacteria on the normal skin (control sites) dropped significantly (p < 0.02) for all time periods (6, 12, 24, 48 h post-infection) except six hours (p < 0.20). On the abraded skin sites the bacteria flourished for all time periods.

**Summary.** Based on the approaches the wounds were produced, the types of animal models of skin abrasion infection can be classified into needle scratch model, blade scarp model, tap stripping model, sand paper model and dermatome model. The needle scratch model is more clinically relevant to shallow skin cuts. Animal species used include mouse, rat and rabbit. Skin abrasions are made either on the dorsal or abdominal surface of the animals. S. aureus is the most common bacterial species investigated, while P. aeruginosa and C. albicans have also been studied. Table 2 summarizes the representative animal models of skin abrasion wound infections.

**Lethal infection models could be developed by using P. aeruginosa,35 while the use of S. aureus usually induces chronic infections in animals.27-29,36 In comparison to the needle/blade/tape approaches, the use of dermatome can result in the wounds with better-controlled, more uniform depths.35,36 To confirm clinically relevant infections are induced in the animals, microscopic observations27,31 are recommended to monitor the infections (e.g., the depth of the injury and the depth of the infected area).**

**Animal Models of Burn Infections**

In previous times serious burns occupying a large percentage of body surface area were an almost certain death sentence because of subsequent infection. A number of factors such as disruption of the skin barrier, ready availability of bacterial nutrients in the burn milieu, destruction of the vascular supply to the burned skin, lack of re-epithelialization of basal epidermal tissue and systemic disturbances leading to immune-suppression combined together to make burns particularly susceptible to infection.1,2

**Boiling water burn model.** An early animal model of burn wound can be traced back to over 40 years ago in 1968. Mason and Walker17 developed a burn model of rat using boiling water to inflict the thermal injury. It was the first animal model burn for research and was used as a standard scald burn model by the US Army Surgical Research Center in San Antonio, TX for various
studies. The template device was made of a thin metal half-cylinder with an aperture of calculated size cut from the central portion of the half-cylinder. The dimensions of the aperture were selected to provide the size of burn desired in animal of known weight (the upper limit of burn size permitting by this approach was approximately 30% of TBSA). The anesthetized animal was shaved on the back and then placed supine in the burning device. The exposed area was immersed in boiling water. It was reported that 10 sec exposure produced a full-thickness burn and 3 sec a partial-thickness burn. The burns per se did not interfere greatly with mobility and the animals ate and drank easily. This model was widely used in the studies on burn infections, including the pathogenesis of candidiasis after thermal injury,38 bacterial translocation in burn infections,39 evaluation of antibiotics40-43 or antimicrobial peptides44,45 for eliminating bacteria in burns, gene therapy of wound infections,46,47 wound dressing for preventing and treating burn infections,48-50 the use of electrolyzed oxidized water functions as a bactericide,51 the ability of insulin to treat infections,52 relationship between prostaglandin synthesis and prostaglandin degradation,53 cardiac contractile responses of burn injury complicated by sepsis,54-56 whether sepsis exacerbates the myocardial inflammatory responses,57 etc.

Table 2. Representative animal models of skin abrasion wound infection

| Animal species | Microorganism & inoculum | Methods used to produce wound and infections | Wound area | End point | Refs |
|----------------|--------------------------|---------------------------------------------|------------|-----------|------|
| Female BALB/c mouse | S. aureus | Mice were pre-treated with cyclophosphamide. Skin abrasion wounds were made on the dorsal surfaces of mice using needles by creating 6 x 6 crossed scratch lines within a defined 1 x 1 cm area. Bacterial suspension was inoculated over the crossed scratches with a pipette tip. | 1 x 1 cm | A stable infection, persisted for over 10 days, was developed in the mouse wounds as characterized by bioluminescence imaging. | 27 |
| Female C57 Black mouse | S. aureus | Twenty-five square millimeter square-shaped wounds were created in the skin of the back by scarification using a needle, run ten times parallel in one direction and another ten times perpendicular to the original tracks. Bacterial suspension was topically placed on each wound. | 25 mm² | This method resulted in a reproducible MRSA wound colonization model, which persisted for up to 5 days post inoculation. | 28 |
| Male CF-1 mouse | S. aureus, S. epidermidis, S. saprophyticus, Micrococcus luteus. | Mice were pre-treated with cyclophosphamide. One drop (0.02 mL) of the prepared culture suspension of S. aureus or S. epidermidis was applied to the shaved skin, which was scraped with a scalpel. | 10 mm² | 89% of the mice (96 of 108) developed large abscesses (15 mm diameter). | 29 |
| Female BALB/c mouse | S. aureus | An area of ca. 2 cm² on the mouse back was tape stripped. Bacterial infection was initiated by topically placing on the skin a 5 µL S. aureus suspension. | 2 cm² | The number of CFU recoverable from the wound 4 hours after bacterial application was 7.03 ± 0.37 log₈. | 31 |
| Male or female New Zealand white rabbit | S. aureus | An electric dermatome set to a thickness of 0.0015 inches created a superficial abrasion (roughly 2.0 x 2.0 cm) on the shaved dorsum. Wounds were inoculated with 50 µL of encapsulated S. aureus suspension. | 2 x 2 cm | On the abraded skin sites the bacteria flourished for all time periods at 6, 12, 24 and 48 h post-infection. | 36 |

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Ethanol bath burn model. Stieritz and Holder\(^9\) reported an ethanol bath burn model in mice. In this model, female CF1 mice weighing 22–24 g were used. Mice were anesthetized and shaved on the back. An asbestos board with a window accounting for approximately 30% of TBSA was pressed firmly against the back of mice. Ethanol was evenly spread over the area outlined by the window, ignited and allowed to burn for 10 sec. The infections were initiated by an immediate subcutaneous injection of 100 CFU of \emph{P. aeruginosa} in the burned area. The infections turned out to be rapidly fatal and the animals appeared moribund 20 h after infection. The model was used to investigate the pathogenesis of \emph{P. aeruginosa}\(^{59-61}\) and \emph{Klebsiella pneumoniae}\(^{62}\) in burn infections as well as the local delivery of polyclonal immunoglobulin against burn infections.\(^{63}\)

Gas flame burn model. A mouse burn model created by using a gas flame was developed by Katakura et al.\(^{64}\) Thermal injury was produced by pressing a custom-made insulated mold (with a 2.5 x 3.5 cm window) firmly against the shaved back of each mouse and subsequently exposing the area to a gas flame for 9 sec. A burner equipped with a flame-dispersing cap was used as a gas flame source. A third degree burn, on approximately 15% of TBSA for a 26 g mouse, was consistently produced by this procedure. Thermally injured SCIDbg mice were exposed to MRSA at a dose of 2 x 10\(^5\) CFU/mouse. This model was used in the studies on pathogenesis of MRSA, \emph{Enterococcus faecalis} translocation\(^{65,66}\) in immunodeficient mice with thermal injury and the effects of a soluble polysaccharide immune-modulator on the resistance to burn infections.\(^{67}\)

Pre-heated double brass block burn model. Stevens et al.\(^{68}\) developed a mouse model of full-thickness burn by applying two pre-heated brass blocks (92–95°C) to the opposing sides of an elevated skin folder on the backs of shaved mice for 5 sec (Fig. 3). Six-week-old male CD1 mice weighing 25–30 g were used. The combined brass block area was 1.8 cm x 1.8 cm, corresponding to a 5% of TBSA. After the infliction of burns, the eschars were immediately injected intradermally with 10–10\(^6\) CFU of \emph{P. aeruginosa}. Survival at 10 days was 100% with burn injury alone and 60% with infected burns. \emph{P. aeruginosa} (consistently 10\(^8\) CFU/g tissue) were recovered from the unburned muscle by 24 hours for all the different inocula of bacteria. This mouse model was employed by Lambrechts et al.,\(^{69}\) Dai et al.,\(^{70}\) Dai et al.\(^{71}\) and Ragas et al.\(^{72}\) with slight modifications to evaluate the efficacy of antimicrobial photodynamic therapy (PDT) and chitosan acetate bandage for burn infections respectively. A similar model using rats with the same burning procedure was described by Busch et al.\(^{73}\) to study Gram-negative bacteremia and sepsis.

A deep partial thickness burn model produced by applying cylindrical aluminum templates heated to 75°C for 5 sec to the depilated moistened skin in guinea pigs was described by Kaufman et al.\(^{80}\) Cylindrical aluminum templates (diameter, 3.76 cm; height, 3.78 cm; length of the handle, 24 cm; total weight, 500 g) were heated in a water bath for 2 h prior to the injury at a constant temperature of 75°C. The anesthetized guinea-pig was restrained and stretched on a metal meshed board and the midline corresponding to the spine was marked on the animal’s back as well as the horizontal upper limits of both sacroiliac joints. The heated and moistened template was applied at right angles to the skin of the animals’ back according to the pre-marked locations. Only minimal pressure was required to ensure a perfect contact between the template surface and the underlying dorsal skin.
For the comparison of different topical drug treatments and dressing materials in a setting that closely mimics clinical reality, a porcine model of full-thickness burn was proposed by Branski et al.81 Yorkshire pigs (40–65 kg) were used in the study. Contact burns were applied paravertebrally under aseptic conditions. This was accomplished by placing a heated aluminum bar on the dorsum of the animal. The aluminum bar was heated to 200°C with a gas burner. The heated bar was placed on the animal for 30 sec. Burn sites of approximately 40 cm² each were made on the dorsum of the animal with 4 cm between each site or from the spine. The total burn size did not exceed 15% TBSA.

Manafi et al.82 introduced a mouse model by applying a 1 x 2 cm piece of hot metal to the thigh, producing a burn of up to 10% of TBSA and extending to all layers of skin but not involving the muscular tissue. After 24 h, 10⁵ CFU of toxigenic strains of P. aeruginosa were inoculated subcutaneously into the burned area. A similar mouse model was described by Kumari et al.83 using a single preheated brass bar as the heater. In this model, the burns were created on the backs of mice followed by topical inoculation of K. pneumoniae culture on the burned area.

A modified model using mice was reported by Calum et al.64 Specifically pathogen-free female 12-week-old C3H/HeN mice (20–25 g) were used. The mice were placed onto a sledge and covered with a heat-resistant blanket with a window (1.7 x 2.6 cm) corresponding to approximately 6% of TBSA. Above the fire blanket a metal plate with a window (1.7 x 2.6 cm) was placed. The sledge with mice, the fire blanket and metal plate were moved into a stream of hot air with a temperature of 330°C delivered by a hot-air blower for 7 sec. The temperature was kept constant using the same time schedule. When the procedure was disrupted, the procedure was restarted following a constant time schedule controlling the temperature. This procedure resulted in a third-degree burn and was confirmed by histological examination. The mice were injected subcutaneously with 100 μL challenge solution (infection dose 10⁷ CFU/mL) with a 27-gauge needle beneath the burn wound.

Other burn models. A model of a partial thickness burn was developed by Bahar et al.85 using male Wistar rats weighing 250–300 g. After the animal was anesthetized, a 2.5 x 2.5 cm, 5 mm thick piece of absorbent lint cloth that had been immersed in boiling water (100°C) was placed over the dorsum. The hot cloth was applied for different time intervals (5 or 12 sec). This method produced reliable superficial and deep dermal burns, as confirmed by the histological features.

Suzuki et al.86 designed a burn model of rat based on skin contact with a glass chamber through which water circulates at a predetermined temperature. Male Wistar rats weighing 450–550 g were used. The glass chamber (applicator) was set in a glass wall to permit direct contact between the chamber base and the skin surface using a total weight of the chamber and water of 10 g/cm². Observations were made on each rat for each combination of temperature (35–60°C) and exposure time (1 min–7 d), which amounted to 162 combinations of temperature-exposure time. The advantage of this model was the possibility of varying temperature and exposure time, as required by the researcher, and also of applying higher or lower contact pressure.

Summary. The animal models of burn infections can be categorized by the heat source used as boiling water, burning ethanol bath, gas flame, pre-heated double brass blocks, pre-heated single metal plate/bar, etc. The animal species used included mouse, rat and pig (guinea pig and Yorkshire pig). Burns were usually made on the shaved backs of animals. The size of the burns ranged from 5% TBSA to 30% TBSA. The time that the heated object or fluid in contact with animal skin mostly ranged from 3 to 30 sec, resulting in partial to full thickness burns. The microorganisms investigated included P. aeruginosa, S. aureus, K. pneumonia, A. baumannii and C. albicans. To induce infections, microorganisms were either topicaly applied or subcutaneously injected to the burned area. Table 3 describes representative animal models of burn wound infections.

In comparison to the use of small animals of mouse and rat, the use of guinea pig and Yorkshire pig as the models of burn infections can mimic clinical reality more closely, as the skin structures of the pigs are more similar to that of human beings than mouse and rat. However, the small animals have the advantage of cheaper cost. For the introduction of the burn infections, to apply the infecting microorganism topically is more clinically relevant than to inject the microorganism under the burn eschar.58,59,68,69,82 Apparently, the minimum inoculum of microorganism required to induce infection by use of subcutaneous injection in lower than that using topical application. Similar to the models SSI and skin abrasion infection, lethal infections can be developed in animals by use of lethal microorganisms (e.g., P. aeruginosa),64,66,71 while chronic infections can be produced using less lethal microorganisms (e.g., S. aureus).60,72

Animal Models of Lacerated Wound Infections

Lacerations are irregular wounds resulting from a blunt trauma (Fig. 4). Lacerations involve separation of skin or other tissues such as tendons, muscles, ligaments, nerves, blood vessels or bone. Laceration wounds are the third most commonly encountered problems in the emergency departments.87 Traumatic laceration is rarely a life-threatening event but improper management increases the chances of wound infection. Deep lacerations with devitalized tissue are more prone to infection. Infection of sutured lacerations may lead to impaired healing, pain, poor cosmesis and loss of function.87

Non-crushed lacerated wounds. The first animal model of lacerated wound infection was reported by Edlich et al.68 Albino guinea pigs (weighing 300–350 g) were selected because of similar susceptibility to the infections as found in humans. Skin preparation consisted of hair clipping, depilation and skin cleaning. Two 3 cm incisions were made parallel and equidistant from the vertebral column, through the subcutaneous tissue down to the fascia, utilizing a modified non-crushing intestinal clamp and a surgical blade. Ten microliters of S. aureus suspension containing 10⁷ CFU was introduced to each wound 5 min after the incision. At the end of the experiment (the authors did not report how long the experiment last), purulent exudates were observed
in over 60% of contaminated wounds and bacterial positive cultures were found in 100% of the contaminated wounds. This model was widely used to study the care and management of contaminated lacerations.  

Using the guinea pig model with slight modifications, Howell et al. 94 compared the effects of different scrubbing and irrigation techniques on eradicating bacteria in contaminated lacerations. Albino guinea pigs weighing 450–500 g were used in the study. Hair on the dorsum was trimmed but not shaved. Four dorsal sites were chosen on each animal, with one on either side of the thoracolumbar spine. Each site was shaved and the surface was prepped. A 3 cm full-thickness laceration was made at each site. Bleeding was controlled with sterile gauze and the surface was prepped. A 3 cm full-thickness laceration was made parallel to the spine and extending to deep fascia. After hemostasis with sterile gauze and pressure, the wounds were then inoculated with 0.4 mL of *S. aureus* at uniform concentrations of 1 to 4 x 10⁸ CFU/mL and allowed to sit undisturbed for 30 min. The model was used by the authors to compare the efficacies of normal saline irrigation with tap water irrigation. No non-treated/infected wounds were used in the study. The contaminated wounds were irrigated either with tap water or normal saline at 30 min after contamination. No long-term follow up was carried out.

### Crushed lacerated wounds

A guinea pig model to mimic an animal bite wound in humans was reported by Lammers et al. 97

It was reasoned that lacerated wounds in prior animal studies were made with a scalpel that left clean edges and no devitalized tissue and wounds were inoculated with a single species of microorganism, whereas bite wounds contain crushed tissues and are contaminated with multiple species. They designed an animal bite wound model in albino guinea pigs by inoculating crushed incisions with three species of bacteria. Wounds were created on the dorsum of anesthetized animals by making four paravertebral lacerations, 2 cm long and 2 cm apart, extending to deep fascia. Wound edges were clamped with hemostat along a 1.5 cm length.
of epidermal/dermal junction for 5 sec to create crushed, devitalized tissue within each wound; 0.4 mL of mixed bacterial inoculum containing *S. aureus*, *B. fragilis* and *Pasteurella multocida* at a density of 4 to 6 x 10^8 CFU/mL was instilled into each laceration. It was observed that 33% animals had either erythema, induration or purulence at 6 d after bacterial inoculation.

A crushed laceration wound model with necrotic tissue using albino rats was reported by Gross et al.98 to study the effectiveness of pulsating water jet lavage in treating contaminated crushed wounds. Albino rats (average weight 350 g) were anesthetized and the left preauricular area of each animal was shaved. A vertical incision through skin was made in the masseter muscle, 1.5 cm long and extending to lateral surface of the mandibular ramus. After the skin was reflected, an identical second incision was made in the muscle, 3 mm distant and parallel to the first one. The wounds were then inoculated with 0.03 mL of moist soil that contained four bacterial species—*S. aureus*, *Proteus mirabilis*, *P. aeruginosa* and *K. pneumoniat* (3 x 10^7 bacteria soil sample). Pliers were inserted into two parallel incisions to the depth of the wound and squeezed firmly causing necrosis of the tissue and also forcing the inoculum into the crushed tissue. No results of non-treated control wounds were reported by the authors. For wounds lavaged with tap water, at 6 d after contamination, positive bacterial cultures were found in all wounds and 13 of 20 wounds were observed purulent.

A similar crushed contaminated wound model in male Wistar albino rats was employed by Erdur et al.99 to investigate the prophylactic efficacies of mupirocin and nitrofurazone on contaminated crush injuries. Two paravertebral linear incisions (2 cm long, 4 cm apart) reaching the deep fascial layer were made. The incision extended for the distance of four vertebral bodies. To stimulate a devitalized crush wound, 1.5 cm of segments of the wound edges were crushed with hemostats for 5 sec. Aliquots of 0.2 mL of (10^6 CFU/mL) *S. aureus* or *S. pyogenes* were dripped into each wound and left to absorb without spilling from the wound edges.

**Summary.** Lacerated wound infection models have been developed in relative large animals, such as guinea pigs and rats, due to the depth of this type of wounds. The wounds are inflicted by making one or more incision of various lengths usually on the dorsum of the animal. This has been considered as a limitation of the available animal models, as most lacerations in humans occur in the highly vascular areas of the body, such as the face and scalp.57 In some models, the tissue was crushed and devitalized by clamping the wound edges with hemostats. The most frequently studied microorganism was *S. aureus*, while *S. pyogenes*, *P. aeruginosa*, *K. pneumoniat* and *P. mirabilis* have also been investigated. Table 4 shows a summary of animal model of laceration wound infections.

Non-crushed laceration is similar to deep surgical incisional wound. The principle difference between non-crushed lacerated wound infection and deep incisional SSI lies in the fact the latter usually occurs in sterilized operational environment and the inocula of the infecting microorganism is much lower.

**Animal Models of Excisional Wound Infections**

Excisional wounds can be induced by excisional surgery. Excisional wounds involve the removal of a significant volume of the target tissue and are frequently complicated by infection and prolonged healing.

**Excisional wounds in mice.** Hamblin et al.100 developed a mouse model of excisional wound infections. Male BALB/c mice weighing 20–25 g were anesthetized, shaved on the back, and then depilated. Four full-thickness excisional wounds were made in a line along the dorsal surface using surgical scissors and forceps. Wounds measured 8 x 12.5 mm and had at least 5 mm of unbroken skin between them. The bottom of the wound was panniculus carnosus, with no visible bleeding. A suspension (50 μL PBS) containing 5 x 10^6 cells of mid-log phase bioluminescent *E. coli* was inoculated into each wound. The next day, infected wounds in living mice had lost, on an average, 90% of the original luminescence signal but with considerable inter-animal variability. The model was rather artificial in that it used a clean excision, a noninvasive bacterial strain and a large inoculum (5 x 10^6 bacteria). Nevertheless, it served to establish a proof-of-principle that antimicrobial photodynamic therapy (PDT) could destroy infection in vivo.

The model was also used by other investigators with slight modifications to investigate antimicrobial and wound healing effects of chitosan acetate bandage on infected wounds.101,102 The effects of peptide on the healing of infected wounds103 and bacterial colonization and the expression of inducible nitric oxide synthase (iNOS) in murine wounds.104 Shi et al.105 made an infection model using MRSA on the skin of diabetic db/db mice. Female mutant diabetic mice (C57BL/ksj db/db) and heterozygous control mice (C57BL/ksj db/+), at 8 weeks of age were used. The dorsal hair was gently clipped. Two round, full-thickness wounds were prepared on the back of each mouse in the anterior-posterior direction using a punch biopsy instrument (3 mm diameter). Each wound was covered with a sterilized transparent dressing and inoculated with 1 x 10^6 CFU of MRSA in 30 μL of PBS by injection into the wounds covered by the sterilized transparent dressing. Hematoxylin and eosin (H&E) staining of the wounds of revealed delayed reepithelialization, inflammatory cell infiltration and edema due to MRSA infection. Gram staining of MRSA-infected wounds showed numerous Gram-positive cocci.

A similar mouse model with punch excisional wound was reported by Schierle et al.106 to investigate the effect of...
Table 4. Representative animal models of laceration wound infections

| Animal species       | Microorganism & inoculum | Methods used to produce wounds and infections                                                                 | End point                                                                 | Refs |
|----------------------|--------------------------|----------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------|------|
| Guinea pig           | *S. aureus* 10^7 CFU     | Two 3 cm incisions were made parallel and equidistant from the vertebral column, through the subcutaneous tissue down to the fascia. Bacterial inoculum was delivered to the selected wound using an ultra-micro blowout pipette. | Purulent exudates were observed in over 60% of contaminated wounds, and bacterial positive cultures were found in 100% of the contaminated wounds. | 88   |
| Guinea pig           | *S. pyogenes* (7.8 x 10^6 CFU) *S. aureus* (9 x 10^6 CFU) | Four dorsal lacerations with 3 cm length were made parallel to the spine and to deep fascia. Bacterial inoculum was placed in each laceration by using a pipette system. | Four days after infection, the bacterial counts were over 10^7 CFU/g-tissue for both bacterial species. | 94   |
| Male lab rat         | *S. aureus* 0.4 to 1.6 x 10^7 CFU | Two 3 cm full-thickness lacerations were made on each animal, with one on either side of the thoracolumbar spine. The wounds were inoculated with bacterial suspension. | No non-treated control wounds were used in the study. No long-term follow up was carried out. | 96   |
| Guinea pig           | Mixed bacterial inoculum containing 1.6 to 2.4 x 10^6 CFU of *S. aureus*, *B. fragilis* and *P. multocida*. | Wounds were created on the dorsum of animals by making four paravertebral lacerations, 2 cm long, and 2 cm apart, extending to deep fascia. Wound edges were clamped with hemostat for 5 sec to create crushed, devitalized tissue. Bacterial inoculum was instilled into each laceration using a sterile pipette system. | At 6 d after bacterial inoculation, 33% animals had either erythema, induration or purulence and the bacterial counts >10^7 CFU/g-tissue. | 97   |
| Albino rat           | Mixed culture of *S. aureus*, *P. mirabilis*, *P. aeruginosa* and *K. pneumoniae* 3 x 10^6 CFU | Two parallel vertical incisions through skin were made in the masseter muscle, 1.5 cm long, 3 mm apart, and extending to lateral surface of mandibular ramus. Pliers were inserted into two parallel incisions to the depth of the wound and squeezed firmly causing necrosis of the tissue. Each wound was then contaminated with 0.03 mL of moist soil which contained equal concentrations of the four bacterial species used. | No results of non-treated control wounds were reported by the authors. For wounds lavaged with tap water, at 6 d after contamination, positive bacterial cultures were found in all wounds, and 13 of 20 wounds were observed purulent. | 98   |
| Male Wistar albino rat | *S. aureus* *S. pyogenes* 2 x 10^6 CFU | Two paravertebral linear incisions (2 cm long, 4 cm apart) reaching the deep fascial layer were made. 1.5 cm of segments of the wound edges were crushed with hemostats for 5 sec. Bacterial Aliquots of 0.2 mL were dripped into each wound and left for resorption without spilling from the wound edges. | Infections were developed in 71.4% animals. Six days after bacterial inoculation, the bacterial counts >10^7 CFU/g-tissue. | 99   |

Staphylococcal biofilms on wound healing. Male C57Bl6/J mice weighing 35–50 g were used. Full-thickness excisional punch wounds were created through the skin down to the panniculus carnosus. Bacterial biofilms were applied at time of wounding. Microscopic analysis of cutaneous mouse wounds inoculated with *S. aureus* and *S. epidermidis* confirmed formation of a discrete biofilm after an incubation period of approximately 3 d. The model was utilized by to Martinez et al.\textsuperscript{107} to investigate applicability of topically applied nitric oxide through nanoparticles (NO-np) to *S. aureus* skin infections.

**Excisional wounds in rats.** A rat model of excisional wound was designed by Nayak et al.\textsuperscript{108} Healthy inbred albino Wistar strain male rats weighing 180–220 g were used. The dorsal fur of the animals was shaved with an electric clipper. A full thickness of the incision wound of circular area 400 mm\textsuperscript{2} and 2 mm depth was created using toothed forceps, a surgical blade and pointed scissors. The entire wound was left open. This model was used to evaluate the effect of helium-neon laser on the healing of the wounds. The model with slight modification was used by other investigators to study the treatment of infected wounds with the antimicrobial peptide\textsuperscript{109} or iron-based hemostatic agent\textsuperscript{110} and collagen-based wound dressing for drug delivery to infected wounds.\textsuperscript{111}

In another similar model reported by Saymen et al. using female Lewis or Sprague Dawley rats weighing 200 to 300 g, a 1 in (2.54 cm) square was outlined by an incision into the skin, dorsolaterally just anterior to the pelvis. The depth of the incision extended through the full thickness of skin but did not include the underlying muscle or panniculus carnosus. The anteriomedial corner of the incised skin was lifted with a toothed forceps and carefully dissected away from the panniculus by using the index finger wrapped in a gauze pad. The resulting full-thickness surface wound provided the basic surgical preparation for all experiments. The surface of the wound was seeded with 10^7 CFU of *P. aeruginosa* by pipetting 0.1 mL of the supernatant culture containing necrosis of the tissue.

**Excisional wounds in pigs.** Fries et al.\textsuperscript{113} reported a model involving dermal excisional wound in pigs. Female specific pathogen-free domestic pigs weighing 80 lb were used. The wound sites over the dorsal trunk area were shaved. Excisional dorsal wounds (n = 10; two sets of 5) were created on the back of each pig. Full-thickness sections of skin (1 x 1 in) were removed during the wounding process. Duragesic (fentanyl transdermal system) patches were placed on the pinna to alleviate pain in response to
Excisional wounds usually occur after the excisional surgery (e.g., excisional debridement of burns). As a result, excisional wound infections, similar to incisional SSIs, develop under sterile operation environment. An animal model of excisional wound infected with large number of bacteria is to some extent artificial. In addition, in most of animal models of excisional wound, the wounds are produced by only removing the full-thickness skin but no injury to the underlying muscle. This is not reasonably relevant to the clinical situations where large volume of tissue could be excised. Nevertheless these models of excisional wound infections are reproducible and are very useful in testing antimicrobial therapies.

Animal Models of Open Fracture Infections

Infection resulting from open fracture is a common problem in orthopedics. Break down of the tissue barrier between the fracture zone and the environment leaves the underlying bone prone to direct contact with contaminating agent. Due to improved aseptic and operative techniques, survival rate of the patients has increased. However, loss of the injured extremity due to wound infection and nonunion fracture is of major concern, especially when combined with a major vascular trauma.

Models of open tibial fractures. Open tibial fractures in rabbits. An early model of open fractures using male New Zealand White rabbits with a minimum weight of 3.5 kg was reported by Worlock et al. The left hind limb of each animal was shaved,
cleaned and draped to provide a sterile field. The medial side of the tibia was exposed and a reproducible fracture of the mid-part of the tibial shaft was created. This was stabilized with a 3 mm diameter Kirschner wire (K-wire). The deep fascia and skin were closed in layers. The animals were inoculated with an isolate of *S. aureus* that had been obtained from a patient who had chronic osteomyelitis. For the inoculation of each fracture, 0.5 mL of the bacterial suspension containing 10^7 CFU was injected into the site of the fracture through the previously placed 23-gauge needle. Another syringe, containing 0.1 mL of a 0.9% solution of saline, was used to flush the inoculum in the needle into the site of the fracture.

Thirty-four rabbits (out of 62) were diagnosed as being infected. At the time of death, thirty-three of them had a positive culture for *S. aureus* as well as radiographic and histological changes that were characteristic of osteomyelitis. The remaining rabbits had no discharge and a culture of a specimen that had been taken from the medullary canal at the time of death was negative. However, the rabbit had typical radiographic and histological signs of infection and the identification of Gram-positive cocci on histological sections confirmed the presence of osteomyelitis.

The model with slight modification was used by other investigators to evaluate the efficacy of local therapy with cefazolin microspheres for the prevention of infection with contaminated open tibial fractures stabilized with internal fixation^119,120^ and the influence of materials and design for fixation implants on local infections.^121,122^

**Open tibial fractures in canines.** In another study conducted by Khodaparast et al.,^123^ canine tibiae were fractured by a penetrating captive bolt device, and an interlocking intramedullary nail was used to repair the community open fractures. The skin overlaying the proximal third of the tibia was incised. A 1 cm longitudinal trough was created in the tibia medullary cavity by using a high-speed drill with a 1 mm carbide burr. The trough was started by burring into the anterior cortex from face) by using a sterile micro-syringe (10 μL volume). When the endosteal surface was reached and resistance felt, the bacterial suspension was inoculated. At 10 min after introduction of bacteria, the troughs were curetted with a dissecting curette.

A 1 cm longitudinal trough was created in the tibia medullary cavity by using a high-speed drill with a 1 mm carbide burr. The trough was started by burring into the anterior cortex from a proximal to distal mark. The burr was placed back into the trough proximally and the process repeated. The endosteal blood supply was disrupted with three to five passes of a high-temperature loop-tipped handheld cautery device.

For contamination, *S. aureus* was introduced into the exposed medullary cavity at the rearmost section of the lumen (dorsal surface) by using a sterile micro-syringe (10 μL volume). When the endosteal surface was reached and resistance felt, the bacterial suspension was inoculated. At 10 min after introduction of bacteria, the troughs were curetted with a dissecting curette.

The troughs then were pulsatile-lavaged using 100 mL of Ringer’s saline (23.7 lb/in^2^). The musculature and skin were closed by simple continuous and interrupted sutures, respectively. It was determined that the minimal inoculum of *S. aureus* that reproducibly infected 50% and 95% of tibiae after 24 h was 1.8 x 10^3^ CFU and 9.2 x 10^3^ CFU, respectively.

**Open tibial fractures in ovines.** An ovine model of open tibial fracture was developed by Hill et al.^125^ to study the outcome following intramedullary nailing of a heavily contaminated fracture. Suffolk-cross female sheep were used (mean weight 64.9 kg, range 55–80) in the study. The left hind limb was shaved and then cleaned. Under sterile conditions, the mid-shaft of the tibia was exposed through a 2 cm longitudinal incision over the subcutaneous border. A chevron osteotomy was created using a Gigli saw under saline irrigation. Soft tissue damage was minimized and the periosteum was not elevated. A standard inoculum (3 x 10^6^ CFU) of *S. aureus* was introduced into the osteotomy on a piece of previously sterile bovine type I collagen (3 x 30 mm). The limb was supported for 6 h following inoculation by means of an external cast. In the non-treated control group the surgical
wounds showed early signs of infection, with soft tissue swelling at the fracture site by the end of the first week and wound breakdown in the second week.

Open tibial fractures in goats. A complex musculoskeletal wound model containing tibial fracture in adult male Spanish-Boer goats was reported by Svboda et al.126 The goat was placed supine on the operating table, and the left lower extremity was shaved and aseptically prepared. A 5 cm skin incision approximately 1 cm lateral to, and at the level of, the tubercle was made and extended distally to the level of the medial periosteum and fascia overlying the anterior and lateral leg compartments. The lateral compartment was elevated from its attachment to the lateral compartment fascia overlying the anterior and lateral leg compartments. The lateral compartment was elevated from its attachment to the lateral aspect of the tibia after the lateral compartment fascia was incised with use of electrocautery. The fascia was also elevated from the superficial surface of the anterior and lateral compartments. The medial tibial periosteum was exposed and incised longitudinally throughout the length of the skin incision and parallel to the incision in the anterior compartment fascia. This incision was measured so that a 6 mm strip of periosteum was left intact on the anteromedial aspect of the tibia. The more posterior portion of periosteum was elevated with a blunt periosteal elevator and retracted medially. A partial medial cortical injury portion of periosteum was elevated with a blunt periosteal elevator and retracted medially. A partial medial cortical injury was created in the tibia with use of a twist drill and a small osteotome. Three Kelly clamps were spaced evenly over a 5 cm segment of the anterior compartment muscles and were left in place for 3 min to induce a standardized crush injury to the anterior compartment muscles. Concurrently, electrocautery was used to create thermal damage to the intervening muscle between the clamps. Thus, the wound rendered was complex, involving injury to muscle, fascia, periosteum and bone (Fig. 5). The wound was inoculated with 1 mL of >10^6 CFU/mL of bioluminescent P. aeruginosa, which was spread evenly over the wound surfaces with a cotton-tipped applicator soaked in the same inoculum. The wound was left open for a five-minute period after which it was dressed open with a cover sponge, a rolled gauze dressing and Vetrap bandaging tape.

Curtis et al.127 reported another goat model of contaminated tibial fractures. After surgical draping, the tibia was exposed through a 10 cm medial incision along the subcutaneous border. The periosteum was reflected for 3 cm to simulate soft-tissue damage and a chevron osteotomy, simulating an open tibial fracture, was then created with a power saw. Saline irrigation was used. S. aureus suspensions containing 10^8 CFU were placed at the fracture site on a 5 x 5 mm piece of absorbable gelatin sponge and the skin was loosely approximated with 3-0 Dexon sutures to prevent further postoperative contamination. The inoculum was placed at the fracture site after stabilization to prevent its dissemination during the surgical manipulation required to stabilize the fracture. The model was used by the authors to compare different treatment modalities of contaminated fractures.

Models of open femoral fractures. Open femoral fractures in rats. A rat model of a blunt trauma open fracture was developed by Lindsey et al.128 using male Sprague-Dawley rats (400–450 g). The model included a blunt trauma injury, an open wound, exposure to common bacteria and definitive surgical fixation. After the rat was anesthetized, the leg was placed over the platforms of the fracture device (ventral side up) with a bluntened blade placed at the mid-shaft of the femur. A weight of 0.94 kg was dropped from 15.3 cm, which impacted the bluntened blade delivering a calculated force of 104.80 N. This method allowed for a reproducible mid-shaft femur fracture with associated soft tissue injury.

Following fracture, an incision was made on the dorsolateral surface of the femur from the area of the greater trochanter to the epicondyles of the femur through the gluteus superficialis. The fracture ends were exposed. S. aureus inoculum containing 10^8 CFU was placed directly into the wound after both ends of the fracture were exposed. The fracture was then left open for 1 h to mimic the “golden hour” of a trauma patient. Fixation was then started immediately. Using a 0.045 K-wire, the investigators drilled in a retrograde fashion starting at the fracture site and coming out in the piriformis fossa. The K-wire was removed and placed in an antegrade fashion through that hole behind the abductors, first into the proximal fragment and then into the distal fragment and partially into the epiphysis for distal fixation. The wound was closed using 4-0 polysorb sutures for myofascial closure and staples for skin closure. A reproducible rate of infection of 90–100% was displayed using this model.

The model was used by the authors to study the effect of interleukin-12 systemic therapy on open fracture infections129 and was used by other investigators with slight modifications for the evaluation of local monocyte chemoattractant protein-1 and interleukin-12 p70 therapies to prevent open fracture infections130 as well as a biodegradable scaffold as a carrier for antibiotics in infected open fractures.131

Another rat model of open femoral fracture was used by Brown et al.132 to determine the effects of earlier debridement...
and antibiotic administration on decreasing infection. Briefly, male Sprague-Dawley rats weighing 388.7 ± 10.68 g were used. Under aseptic conditions, a longitudinal incision was made over the left anterolateral femur and the entire femoral shaft was exposed using blunt dissection. A polycrystalline plate (length 25 mm, width 4 mm and height 4 mm) was fixed to the surface of the femur using six threaded K-wires. A 6 mm mid-diaphyseal full-thickness defect was created with a small reciprocating saw blade under continuous irrigation with sterile saline. The defects in all animals were implanted with 30 mg of type I bovine collagen ethanol sterilized and wetted with 10^7 CFUs of bioluminescent *S. aureus* suspended in 0.1 mL of sterile normal saline. The contaminated collagen was packed into the defect and the wound was closed in a layered fashion. A high resolution radiograph of each femur with stabilized defect was obtained at initial surgery to confirm appropriate placement of the implant and adequate creation of the defect. The bacteria quantity in wounds was monitored using a photo counting camera together with quantitative cultures. Purulence and pin loosening were observed in four of the 10 wounds without antibiotic treatment. Bacteria in the bone increased significantly between the 2 and 6 h time points. There was a further increase between the 6 and 24 h time points.

**Open femoral fractures in hamsters.** An open fracture model using Syrian hamsters was created by Merritt and Dowd.138 A skin incision was made on the lateral aspect of the left thigh of the animal. The muscle bundles were gently separated to expose the femur. The wound was deflected with retractors. An osteotomy was made with an osteotomy blade on an air saw. As the bone fractured instantly, no irrigation was used. Internal fixation chosen was an intramedullary 0.9 mm K-wire. *S. aureus* (10⁴ CFU), *P. mirabilis* (10⁶ CFU) or a mixture of the two organisms was injected into the muscle at the site of the wound after the osteotomy. Animals killed at 1 week had many organisms in the muscle at the site of the osteotomy indicating that tissue colonization was still clearing and osteomyelitis had not yet fully developed. The animals killed at 2 weeks rarely had organisms in the tissue, and thus the organisms isolated from the bone reflected a true infection of the bone and not contamination with adhering tissue. In addition, organisms were isolated when the rod was cultured indicating that the organisms were in the bone and causing osteomyelitis. It was also observed that that the presence of mixed organisms altered the infection rate. The presence of the Gram-positive organism markedly increases the infection rate with the Gram-negative organism. The presence of the Gram-positive organism markedly increases the infection rate with the Gram-negative organism.

**Open femoral fractures in rabbits.** Grewe et al.134 developed an open femoral fracture model in New Zealand white rabbits. An incision was made over the proximal third of femur, and using a sharp 2.5 mm drill, a hole was placed through both cortices of the femur. The hole was then tapped with a 3.5 mm tap. Half of the rabbits received a 3.5 mm stainless screw, and for the other half the hole was left empty. The wound was inoculated with 0.5 mL of culture medium containing 10⁸ CFU *S. aureus* and closed with single running suture. No buried suture was used. Results showed that in the study group received screws, 30 out of 49 rabbits (61.2%) became infected, while in the control group without screw, 19 out of 53 (35.8%) became infected.

**Summary.** The animal models of open fracture infections can be categorized as open tibial fracture infection and open femoral fracture infection. The animal species used included rat, canine, rabbit and sheep or goat. The open fractures were inflicted using saw, weight drop in combination with blunted blade or high speed drill. The most commonly investigated pathogenic microorganism is *S. aureus*. A summary of representative animal models of open fracture infections is depicted in Table 6.

To closely mimic the clinical reality, an ideal model of open fracture is the one involved with bone, soft tissue, periosteum and fascia injuries.123,124,126-128 Besides the Gram-positive *S. aureus*, the microorganisms commonly causing fracture infections also include Gram-negative species *A. baumannii*, *P. aeruginosa*, etc.135,136 These microorganisms should be investigated especially when evaluating the efficacy of antimicrobial therapy, because Gram-negative species are more resistant to many antimicrobials than their Gram-positive counterparts. It is reported that fractures in rabbits are more prone to infections than other animals.137 Rats are much less expensive than rabbits; however, their small size does not facilitate the testing of larger fixation devices or implants and does not provide for easy intravenous antibiotic administration.137 In addition, the infection rate, dose of organisms, and type of organisms causing infection are very different in the rat from those in human infections.133

**Discussions**

In brief, animal models of external traumatic wound infections are used for two major purposes: (1) to study the basic pathogenesis and virulence; (2) to study treatment modalities. Basic approaches of evaluation include clinical observation, radiography, microbiology and histology.

Animal species commonly used in external traumatic wound infection models include mouse, rat, pig, rabbit, as well as dog, goat and sheep. The mouse strains used include BALB/c, CD-1, CF-1, C57/BL, as well as mutant diabetic mouse, SCID bg mouse. The major strains of rat used are Sprague-Dawley, Wistar and Lewis. The strains of pig used include guinea pig, domestic pig and Yorkshire pig. The major strain of rabbit used is New Zealand white rabbit. The most commonly investigated pathogenic microorganisms are Gram-positive *S. aureus* and Gram-negative *P. aeruginosa*.

Except for laceration wound and open fracture infection models, the mouse is the most frequently used species in external traumatic wound infection models. Mice are an ideal organism in which to understand human infectious diseases. Despite some differences, the immune systems of mice and humans are similar and they can often be challenged with the same or similar pathogens.138,139 Another advantage of using mice is the availability of genetically modified animals or knockouts. Knockout mice, in which a specific gene has been deleted, have proved extremely useful in studying mechanisms of infectious disease.140 In addition, mouse models have the advantage of using animals
that are inexpensive to purchase and maintain, thereby allowing one to perform studies with enough samples to yield statistically significant conclusions. The wounding procedure is quick, simple to perform, requires no specialized equipment and yields a highly reproducible result. The principal disadvantages of simple to perform, requires no specialized equipment and yields cally significant conclusions. The wounding procedure is quick,ing one to perform studies with enough samples to yield statisti - that are inexpensive to purchase and maintain, thereby allowing pathogen and low light imaging cameras has greatly eased the recent availability of genetically engineered bioluminescent pathogens and low light imaging cameras has greatly eased the non-invasive real-time monitoring of progress of infections in small animals such as mice. In contrast, larger animals (e.g., rabbit, sheep, pig, etc..)address many of the deficiencies of small animals using as external traumatic wound inaction models. For example, the larger size allows for serial sampling of blood, other fluids and tissues. Furthermore, in contrast to rodent models, the ability to obtain

| Animal species               | Microorganism & inoculum | Methods used to produce infections                                                                 | End point                                                                 | Refs |
|------------------------------|--------------------------|-----------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------|------|
| Male New Zealand White rabbit| S. aureus 10⁶ CFU         | The medial side of the tibia was exposed and a reproducible fracture of the mid-part of the tibial shaft was created. Bacterial suspension was injected into the site of the fracture through a previously placed needle. | Thirty-four rabbits (out of 62) were diagnosed as being infected. At the time of death, thirty-three of them had a positive culture for S. aureus. | 118  |
| Canine                      | S. aureus 10⁶ CFU         | Canine right tibiae were fractured with a penetrating, captive-bolt device.                          | In the presence of S. aureus, VEGF mRNA expression on day 7 decreased from initial day 0 levels. | 123  |
| Male Sprague-Dawley rat      | S. aureus 10⁷ CFU to 9.2 x 10⁷ CFU | A 1 cm longitudinal trough was created in the tibia medullary cavity by using a high-speed drill. S. aureus was introduced into the exposed medullary cavity at the rearmost section of the lumen by using a micro-syringe. | The minimal inoculum of S. aureus that reproducibly infected 50% and 95% of tibiae after 24 h was 1.8 x 10⁷ CFU and 9.2 x 10⁷ CFU, respectively. | 124  |
| Sulkolk-cross female sheep   | S. aureus 3 x 10⁵ CFU     | The mid-shaft of the tibia was exposed through a 2 cm longitudinal incision over the subcutaneous border. A chevron osteotomy was created using a Gigli saw. S. aureus was introduced into the osteotomy on a piece of sterile bovine type I collagen. | The wounds showed early signs of infection, with soft tissue swelling at the fracture site by the end of the first week. | 125  |
| Goat                        | S. aureus 10⁷ CFU         | A chevron osteotomy was created with a power saw. S. aureus suspensions were placed at the fracture site on a 5 x 5 mm piece of absorbable gelatin sponge. | There were significantly fewer and less severe infections in fractures fixed with external fixation than in those fixed with an intramedullary nail with or without reaming. | 127  |
| Male Sprague Dawley rat      | S. aureus 10⁷ CFU         | Fracture device with a blunted blade placed at the mid-shaft of the femur. A weight of 0.94 kg was dropped from 15.3 cm. S. aureus inoculum was placed directly into the wound after both ends of the fracture were exposed. | A reproducible rate of infection of 90–100% was displayed using this model. | 128  |
| Male Sprague Dawley rat      | S. aureus 10⁷ CFU         | A 6 mm mid-diaphyseal full-thickness defect was created with a small reciprocating saw blade. The defects in all animals were implanted with 30 mg of type I bovine collagen and wetted with S. aureus suspension. | Purulence and pin loosening were observed in 4 of the 10 wounds. Bacteria in the bone increased significantly between the 2 and 6 h time points. There was a further increase between the 6 and 24 h time points. | 132  |
| Syrian hamsters              | S. aureus (10⁴ CFU), P. mirabilis (10⁵ CFU) | An osteotomy was made with an osteotomy blade on an air saw. Bacterial suspension was injected into the muscle over the femur. | Animals killed at 1 week had many organisms in the muscle at the site of the osteotomy. The animals killed at 2 weeks rarely had organisms in the tissue but only in the bone. | 133  |
| New Zealand white rabbits    | S. aureus 10⁵ CFU         | Using a 2.5 mm drill, a hole was placed through both cortices of the femur. The hole was then tapped with a 3.5 mm tap. Half of the rabbits received a 3.5 mm stainless screw, and for the other half the hole was left empty. The wound was inoculated with S. aureus suspension. | In the study group received screws, 30 out of 49 rabbits (61.2%) became infected, while in the control group without screw, 19 out of 53 (35.8%) became infected. | 134  |
vast amounts of time-dependent data for a wide variety of variables allows for great statistical power and ability to derive correlations with relatively few animals. In addition, for some traumatic wounds, like open fractures, mouse is not a suitable candidate due to the size of animal. Food and Drug Administration (FDA) guidelines suggest that all new drug applications include data from two species of animals, one of which must be a non-rodent large animal.

Despite these advantages, challenges remain in applying large animal models in the study of external traumatic wound infections. Experimental therapies for large animal models require at least 100 to 1,000 times more compound than mice because the large sizes make each experiment more expensive. The animal housing regulations for large animals is quite rigorous, detailed and expensive. Use of species more closely related to humans (non-human primates) presents extraordinary difficulties: These and expensive. Use of species more closely related to humans (non-human primates) presents extraordinary difficulties: These reagents and genetic information are vital for the assessment and understanding of particular phenomena such as pathology of infection, bacterial virulence and host resistance.

We have developed a method to monitor bacterial numbers and viability in real-time in living animals using genetically engineered bacteria that emit luminescence together with sensitive low-light imaging cameras. The rate of luciferase enzyme turnover in the presence of substrate allows for real-time measurements and the enzyme is active at the body temperature of mammals. This method is a significant improvement on the traditional use of survival or body fluid sampling and subsequent plating and colony counting. The first method suffers from the disadvantage of being wasteful of animals, and does not really address the question of where the bacteria are in the animal, while the second method suffers from the disadvantage that tissue sampling introduces another source of experimental error, is laborious and does not give real-time results. The in vivo bioluminescence imaging technique has now been widely used in the studies of external traumatic wound infections, especially in rodents. 25,30-71,101,132,146-149

Due to reason for cost, almost universally, animal models of external traumatic wound infections use young animals with physiologic ages equivalent to humans less than 18 years old. Older wound patients are also more likely to develop infections and die from sepsis. Gender of animals may also affect the outcome of the studies. In many studies, only a single gender of animals was used.

The main determinants of the severity of external traumatic wound infections and whether the animals develop sepsis and die are as follows: the number of bacteria applied to the wound, the virulence of the particular bacterial strain, the size of the wound expressed as % of TBSA and the immune status of the animals. For burn infections, whether the bacteria are applied to the surface or injected into or beneath the burn, and the length of time the heated object or liquid is in contact with the skin also affect the infection severity. Animal models of external traumatic wound infections reported by different instigators vary significantly in the above parameters. To enable outcomes from different research sites to be compared, standard protocols for establishing animal models of external traumatic wound infections as well as basic approaches of evaluation are warranted.

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References

1. Pruitt BA Jr, Manusun AT, Kim SH, Goodwin CW. Burn wound infections: current status. World J Surg 1998; 22:135-45.
2. Murray CK. Infections in burns. J Trauma 2007; 62:73.
3. Cheadle WG, Tiutina M. Infection and organ failure in the surgical patient: a tribute to seminal contributions by Polk HC Jr. Am J Surg 2005; 190:173-7.
4. Sebeny PJ, Riddle MS, Petersen K. Acinetobacter baumannii skin and soft-tissue infection associated with war trauma. Clin Infect Dis 2008; 47:444-9.
5. Evans RP. Surgical site infection prevention and control: an emerging paradigm. J Bone Joint Surg Am 2009; 91:2-9.
6. Neubauer T, Bayer GS, Wagner M. Open fractures and infection, Acta Chir Orthop Traumatol Cech 2006; 73:501-12.
7. Brock EC, van Aselt AD, Bruggeman CA, van Tiel FH. Surgical site infections: how high are the costs? J Hosp Infect 2009; 72:193-201.
8. Zak O, O’Reilly T. Animal models in the evaluation of antimicrobial agents. Antimicrob Agents Chemother 1991; 35:1527-31.
9. Kaiser AB, Kernodle DS, Parker RA. Low-inoculum model of surgical wound infection. J Infect Dis 1992; 166:393-9.
10. McKenry R, Whitney RR. Characterization and quantitation of experimental surgical-wound infections used to evaluate topical antibacterial agents. Antimicrob Agents Chemother 1976; 10:38-44.
11. Gilpin DA. Calculation of a new Meeh constant and experimental determination of burn size. Burns 1996; 22:607-11.
12. Ritenourer S, Singly C, Hoover J, Page R, Payne D. Use of the surgical wound infection model to determine the efficacious dosing regimen of retapamulin, a novel topical antibiotic. Antimicrob Agents Chemother 2006; 50:3886-8.
13. Rupp ME, Ulphani JS, Fev PD, Barscht K, Mack D. Characterization of the importance of polysaccharide intercellular adhesin/hemagglutinin of Staphylococcus epidermidis in the pathogenesis of biomaterial-based infection in a mouse foreign body infection model. Infect Immun 1999; 67:2627-32.
14. Kuldin NA, Pancari GD, Toberty TW, Cope L, Jackson J, Gill C, et al. Real-time monitoring of bacterial infection in vivo: development of bioluminescent staphylococcal foreign-body and deep-thigh-wound mouse infection models. Antimicrob Agents Chemother 2003; 47:2740-8.
15. Epperson F, Frimodt-Moller N, Cornelissen L, Riber U, Rosdahl VT, Skinhoj P. Effect of treatment with mexitelmin and gentamicin in a new experimental mouse model of foreign body infection. Antimicrob Agents Chemother 1994; 38:2047-53.
16. Acton P, Grappler SF. Efficacy of ceftriaxone and related compounds in animals models of infection. J Antimicrob Chemother 1982; 10:81-9.
17. Ford CW, Hamel JC, Stupart D, Yancey RY. Establishment of an experimental model of a Staphylococcus aureus abscess in mice by use of dextran and gelatin microcarriers. J Med Microbiol 1989; 28:259-66.
18. Bunce C, Wheeler L, Reed G, Mussel J, Barg N. Murine model of cutaneous infection with gram-positive cocci. Infect Immun 1992; 60:2636-40.
19. Yarboro SR, Baum EF, Dahners LE. Locally administrated antibiotics for prophylaxis against surgical wound infection. An in vivo study. J Bone Joint Surg Am 2007; 89:929-33.
20. Cavanaugh DL, Berry J, Yarboro SR, Dahners LE. Better prophylaxis against surgical site infection with local as well as systemic antibiotics: An in vivo study. J Bone Joint Surg Am 2009; 91:1907-12.
21. Van Wijngaarden E, Peetersmans WE, Vandermassen J, Van Liesse S, Bobbaers H, Van Elder J. Foreign body infection: a new rat model for prophylaxis and treatment. J Antimicrob Chemother 1999; 44:669-74.
22. Bergmans TM, Lumont PM, Cheadle WG, Polk HC Jr. Combined topical and systemic antibiotic prophylaxis in experimental wound infection. Am J Surg 1984; 147:753-6.

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Research in the Hamblin laboratory is supported by the NIH (grant RO1AI050875) and the US Air Force MREF program (contract FA9550-04-1-0079). T.D. was partially supported by a Bullock-Wellman Fellowship Award and an Airlift Research Foundation Extremity Trauma Research Grant (grant #109421).
23. Fallon MT, Shafer W, Jacob E. Use of ceftazolin microspheres to treat localized methicillin-resistant Staphylococcus aureus infections in rats. J Surg Res 1999; 86:97-102.

24. Moegaard E, Lykkegaard Nielsen MH, Juttesten T. Bacterial model of surgical wound infection applicable to antibiotic prophylaxis. Eur J Clin Microbiol 1983; 2:439-62.

25. Stratford AF, Zourman DE, Davidson JS. Effect of lidocaine and epinephrine on Staphylococcus aureus in a guinea pig model of surgical wound infection. Plast Reconstr Surg 2002; 110:1275-9.

26. Mangram AJ, Horan TC, Pearson ML, Silver LC, Jarvis WR. Guidelines for prevention of surgical site infection, 1999. Hospital Infection Control Practices Advisory Committee. Infect Control Hosp Epidemiol 1999; 20:250-78.

27. Dai T, Tegos GP, Zhifyentayev T, Myolkas E, Hamblin MR. Photodynamic therapy for methicillin-resistant Staphylococcus aureus infection in a mouse skin abrasion model. Lasers Surg Med 2010; 42:38-44.

28. Zolfighri PS, Packer S, Singer M, Nair SP, Bennett J, Street C, et al. In vivo killing of Staphylococcus aureus using a light-activated antimicrobial agent. BMC Microbiol 2009; 9:27.

29. Kraft WG, Johnson PT, David BC, Morgan DR. Cutaneous infection in normal and immunocompromised mice. J Invest Dermatol 1986; 52:707-13.

30. Hahn BL, Bischof TS, Soehle PG. Superficial exudates of neutrophil prevent invasion of Bacillus anthracis bacilli into abraded skin of resistant mice. Int J Exp Pathol 2008; 89:180-7.

31. Kugelberg E, Nordenstam T, Petersen TK, Davtov T, Anderson DH, Huggett DF. Establishment of a superficial skin infection model in mice by using Staphylococcus aureus and Streptococcus pyogenes. Antimicrob Agents Chemother 2005; 49:3435-41.

32. Hu Y, Shamaei-Tousi A, Liu Y, Coates A. A new approach for the discovery of antibiotics by targeting non-multiplying bacteria: a novel topical antibiotic for staphylococcal infections. PLoS One 2010; 5:e11818.

33. Onunwoke CC, Hahn BL, Soehle PG. Clearance of experimental cutaneous Staphylococcus aureus infections in mice. Arch Dermatol Res 2010; 302:375-82.

34. Gaspar AA, Burns R, Naair A, Ramirez D, Barth RK, Haidaris CG. CD86 (B7-2), but not CD80 (B7-1), expression in the epidermis of transgenic mice enhances the immunogenicity of primary cutaneous Candida albicans infections. Infect Immun 1998; 66:4480-9.

35. Mierse AM, Leaney AG, Buid B. A simple trauma model in the rat. J Trauma 1973; 13:827-8.

36. Jeray KJ, Banks DM, Pheffer LS, Middlebrooks ES, Frankenburg KP, Hudson MC, et al. Evaluation of standard surgical preparation performed on superficial dermal abrasions. J Orthop Trauma 2000; 14:206-11.

37. Walker HL, Mason AD Jr. A standard animal burn. J Trauma 1960; 10:8419-51.

38. Fader RC, Nunez D, Unbehagen J, Linares HA. Experimental candidiasis after thermal burns. Wound Repair Regen 2002; 10:372-7.

39. Ecrits SA, Demidova TN, Aalders MC, Hasan T, Hamblin MR. Photodynamic therapy for Staphylococcus aureus infected burn wounds in mice. Photochem Photobiol Sci 2005; 4:503-9.

40. Dai T, Tegos GP, Lu Z, Huang L, Zhifyentayev T, Franklin MJ, et al. Photodynamic therapy for Acinetobacter baumannii burn infections in mice. Antimicrob Agents Chemother 2009; 53:3929-34.

41. Dai T, Tegos GP, Burkatovsky M, Castano AP, Hamblin MR. Chitosan acetate bandage as a topical antimicrobial dressing for infected burns. Antimicrob Agents Chemother 2009; 53:393-400.

42. Raga S, Sanchez-Garcia D, Ruiz-Gonzalez R, Dai T, Agut M, Hamblin MR, et al. Cationic porphyrins as potential photostimulants for antimicrobial photodynamic therapy. J Med Chem 2010; 53:7766-83.

43. Busch NA, Zanotti EM, Loiselle PM, Carter EA, Allaire JE, Yarmish ML, et al. A model of infected burn wounds using Escherichia coli O18K1:H7 for the study of Gram-negative bacteria and sepsis. Infect Immun 2000; 68:3549-51.

44. Orenstein A, Klein D, Kopolovic J, Winkler E, Malik Z, Keller N, et al. The use of probiotics for eradication of Staphylococcus aureus in burn wound infections. FEBS Immunol Microbiol Microbiol 1997; 19:307-40.

45. Stinnett JD, Loose LD, Miskell P, Tenney CL, Gonce SJ, Alexander JW. Synthetic immunomodulators for prevention of fatal infections in a burned guinea pig model. Ann Surg 1983; 198:53-7.

46. Singert AJ, McClain SA. Persistent wound infection delays epidermal maturation and increases scarring in thermal burns. Wound Repair Regen 2002; 10:572-7.

47. Breuing K, Kaplan S, Liu P, Onderdonk AB, Erikson AL. Wound fluid bacterial levels exceed tissue bacterial counts in controlled porcine partial-thickness burn infections. Plast Reconstr Surg 2003; 111:781-8.

48. Wolfe RR, Miller HI. Burn shock in untreated and saline-resuscitated guinea pigs. Development of a model. J Surg Res 1976; 21:269-76.

49. Herndon DN, Willmore DW, Mason AD Jr. Development and analysis of a small animal model simulating the human postburn hypermetabolic response. J Surg Res 1978; 25:394-403.
80. Kaufman T, Lusthaus SN, Sager U, Wiedler MR. Deep partial skin thickness burns: a reproducible animal model to study burn wound healing. Burns 1990; 16:13-6.
81. Branicki LF, Mittermayr R, Herndon DN, Norbury WA, Elder OE, Hofmann M, et al. A reproducible model of full-thickness burn, excision and skin autografting. Burns 2008; 34:1119-27.
82. Manafi A, Kohanteb J, Mehrabani D, Japoni A, Amini M, Naghmachi M, et al. Active immunization using exotoxin A confers protection against Pseudomonas aeruginosa infection in a mouse burn model. BMC Microbiol 2009; 9:23.
83. Kumari S, Harjai K, Chhibber S. Topical treatment of Klebsiella pneumoniae B5055 induced burn wound infection in mice using natural products. J Infect Dev Ctries 2010; 4:367-77.

84. Calum H, Moser C, Jensen PO, Christophersen L, Maling DS, van Gennip M, et al. Thermal injury induced impaired function in polymorphonuclear neutrophil granulocytes and reduced control of burn wound infection. Clin Exp Immunol 2009; 156:102-10.
85. Balas T, Bilezikci B, Maral T, Borman H. A modified partial-thickness burn model in rats. Burns 2007; 33:52-3.
86. Suzuki T, Hirayama T, Aikara K, Hirohata Y. Experimental studies of moderate temperature burns. Burns 1968; 17:345-56.
87. Hollandier JE, Singer AJ, Valentine SM, Shofner FS. Risk factors for infection in patients with traumatic lacerations. Acad Emerg Med 2001; 8:716-20.
88. Edlich RF, Tsung MS, Rogers W, Rogers P, Wangensteen OH. Studies in management of the contaminated wound. I. Technique of closure of such wounds together with a note on a reproducible experimental model. J Surg Res 1968; 8:585-92.
89. Edlich RF, Prusak M, Panek P, Madden J, Wangensteen OH. Thul J. Studies in the management of the contaminated wound. 8. Assessment of tissue adhesives for repair of contaminated tissue. Am J Surg 1971; 122:394-7.
90. Edlich RF, Madden JE, Prusak M, Panek P, Thul J, Wangensteen OH. Studies in the management of the contaminated wound. VI. The therapeutic value of gentle scrubbing in prolonging the limited period of effectiveness of antibiotics in contaminated wounds. Am J Surg 1971; 121:608-72.
91. Custer J, Edlich RF, Prusak M, Madden J, Panek P, Wangensteen OH. Studies in the management of the contaminated wound. V. An assessment of the effectiveness of p-Hexol and Betadine surgical scrub solutions. Am J Surg 1971; 121:572-5.
92. Edlich RF, Custer J, Madden J, Dajani AS, Rogers W, Wangensteen OH. Studies in management of the contaminated wound. 7. Assessment of tissue adhesives for repair of contaminated tissue. Am J Surg 1970; 119:213-10.
93. Edlich RF, Rogers W, Kasper G, Kaufman D, Tsung MS, Wangensteen OH. Studies in the management of the contaminated wound. I. Optimal time for closure of contaminated open wounds. II. Comparison of resistance to infection of open and closed wounds during healing. Am J Surg 1969; 117:323-9.
94. Howell JM, Dhindia HS, Stair TO, Edwards BA. Effect of scrubbing and irrigation on staphylococcal and streptococcal counts in contaminated lacerations. Antimicrob Agents Chemother 1993; 37:2754-5.
95. Howell JM, Bresnahah NA, Stair TO, Dhindia HS, Edwards BA. Comparison of effects of suture and cya-noacrylate tissue adhesive on bacterial counts in contaminated lacerations. Antimicrob Agents Chemother 1995; 39:559-60.
96. Moscati R, Mayrone J, Fincher L, Jehle D. Comparison of normal saline with tap water for wound irrigation. Am J Emerg Med 1998; 16:379-81.
97. Lammers R, Henry C, Howell J. Bacterial counts in experimental, contaminated crush wounds irrigated with various concentrations of cepazolin and penicillin. Am J Emerg Med 2001; 19:1-5.
98. Gross A, Curtright DE, Bhaskar SN. Effectiveness of pulsatile lavage irrigation in treatment of contaminated crush wounds. Am J Surg 1972; 124:373-7.
99. Erdur B, Ersoy G, Yılmaz O, Özkunak A, Sis B, Karcıoglu O, et al. A comparison of the prophylactic uses of topical murinepurin and nitrofurazone in murine crushed wound wounds. Am J Emerg Med 2000; 18:1637-43.
100. Hamblin MR, O’Donnell DA, Murthy N, Contag CH, Hasan T. Rapid control of wound infections by targeted photodynamic therapy monitored by in vivo bioluminescence imaging. Photochem Photobiol 2002; 75:51-7.
101. Burktazarova M, Cartano AB, Demidova-Biehn TN, Tegos GP. Hamblin MR. Effect of chitosan acetate bandage on wound healing in infected and noninfected wounds in mice. Wound Repair Regen 2008; 16:425-31.
102. Simoniotti C, Cirizone G, Ghiselli R, Goteri G, Scalise A, Orlando E, et al. RNAi-inhibiting peptide enhances healing of infected wounds infected with meticillin-resistant Staphylococcus aureus. Antimicrob Agents Chemother 2008; 52:2205-11.
103. Mahoney E, Reichner J, Bostom LR, Mastrofrancesco B, Henry W, Albina J. Bacterial colonization and the expression of inducible nitric oxide synthase in murine wounds. Am J Pathol 2002; 161:2148-52.
104. Shi CM, Nakao H, Yanzadi M, Tsaboh R, Ogihara F. Mixture of sugar and povidone-iodine stimulates healing of MRSA-infected skin ulcers on db/db mice. Arch Dermatol Res 2002; 294:449-56.
105. Schirger CE, De la Garza M, Musto TA, Galiano RD. Staphylococcal biofilms impair wound healing by delaying re-epithelialization in a murine cutaneous wound model. Wound Repair Regen 2009; 17:354-9.
106. Martinez LR, Han G, Chacko M, Mihu MR, Jacobson M, Galanella P, et al. Antimicrobial and healing efficacy of sustained release nitric oxide nanoparticles against Staphylococcus aureus skin infection. J Invest Dermatol 2009; 129:2463-9.
107. Nayak BS, Mayia A, Kumar P. Influence of Helium-Neon laser photo therapy on wound healing in Wistar Rats. OnLine J Biol Sci 2007; 7:89-92.
108. Chalekson CP, Neumeister MW, Jaynes J. Treatment of infected wounds with the antimicrobial peptide D2A21. J Trauma 2003; 54:770-4.
109. Bracho DO, Barsan L, Arekapudi SR, Thompson JA, Hen J, Sterin SA, et al. Antibacterial properties of an iron-based hemostatic agent in vitro and in a rat wound model. Acad Emerg Med 2009; 16:656-60.
110. Adhijrajan N, Shanmuganandaram N, Shanmuganathan S, Babu M. Collagen-based wound dressing for diabetic wound healing. Int J Pharm Sci 2004; 61:167-171.
111. Saymen DG, Nathan P, Holder IA, Hill EO, Macmillan J, Galiano DS, Mokhtar M, De la Garza M, Mustoe TA, Galiano DB. Treatment of infected wounds with the antimicrobial peptide E20:648-53.
112. Svoboda SJ, Bice TG, Gooden HA, Brooks DE, Thomas DB, Wenke JC. Comparison of bulb syringe and pulsed lavage irrigation with use of a biosilumin- cent muscleskeletal wound model. J Bone Joint Surg Am 2007; 119:213-8.
113. Hill PF, Clasper JC, Parker SJ, Watkins PE. Early intratrheamic catheterization in a murine model of a heavily contaminated fracture of the tibia. J Orthop Res 2002; 20:648-53.
114. Sato S, Tsuchiya T, Namba Y. Collagen-based wound dressing for diabetic wound healing of MRSA-infected skin ulcers on db/db mice. Arch Dermatol Res 2002; 294:449-56.
115. Edlich RF, Madden JE, Prusak M, Panek P, Thul J, Wangensteen OH. Studies in the management of the contaminated wound. VI. The therapeutic value of gentle scrubbing in prolonging the limited period of effectiveness of antibiotics in contaminated wounds. Am J Surg 1971; 122:394-7.
116. Page MD, Tao Y, Custer J, Wangensteen OH. Studies in the management of the contaminated wound. V. An assessment of the effectiveness of p-Hexol and Betadine surgical scrub solutions. Am J Surg 1971; 121:572-5.
117. Edlich RF, Custer J, Madden J, Dajani AS, Rogers W, Wangensteen OH. Studies in management of the contaminated wound. 8. Assessment of tissue adhesives for repair of contaminated tissue. Am J Surg 1970; 119:213-10.
118. Edlich RF, Rogers W, Kasper G, Kaufman D, Tsung MS, Wangensteen OH. Studies in the management of the contaminated wound. I. Optimal time for closure of contaminated open wounds. II. Comparison of resistance to infection of open and closed wounds during healing. Am J Surg 1969; 117:323-9.
119. Howell JM, Dhindia HS, Stair TO, Edwards BA. Effect of scrubbing and irrigation on staphylococcal and streptococcal counts in contaminated lacerations. Antimicrob Agents Chemother 1993; 37:2754-5.
120. Howell JM, Bresnahah NA, Stair TO, Dhindia HS, Edwards BA. Comparison of effects of suture and cyanoacrylate tissue adhesive on bacterial counts in contaminated lacerations. Antimicrob Agents Chemother 1995; 39:559-60.
121. Moscati R, Mayrone J, Fincher L, Jehle D. Comparison of normal saline with tap water for wound irrigation. Am J Emerg Med 1998; 16:379-81.
135. Mody RM, Zapor M, Hartzell JD, Robben PM, Waterman P, Wood-Morris R, et al. Infectious complications of damage control orthopedics in war trauma. J Trauma 2009; 67:758-61.
136. Johnson EN, Burns TC, Hayda RA, Hospenthal DR, Murray CK. Infectious complications of open type III tibial fractures among combat casualties. Clin Infect Dis 2007; 45:409-15.
137. An YH, Kang QK, Arciola CR. Animal models of osteomyelitis. Int J Artif Organs 2006; 29:407-20.
138. Flajnik MF. Comparative analyses of immunoglobulin genes: surprises and portents. Nat Rev Immunol 2002; 2:688-98.
139. Buer J, Balling R. Mice, microbes and models of infection. Nat Rev Genet 2003; 4:195-205.
140. Zanetti-Cavazzoni SL, Goldfarb RD. Animal models of sepsis. Crit Care Clin 2009; 25:763-19.
141. Neely AN, Holder IA, Warden GD. Then and now: studies using a burned mouse model reflect trends in burn research over the past 25 years. Burns 1999; 25:603-9.
142. Rocchetta HL, Boylan CJ, Foley JW, Iversen PW, LeTourneau DL, McMillian CL, et al. Validation of a noninvasive, real-time imaging technology using bioluminescent Escherichia coli in the neutropenic mouse thigh model of infection. Antimicrob Agents Chemother 2001; 45:129-37.
143. Francis KP, Joh D, Bellinger-Kawahara C, Hawkinson MJ, Purchio TF, Contag PR. Monitoring bioluminescent Staphylococcus aureus infections in living mice using a novel luxABCDE construct. Infect Immun 2000; 68:3594-600.
144. Francis KP, Yu J, Bellinger-Kawahara C, Joh D, Hawkinson MJ, Xiao G, et al. Visualizing pneumococcal infections in the lungs of live mice using bioluminescent Streptococcus pneumoniae transformed with a novel gram-positive lux transposon. Infect Immun 2001; 69:3350-8.
145. Demidova TN, Gad F, Zahra T, Francis KP, Hamblin MR. Monitoring photodynamic therapy of localized infections by bioluminescence imaging of genetically engineered bacteria. J Photochem Photobiol B 2005; 81:15-25.
146. Thorn RM, Greenman J. A novel in vitro flat-bed perfusion biofilm model for determining the potential antimicrobial efficacy of topical wound treatments. J Appl Microbiol 2009; 107:2070-9.
147. Jawhara S, Mordon S. Monitoring of bactericidal action of laser by in vivo imaging of bioluminescent Escherichia coli in a cutaneous wound infection model for evaluation of an antibiotic therapy. Antimicrob Agents Chemother 2004; 48:3436-41.
148. Enjalbert B, Rachini A, Vediyappan G, Pietrella D, Spaccapelo R, Vecchiarelli A, et al. A multifunctional, synthetic Gaussia princeps luciferase reporter for live imaging of Candida albicans infections. Infect Immun 2009; 77:4847-58.