A Scanning and Transmission Electron Microscopic Study of an Infected Endocardial Pacemaker Lead

THOMAS J. MARRIE, M.D., JOYCE NELLOGAN, AND J. WILLIAM COSTERTON, PH.D.

SUMMARY We studied the pacemaker lead that had been removed from a patient who suffered three sequential episodes of Staphylococcus aureus bacteremia. This organism was recovered from the surface of the lead. Scanning electron microscopy showed differential colonization of the pacemaker lead. The metal tip, the inner surface and the internal wires were covered with a heavy biofilm of bacteria. The outer silastic surface had no biofilm adherent to it; instead, well-spaced bacterial cells were seen. These observations illustrate why infection of implantable devices persists despite intensive antibiotic chemotherapy.

APPROXIMATELY 1% of patients with endocardial pacemakers develop septicemia.1 Staphylococcus aureus is the organism most frequently isolated from the blood of these patients. In most instances, removal of the endocardial system is required.1–3 We used scanning electron microscopy to study the intracardiac portion of a pacemaker lead removed from a patient with recurrent S. aureus bacteremia. The extensive colonization of the various components of this system illustrates why antibiotic therapy alone is usually ineffective.

Case Report

A 56-year-old male was admitted to our hospital on August 28, 1981 with a 4-day history of anorexia, nausea, vomiting and shaking chills. Three weeks before admission, he had injured his left elbow and on two occasions he had expressed pus from it. He had undergone surgery for peptic ulcer disease, a history of ethanol abuse and syncopal attacks. In May 1975 a Medtronic bipolar transvenous pacemaker had been inserted when investigation of his syncopal attacks revealed prolongation of conduction through the atrioventricular node.

Physical examination revealed a temperature of 39.2°C, a resolving indurated lesion on his left elbow, and tenderness in his right upper quadrant. Blood cultures grew S. aureus. He was treated with cloxacillin, 12 g/day i.v. for 4 weeks. During the third week in the hospital, his gall bladder was removed.

One week after discharge he developed nausea, vomiting, fever and sweating. Blood cultures again grew S. aureus. He was treated for 6 weeks with cloxacillin, 12 g/day i.v., and rifampin, 600 mg/day orally. No signs of endocarditis were evident. There was no infection of the pacemaker pocket and serial echocardiograms showed normal cardiac values. He promptly responded to the antibiotic therapy, but was readmitted a third time 9 days after discharge with the same symptoms. Again, blood cultures grew S. aureus. On this occasion the entire pacing system was removed and replaced by an epicardial pacemaker. Intravenous cloxacillin was continued for 4 weeks after removal of the infected pacemaker lead. He has since remained well.

Materials and Methods

The pacemaker was aseptically removed under general anesthesia. The distal 10 cm (intracardiac portion) was cut off and transported to the laboratory in a sterile container. The outer surface was swabbed using cotton-tipped swabs that were used to inoculate blood agar plates (5% sheep blood in trypticase soy agar).

Scanning Electron Microscopy

The distal 5 cm of the lead was placed in a fixative solution consisting of 5% glutaraldehyde in cacodylate buffer (0.067% pH 6.2) with 0.15% ruthenium red for 24 hours at 20°C. The specimen was bisected and then “metallized” with osmium tetroxide and thiocarbohydrazide,4 dehydrated in ethanol and Freon 113, before critical point drying5 and examined using a Hitachi S450 (Hitachi) scanning electron microscope.

Transmission Electron Microscopy

A sterile scalpel blade was used to scrape material from the surface of the pacemaker lead. This material was fixed in 5% glutaraldehyde in cacodylate buffer (0.067% pH 6.2) with 0.15% ruthenium red for 24 hours at 20°C. The material was then washed five times in the buffer, postfixed in 2% OsO4 in buffer, washed five more times in the buffer, and dehydrated through a series of acetone washes. All of the solutions used in processing the specimen, from the washes after glutaraldehyde fixation to dehydration with the 70% acetone solution, contained 0.05% ruthenium red. (Ruthenium red was omitted from the 90% and 100% acetone solutions because of its limited solubility in these solutions.) After further dehydration in propylene oxide, the specimen was embedded in Spurr® low-viscosity embedding resin (Electron Microscopy Sciences), sectioned, stained with uranyl acetate and lead citrate,7 reinforced with evaporated carbon, and examined with an electron microscope (AEI Model No. 801) at an acceleration voltage of 60 kV.

Results

S. aureus was isolated from several sites on the surface of the pacemaker lead. Figure 1 is a schematic drawing of a sagittal section of the pacemaker lead.
showing the sites that were examined by scanning electron microscopy. Figures 2 and 3 are progressively higher magnification views of the tip of the pacemaker lead. An extensive biofilm is evident (fig. 2, bottom) and confluent masses of coccoid bacteria are present (fig. 3, top). The surfaces of many of these bacteria were covered by a floccular material. This probably represents exopolysaccharide that has condensed down onto the surface of the bacteria during the dehydration process. Individual bacterial cells are embedded in a background matrix, as evidenced by the pits (fig. 3, bottom) left when individual cells moved during the processing for electron microscopy.

The inner surface of the pacemaker lead was also heavily colonized. In contrast, the outer surface (apart from the tip) showed an adherent population of well-spaced individual bacterial cells. These cells appeared intact despite the preceding intensive antibiotic therapy.

The electrodes that ran down the inside of the pacemaker lead in a circular manner had a massive biofilm covering the coils and their interstices (fig. 4, top). This biofilm was composed of a thick mass of bacterial cells (fig. 4, bottom). Layering of the bacterial cells is also evident in this micrograph.

Finally, transmission electron micrographs of material scraped from the surface of the pacemaker lead showed that these bacteria have a gram-positive cell wall and an extensive surface matrix, probably polysaccharide in nature.

Discussion

The source of S. aureus bacteremia in our patient most likely was his infected olecranon bursa. The bacteria then seeded his pacemaker lead. Examination of the pacemaker lead by scanning electron microscopy revealed differential colonization. Areas with crevices seemed to have massive buildup of a biofilm of S. aureus. This may explain why therapy with antibiotics without removal of the foreign body is usually unsuccessful. One factor important in the pathogenesis of infections involving prosthetic devices is that bacteria in natural and industrial aquatic systems grow predominantly in glycopolymer-enclosed biofilms adherent to surfaces.8 This mode of growth affords these adherent microorganisms some protection from antibiotics, bacteriophages and phagocytes.9 Differential bacterial adhesion has been demonstrated for various epithelial surfaces; for example, within the oral cavity there is preferential bacterial colonization of either tooth or mucosal surfaces.10,11 Similarly, Sugarman and Musher12 showed that adherence of S. aureus and Enterobacteriaceae was 100 times greater to gut suture material than to nylon. Substantial changes have occurred in the construction of pacemaker leads since 1975: They are smaller in diameter and polyurethane has been substituted for Silastic as the insulation material (personal communication: Medtronic representative). Whether such a change will reduce the incidence of secondary bacterial seeding of these leads remains to be seen. The type of infection is also important. S. epidermidis infections of pacemaker implantation sites could be successfully treated conservatively, whereas reimplantation of a new unit in a new clean site was required for infection with all other organisms.13 The reasons for this differential response to antimicrobial therapy need further investigation.

Impregnation of implantable devices with compounds that block adhesion of bacteria to these sur-

![Figure 1](image1.png)

**Figure 1.** Sagittal section of the intracardiac portion of a demand pacemaker lead showing the sites (a–f) examined by scanning electron microscopy.

![Figure 2](image2.png)

**Figure 2.** (Top) Scanning electron micrograph of the pacemaker tip (site a in figure 1). Note the irregularity of the convex Silastic surface and a film of material coating the central metal tip. The bar represents 500 μm. (Bottom) A higher magnification of the area designated by the quadrangle in figure 2 (top). The thickness of the biofilm on this metal surface is indicated by the depths of the cracks that have formed during dehydration. The bar represents 50 μm. Original magnification: (top) × 50; (bottom) × 20.
faces may prevent such infections; antibiotic bonding to polytetrafluoroethylene vascular grafts prevents vascular prosthetic infection in dogs.14

References

FIGURE 3. (top) Scanning electron micrograph of the area delineated by the quadrangle shown in figure 2 (bottom). Note the confluent mass of coccoid bacterial cells. Staphylococcus aureus was recovered from several sites on this pacemaker lead. The bar represents 5 μm. (bottom) A higher magnification of the same area as shown in figure 2 (top) showing floccular material covering the surface of many of the S. aureus cells. Note the pits (arrows), which probably represent areas where individual S. aureus cells had been embedded in the background matrix. The bar represents 5 μm. Original magnification: (top) × 3000; (bottom) × 7000.

FIGURE 4. (top) Scanning electron micrograph of one of the pacemaker wires that runs through the core of the pacemaker lead (site e in figure 1). A massive biofilm covers the coils and their interstices. The cracks in the biofilm indicate the thickness of this material. The bar represents 300 μm. (bottom) A higher magnification of the biofilm covering the surface of the wire. It is composed of a thick mass of bacterial cells that appear confluent. The layering of bacteria is readily evident. The bar represents 5 μm. Original magnification: (top) × 120; (bottom) × 7500.
A scanning and transmission electron microscopic study of an infected endocardial pacemaker lead.
T J Marrie, J Nelligan and J W Costerton

Circulation. 1982;66:1339-1341
doi: 10.1161/01.CIR.66.6.1339

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/66/6/1339

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/