Animal Model

Under general anesthesia, the sheep underwent a unilateral mid-diaphyseal tibial osteotomy using a medial approach. The osteotomy was repaired with an eight or nine-hole locking compression plate. A temporary indwelling silicone catheter was inserted into the osteotomy site next to the plate during soft-tissue closure. Following complete closure, the catheter was used to inoculate the osteotomy site with a 2.5-mL suspension of 10^6, 10^8, or 10^{10} CFU/mL of *Staphylococcus aureus* ATCC 25923; eight animals received each inoculum concentration in the model development trials. The ATCC 25923 strain is a clinical isolate known to form biofilms, and it is susceptible to commonly used antimicrobials; the minimum inhibitory concentration for vancomycin is 1 to 2 µg/mL. The most commonly used staphylococcal strains in animal models of osteomyelitis include ATCC 29213, 25923, and 6538P. Each animal underwent a daily clinical examination and digital radiography to evaluate the presence of signs consistent with septic osteomyelitis[27-32]. The animals were killed at thirty days postoperatively, and ex vivo gross pathologic and histologic analyses and microcomputed tomography were performed to evaluate fracture-healing. We determined that using 2.5 mL of a 10^6 CFU/mL suspension of *Staphylococcus aureus* yielded reproducible infections in this model[25,26].

TABLE E-1 Outcome Scoring System*

	Criterion	Score
Digital radiographs		
Periosteal reaction or lifting	+/present	1
	−/absent	0
Osteolysis	+/present	1
	−/absent	0
Sequestrum formation	+/present	1
	−/absent	0
Implant loosening	+/present	1
	−/absent	0
Soft-tissue swelling	+/present	1
	−/absent	0
Callus	None	3
	Early callus (nonbridging)	2
	Bridging callus <50%	1
	Bridging callus >50%	0
Histology		
Bacteria	None	0
	Focal or few colonies	1
	Moderate to many colonies	3
Inflammation	None	0
	Minimal or mild; no suppuration	1
	Minimal; mild suppuration	2
	Moderate; severe suppuration	3
Osteolysis	None or minimal	0
	Mild localized expansion of osteonal canals	1
	Moderate	2
	Severe	3
Periosteal callus formation	Minimal or mild	0
	Prominent, well organized	1
	Prominent, disorganized	2
	Incomplete union	3
Bridging callus at osteotomy site	Complete	0
	Partial, >50%	1
	Partial, <50%	2
	None	3
Pathology		
Soft-tissue envelope	Healed	0
	Inflammation	1
	Mild necrotizing inflammation	2
	Severely necrotic debris or devascularized	3
Plate in situ	No abscess	0
	Microabscess formation within plate holes	1

	Abscess covering <50% of plate	2
	Abscess covering >50% of plate	3
Osteotomy after plate removal	Bridging, stable callus	0
	Bridging, unstable callus	1
	Nonbridging, unstable callus	2
	No callus	3

*These grading systems used for the in vivo part of the study have been used extensively in large-animal models for orthopaedic research[30].

TABLE E-2 Optimization of the Surface Modification*

	Adherent Live Bacteria†		
Treatment	1:10 Dilution	1:100 Dilution	1:1000 Dilution
1 (control; untreated)	1753.3 ± 1420.8	148.33 ± 193.4	12 ± 11.5
2 (NHF acid)	2052 ± 177.5	239 ± 143.8	19 ± 10.0
3 (NHF acid, Ti 101)	1336 ± 105.9	94.67 ± 75.4	10.33 ± 8.1
4 (NHF acid, Ti 101, NHF acid)	549.67 ± 153.3	52.67 ± 9.3	3.67 ± 2.9

*Three different surface treatments of titanium slugs were compared with no treatment to optimize surface derivatization with vancomycin. NHF acid = nitrohydrofluoric acid. All specimens were evaluated for bactericidal efficacy by bacterial challenge with 10^4 CFU of *Staphylococcus aureus* ATCC 25923. Adherent bacteria were visualized with the LIVE/DEAD BacLight Bacterial Viability Kit. The number of adherent bacteria was determined by plating bacterial sonicate samples at dilutions of 1:10, 1:100 and 1:1000. Assays, including positive controls (bacterial challenge without coating) and negative controls (coating without bacterial challenge), were performed in triplicate for each surface derivatization. The surface modification resulting from treatment 4 was consistently associated with highest bactericidal efficacy (the lowest colony counts compared with the other surface modifications, $p = 0.001$) and was chosen as the treatment for the LCP plates used in the in vivo study. †Values are given as the mean and the standard deviation.

TABLE E-3 Histologic Scores in the Study Animals*

	Animal								
Histologic Criterion	Vancomycin 1786	Vancomycin 1862	Vancomycin 1785	Vancomycin 1860	Vancomycin 1777	Control 1866	Control 271	Control 1830	Control 1838
Infection	0	0	0	0	0	3	3	3	3
Inflammation	0	0	1	0	1	2	1	2	3
Osteolysis	1	1	1	2	1	2	2	2	2
Osteotomy callus	0	0	0	0	0	1	1	1	3
Periosteal callus	1	1	1.5	0	1.5	2.5	1	1	2.5
Total score	2	2	3.5	2	3.5	10.5	8	9	13.5

*The semiquantitative histological observations were converted to point scores as outlined in Table E-1, and the total score was determined for each animal. A low score was more desirable and consistent with normal fracture-healing, whereas a high score was reflective of osteomyelitis.

TABLE E-4 Culture Results from the Osteotomy Site in the Study Animals

Animal	Culture Result
Vancomycin 1785	No growth
Vancomycin 1786	No growth
Vancomycin 1777	No growth
Vancomycin 1860	No growth
Vancomycin 1862	Light *Staphylococcus aureus* growth*
Control 271	Light *Staphylococcus aureus* growth*
Control 1866	Moderate *Staphylococcus aureus* growth*
Control 1830	No growth
Control 1838	Moderate *Staphylococcus aureus* growth*

*A pure growth of *Staphylococcus aureus* was achieved in all cases.