Supplemental Methods

Bacterial Load Quantification

Bacterial load was measured using the method described previously (1). The necrotic tissue was excised, and then 2 mm (width) × 2 mm (length) ×1-5 mm (depth) tissue on the surface of the wound was dissected. The tissue was weighed and homogenized in 99× weight sterile 0.85% sodium chloride. The supernatant was then gradient diluted (10^{-2} to 10^{-7}) and 100 μl of the diluted supernatant was streaked onto a sheep blood agar plate for aerobic bacteria detection and an anaerobic blood agar plate for anaerobic bacteria detection. The bacterial load was quantified by counting CFUs on each plate. The bacterial load was quantified using the following formula: bacterial load (CFU/g) = (number of CFUs on plate $\times 10^{3}$) / dilution.

Bacterial Species Detection

Aerobic and anaerobic bacteria species were detected with MicroScan WalkAway 96 CI (Siemens AG, Munich, Germany) as previously described(2-3). Briefly, the tissue dissected from the wound was weighed and homogenized, and the homogenate was inoculated in a sheep blood agar plate for aerobic bacteria detection and in an anaerobic blood agar plate for anaerobic bacteria detection. For the aerobic bacteria detection, the plate was observed by an experienced Medical Lab Technician, the colonies were categorized, and the percentage of each type of colony was calculated. Colonies beyond 5% were chosen to inoculate in the MicroScan® - Neg Combo Panel Type 31 (Cat. No. B1017 – 301, Siemens AG, Munich, Germany) and MicroScan® - Pos Combo Panel Type 20 (Cat. No. B1017 – 200, Siemens AG, Munich, Germany) to determine the specific bacteria species. Colonies that were under 5%

were not processed. For the anaerobic bacteria, the plate and the bacteria colony were processed the same way as the aerobic bacteria, except that the colonies that were beyond 5% were inoculated in the Rapid Anaerobe ID B1017-2 (Siemens AG, Munich, Germany) plates. For each specimen, the process was repeated by three technicians.

Specimens on the floor of the animal house were collected using sterile cotton applicators and the skin scurf on the back of swine was collected by scraping the skin using a sterile surgical blade. The applicators and the skin scurf were then added to 100μl PBS for 10 minutes which were used for determination of bacterial composition by the method mentioned above.

Supplemental Results

Bacteria flora in the animal house and the swine skin

Since no bacteria were put in the blast injury model in this study and the bacteria in the infected injury wounds were from the animal house and the skin of the swine. 18 specimens were collected from the floor of the 3 animal houses early in the morning before the daily cleaning and disinfection; the most common aerobic bacteria isolated were *E. Coli, group C streptococci* and *Staphylococcus epidermidis,* the percentages of which were 62.4 ± 8.97%, 13.1 ± 4.53% and 9.51 ± 3.26% respectively. *Staphylococcus aureus* and *group A streptococci* were beyond 5% in 14 and 7 specimens respectively, and *Bacillus cereus* and *Micrococcus* were isolated in some of the specimens. No anaerobic bacteria were isolated in these specimens.

The aerobic bacterial composition of the 12 scurf specimens, which were collected from the back of 12 pigs, was similar to that in the infected soft tissue blast injury. The most common aerobic bacteria in the scurf were *E. Coli*, *Staphylococcus epidermidis*, *group C streptococci*, *group A streptococci* and *Staphyloccocus aureu*, the percentage of which were 55.4 ± 9.82%, 15.5 ± 6.21%, 9.43 ± 3.56%, 7.33 ± 5.68% and 4.68 ± 6.84% respectively. No anaerobic bacteria were detected. Since both the specimens from animal houses and the skin of pigs have no anaerobic bacteria was detected, the anaerobic bacteria in infected soft tissue blast injury might derived from hair follicles, sweat glands, and sebaceous glands.

Supplemental Table I

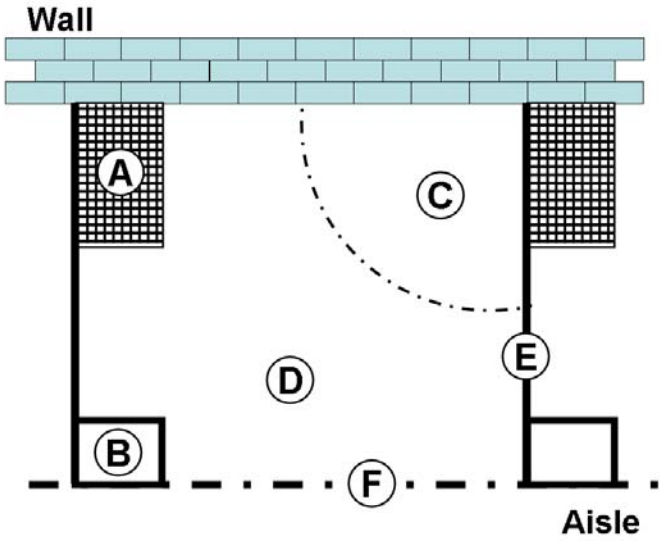
A. Aerobic bacterial composition in infected soft tissue blast (n=8, % of aerobic bacterial load)

Staphylococcus epidermidis	*Staphylococcus aureus Rosenbach*	*group C streptococci*	*group A streptococci*	*E. Coli*	*Klebsiella pneumoniae*
24.5±6.42	8.63±3.31	16.5±3.84	9.72±1.64	34.3±5.56	3.02±5.22

B. Anaerobic bacterial composition in infected soft tissue blast injury wounds (n=8, % of anaerobic bacterial load)

peptostreptococcus	*Clostridium*	*Veillonella*
65.3±10.8	22.4±8.75	8.45±7.62

Note: The soft tissue blast injury wounds were created by explosion of the electric detonators, and the wounds were left untreated for 48 hours and were subsequently infected. The necrotic tissue was debrided and the superficial layer of the debrided wounds were processed to determination of bacterial composition. A & B, Aerobic and Anaerobic bacterial composition in infected soft tissue blast injury wounds (*n*=8, %).



Supplemental Figure 1. A scheme of the animal house for 3 to 5 pigs weighing 10-15 kg. The house is 2 m wide and 2 m long with concrete floor. A, excrement and urine area. This area is connected with the sewerage system and covered with a steel mesh. The opening of the steel mesh is 1.5 cm×1.5 cm. B, food and water tank. C, sleeping area. This area is covered with gamma-ray irradiated sawdust. D, playing area. E, steel plate. The steel plate is 2.5 mm thick and 1.4 m high. F, steel picket fencing door. The door is 1.4 m high and the spacing between the pickets is 8 cm. The floor is cleaned with high pressure water hose and disinfected with peroxyacetic acid daily, and the sawdust in the sleeping area is changed daily too.

References

1. Xu L, McLennan SV, Lo L, et al. Bacterial load predicts healing rate in neuropathic diabetic foot ulcers. *Diabetes Care* 2007;30:378-380.
2. Burns JL SL, Whittier S, Krzewinski J, Liu Z, Larone D, Marshall SA, Jones RN. Comparison of two commercial systems (Vitek and MicroScan-WalkAway) for antimicrobial susceptibility testing of Pseudomonas aeruginosa isolates from cystic fibrosis patients. *Diagn Microbiol Infect Dis* 2001;39:4.
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