Role of biofilm in *Staphylococcus aureus* and *Staphylococcus epidermidis* ventricular assist device driveline infections

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Objective: Infections, especially those involving drivelines, are among the most serious complications that follow ventricular assist device implantation. Staphylococci are the most common causes of these infections. Once driveline infections are established, they can remain localized or progress as an ascending infection to cause metastatic seeding of other tissue sites. Although elaboration of biofilm appears to be critical in prosthetic device infections, its role as a facilitator of staphylococcal infection and migration along the driveline and other prosthetic devices is unclear.

Methods: A murine model of driveline infection was used to investigate staphylococcal migration along the driveline. A biofilm-producing strain of *Staphylococcus epidermidis* and a *Staphylococcus aureus* strain and its intercellular adhesion gene cluster *(ica)*–negative (biofilm-deficient) isogenic mutant were used in these studies. Bacterial density on the driveline and the underlying tissue was measured over time. Scanning electron microscopy was used to examine the morphology of *S epidermidis* biofilm formation as the infection progressed.

Results: The biofilm-deficient *S aureus* mutant was less effective at infecting and migrating along the driveline than the wild-type strain over time. However, the *ica* mutation had no effect on the ability of the strain to infect underlying tissue. *S aureus* exhibited more rapid migration than *S epidermidis*. Scanning electron microscopy revealed the deposition of host matrix on the Dacron material after implantation. This was followed by elaboration of a bacterial biofilm that correlated with more rapid migration along the driveline.

Conclusions: Biofilm formation is a critical virulence determinant that facilitates the progression of drivelines infections. (J Thorac Cardiovasc Surg 2011;141:1259-64)

Ventricular assist devices (VADs) are used to improve cardiac function in patients with end-stage congestive heart failure.[1-3] Originally designed as a bridge to transplantation, the use of VADs as destination therapy (for patients who are not transplant candidates or who have refractory heart failure) is increasing.[1,2] Infections are among the most common and serious complications in patients (10%–40%) after VAD implantation.[4] The presence of these infections might delay transplantation in bridge-to-transplantation patients and is a major cause of death in destination patients. Despite a reduction in the overall number of VAD-related infections, the driveline remains a common site of infection (21%, as reported by the INTERMACS Registry at www.intermacs.org).[3] The driveline exit site is accessible to commensal flora and susceptible to trauma that can interfere with tissue integration of the driveline.[5] The ability of bacteria to cause these infections is due in part to their ability to adhere to and colonize both skin and device surfaces by using the transcutaneous driveline of VADs as a portal of entry.[6,7]

The predominant pathogens associated with device infections are biofilm-forming *Staphylococcus epidermidis* and *Staphylococcus aureus*; both are part of the host commensal flora.[8] Infection results from the ability of these bacteria to initially colonize the device surface and then migrate along its surface into the underlying host tissue.[6,9,10] Biofilms appear to contribute to this process.[5,11,12] They constitute a consortium of surface-attached bacteria encased in a self-produced extracellular polymeric substance.[5,12-14] When a VAD is implanted, a series of host molecules, such as fibronectin, fibrin, collagen, lipids, and inorganic ions, are deposited on the different VAD surfaces, creating a host tissue matrix.[5,7,12-14] This matrix facilitates the attachment of biofilm-forming bacteria.

In staphylococci, the intercellular adhesion gene cluster *(ica)* comprises 4 genes *(icaADBC)* that direct the production of a polysaccharide that mediates intercellular adhesion and is regarded as one of the essential factors for biofilm formation.[15]

Although most driveline infections remain localized to the entry site, they can also spread to other tissue sites. One hypothesis for this process is that there is bacterial biofilm expansion that enables bacteria to migrate along the driveline. With this in mind, we investigated the contribution *S aureus* and *S epidermidis* biofilm to the initiation and progression of driveline infections using an *in vivo* model of driveline infection.

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Abbreviations and Acronyms
CFU = colony-forming units
ica = intercellular adhesion gene cluster
VAD = ventricular assist device

MATERIALS AND METHODS

Bacterial strains and growth conditions

S epidermidis strain 9491 (SE9491)[16] and *S aureus* isogenic strains RN450 (SA450) and RN450 (Δ*ica*; SAΔica; kindly provided by Chia Y. Lee)[17,18] were grown in tryptic soy broth (supplemented with glucose 0.25% for SE9491) at 37°C with aeration (Table 1). The presence of *ica* in SA450 and SE9491 was confirmed by means of polymerase chain reaction by using the following primers: *ica* forward primer, 5′-GAT TGT ATT AGC TGT AGC TAC-′3, and *ica* reverse primer, 5′-CAA CTG CAG CTG ATT TCG CCC ACC GCG TG-′3. For plate counts, mannitol salt agar plates or tryptic soy agar plates were incubated overnight at 37°C. A growth curve (tryptic soy broth at 37°C) was performed to assess the effect of the *ica* deletion on SAΔica. No difference was found in the rate of growth between the isogenic pair.

In Vivo Infection Model

The murine model of driveline infection has been previously described.[7,19] Briefly, 15-mm-long Dacron (kindly provided by Thoratec, Pleasanton, Calif)–coated drivelines were implanted transcutaneously in the backs of C57BL/6J mice. The model is shown in Figure 1. The internal (subcutaneous) part of the drivelines was 10 mm in length. The day of implantation was considered day –2. Two days after implantation (day 0), the skin surrounding the exposed driveline was inoculated with a bacterial suspension (50 μL) containing 5×10^7 colony-forming units (CFU) of SE9491 or 5×10^6 CFU of *S aureus* (suspended in phosphate-buffered saline). No perioperative antibiotics were administered to the mice. Two days after bacterial challenge (day 2), the drivelines were explanted, the external portion was sterilely separated, and the internal portions were processed (see below). These studies were reviewed and approved by the Columbia University Institutional Animal Care and Use Committee.

The drivelines were washed twice with phosphate-buffered saline (1 mL), and then adherent bacteria were harvested by vortexing in 0.05% trypsin–ethylenediamine tetraacetic acid (0.5 mL). Dilutions of the samples were plated onto mannitol salt agar plates, and bacterial cell counts were determined. The tissue underlying the driveline was also collected from each mouse and weighed. The homogenized samples were weighed and then plated onto mannitol salt agar plates; bacterial counts were determined and then normalized by sample weight. Two independent experiments were performed with a total of 20 mice per strain.

For the bacterial migration studies, the driveline size was increased to 20 mm in length, increasing the subcutaneous portion to 15 mm in length. To measure bacterial migration along the driveline over time, we divided the driveline into three 5-mm segments. The 3 subcutaneous sections were defined as proximal, medial, and distal based on their position with respect to the transcutaneous entry point. Infection of the driveline was carried out as outlined above. The bacterial inoculum was increased to 1×10^7 CFU. Drivelines were explanted at days 0, 2, 6, 9, and 12. Each section (proximal, medial, and distal) was processed separately. Results were expressed as a ratio by comparing the bacterial counts of each section to the total number of bacteria found per driveline (proximal + medial + distal).

Tissue samples of kidney, liver, and spleen were collected and processed in a similar manner as the underlying muscle tissue described above to assess for possible metastatic seeding.

Scanning Electron Microscopy

Sections of explanted drivelines were fixed with glutaraldehyde (2%) in cacodylate buffer, as previously described.[20] A JEOL JSM6400 Scanning Electron Microscope (Peabody, Mass) was used. Three micrographs were obtained from each section of 2 different drivelines. Images of control driveline samples (nonimplanted and implanted but noninfected) were compared with images obtained from infected drivelines at different time points (days 6 and 12 after infection).

Statistical Analysis

Data were analyzed by using an unpaired Student *t* test.

RESULTS

Role of Biofilm in Driveline Infections

The role that biofilm contributes to bacterial density on the drivelines and in the surrounding tissue was assessed by using both the isogenic strain of *S aureus* and a biofilm-positive *S epidermidis* strain. The average bacterial counts on the explanted drivelines for the biofilm-forming strain SA450 was 5×10^4 CFU per driveline; this was significantly higher than those for the biofilm-negative strain SAΔica (2×10^4 per driveline, Figure 2, $P < .05$). Despite the difference in adherence to the driveline, the presence of bacteria in the underlying tissue was similar for the isogenic strains ($P > .05$, Figure 2). Although biofilm positive, SE9491 had fewer bacterial counts than SA450 on both the drivelines (3×10^4 per driveline) and in the underlying tissue (1×10^4 CFU/g, $P < .05$, Figure 2). This was despite the use of a higher initial bacterial inoculum.

Role of Biofilm as a Facilitator of Migration Along the Driveline

Bacterial migration along the driveline was studied over time (Figure 3). With SA450, total bacterial density went from 2.6×10^4 CFU per driveline at day 2 to 4.8×10^5 CFU per driveline by day 12, almost doubling every 2 days (Figure 4, *A*). In contrast, the bacterial density for

TABLE 1. Description of bacterial strains used in this study

Species	Strain	Phenotype	Reference
Staphylococcus aureus	RN450	Biofilm-forming wild type	Chia Y. Lee, University of Arkansas
	SA450		
	RN450 (Δica)	Biofilm-deficient strain	Chia Y. Lee, University of Arkansas
	SAΔica		
Staphylococcus epidermidis	9491	Biofilm-positive laboratory strain	McCrea, 2000
	(SE9491)		