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Scanning Electron Microscopy of Bacteria Adherent to Intravascular Catheters

TIMOTHY R. FRANSON,[1]* NEELA K. SHETH,[2] HAROLD D. ROSE,[1] AND PETER G. SOHNLE[1]

Departments of Medicine[1] and Pathology,[2] The Medical College of Wisconsin, Milwaukee, Wisconsin 53226, and the Wood Veterans Administration Medical Center, Milwaukee, Wisconsin 53193

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Scanning electron microscopy was used to assess the morphological features of coagulase-negative staphylococci adherent to polyvinylchloride intravascular catheter specimens. Clinical specimens were obtained by using patient catheters from which coagulase-negative staphylococci (≥15 colonies per catheter) grew on semiquantitative blood agar roll cultures. In vitro specimens were prepared by a previously published technique in which sterile polyvinylchloride catheters were immersed in 10^6 CFU of coagulase-negative staphylococci per ml suspended in phosphate-buffered saline. Unused sterile polyvinylchloride catheters were also examined. Scanning electron microscopy of unused sterile polyvinylchloride catheters demonstrated multiple linear surface irregularities. Scanning electron microscopy of infected patient catheters showed a diffuse amorphous material covering the entire surface and the presence of bacteria which appeared anchored to that surface by several different means. These included a slime layer, "foot" processes, and lodgement in surface irregularities. Scanning electron microscopy of in vitro specimens demonstrated no background surface coating, but it did show attachment of cocci to the surface by the same mechanisms as described for clinical specimens. These observations of similar means of attachment in clinical and in vitro specimens suggest that intrinsic catheter surface properties, bacterial surface features, and perhaps coating with host substances may all play a role in bacterial attachment to intravascular catheters. More sophisticated analysis of these interactions may clarify mechanisms of pathogenesis.

Infections associated with the use of intravascular devices can be a serious complication of parenteral therapy in hospitalized patients. Intravenous catheter-associated infections have been reported to be related to local skin conditions, catheter placement techniques, and the duration of catheter use at one site (7, 15). However, the initiating event and evolution of the mechanisms leading to clinical infection have yet to be elucidated.

Most authorities now suspect that organisms from either exogenous sources (such as the hands of personnel) or microflora indigenous to patients invade via the catheter insertion wound and migrate along the external catheter surface, eventually colonizing the intravascular catheter segment (13, 14). Bacteremia from infections at distant sites may also seed the catheter surface (17). In either event, the encasement of fibrin, plasma proteins, platelets, and other undefined substances around the external catheter surface may provide a nidus conducive to bacterial implantation. The kinetics of this catheter-organism interaction are not clear, and the catheter per se has been previously thought to be only an inert, innocent bystander in this process. However, recent studies from our laboratory indicate that different catheter materials have varying affinities for bacterial adherence (20).

Studies by Locci et al. (12) and Peters et al. (18) have addressed the morphology of bacterial adherence to catheter lumen and external surfaces in vitro and in vivo by using scanning electron microscopy (SEM). In their studies, it was noted that there were abundant "amorphous deposited substances" surrounding bacteria adherent to these surfaces. Christensen et al. also observed that slime-producing strains of coagulase-negative staphylococci demonstrated increased adherence to plastic surfaces as compared with non-slime-producing strains (G. D. Christensen, W. A. Simpson, E. H. Beachey, A. L. Bisno, and F. F. Barrett, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother., 22nd, Miami Beach, Fla., abstr. no. 649, 1982).

The following study presents findings of a SEM study of coagulase-negative staphylococci adherent to polyvinylchloride (PVC) catheters with clinically-infected specimens and those produced in an in vitro model system.

MATERIALS AND METHODS

Catheters. PVC 16-gauge catheters (Intracath; Deseret Pharmaceutical Co., Sandy, Utah) were used in all phases of study. Two sterile unused PVC catheters were processed for SEM directly upon removal from commercial packaging. Four clinically infected catheters (submitted to the microbiology laboratory for routine processing) which were obtained from four patients showed growth of ≥15 colonies per catheter of coagulase-negative staphylococci on semiquantitative blood agar roll cultures performed by using the method described by Maki et al. (15). Four other in vitro catheter specimens were prepared by using a previously published technique (20), in which sterile 4-cm segments of PVC catheters were immersed for 2 min to 18 h in 10^6 CFU of coagulase-negative staphylococci per ml suspended in phosphate-buffered saline at pH 7.0, washed, and then fixed. All clinical and in vitro specimens were fixed in 2% glutaraldehyde before preparation for SEM.

Bacteria. All clinical isolates of coagulase-negative staphylococci were identified by standard microbiological techniques (8). One clinical strain and one strain of standard American Type Culture Collection (ATCC 12228) coagulase-negative staphylococci (*Staphylococcus epidermidis*) were used for in vitro catheter studies. Strains for in vitro studies were cultured for 4 to 6 h in tryptic soy broth at 37°C, harvested by centrifugation (3,000 rpm for 10 min), and

* Corresponding author.

FIG. 1. SEM of unused sterile PVC catheter surface (magnification, ×100).

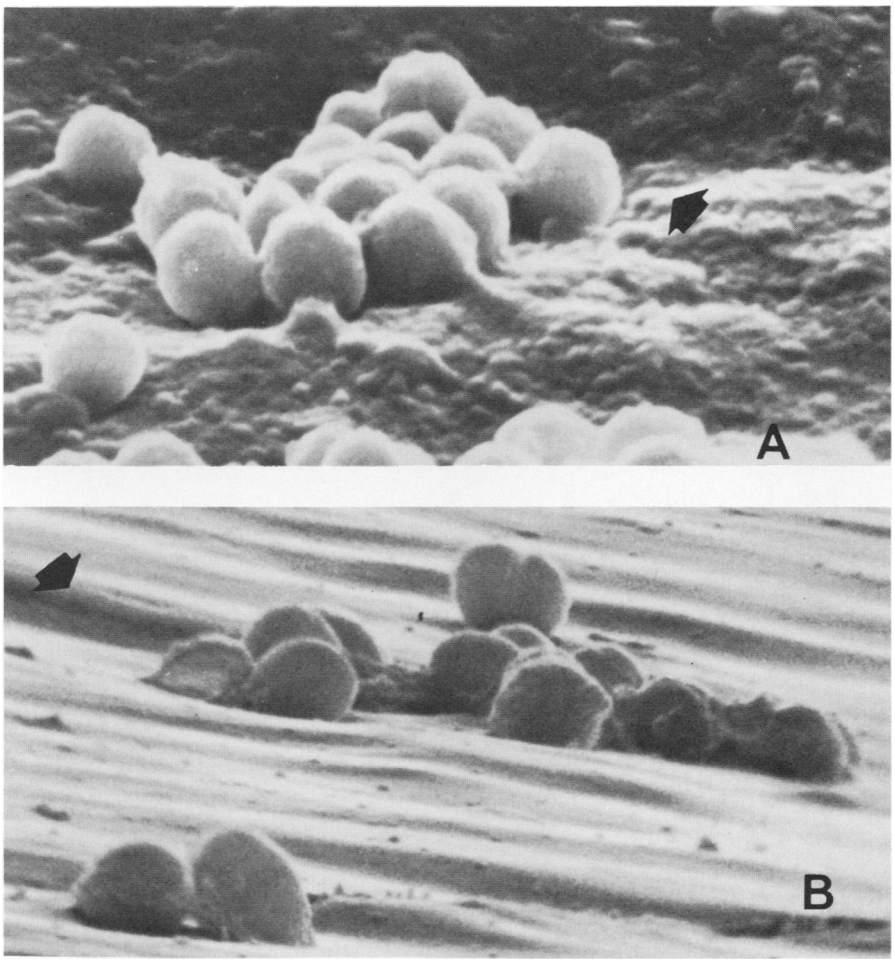

FIG. 2. SEM of coagulase-negative staphylococci on the surface of clinically infected (A) and in vitro prepared (B) PVC catheters: lodgement of organisms in surface irregularities (magnification, ×10,000) (arrow, ridge-like irregularity on catheter surface).

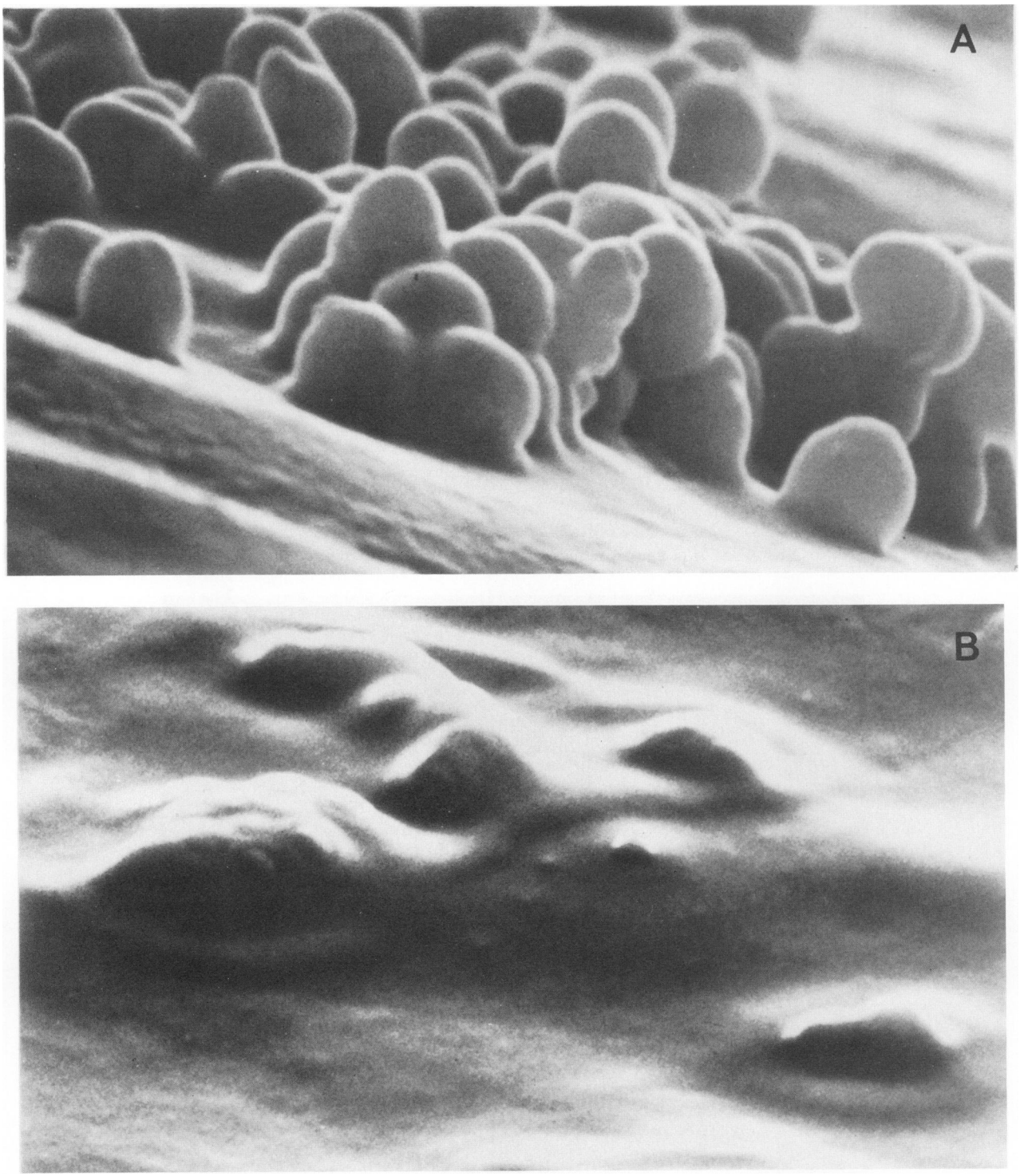

FIG. 3. SEM of coagulase-negative staphylococci on the surface of clinically infected (A) and in vitro prepared (B) PVC catheters: slime layers (magnification, ×16,000 [A]; ×10,000 [B]).

washed twice in 0.01 M phosphate-buffered saline at pH 7.0. Organisms were then resuspended at a concentration of ca. 10^6 CFU/ml in phosphate-buffered saline by visual comparison with a one-half McFarland $BaSO_4$ standard. Samples of the final suspension were plated at various concentrations to obtain exact inoculum counts.

SEM methods. After fixation for a minimum of 4 h in 2% glutaraldehyde, each clinical or in vitro catheter specimen was washed three times in phosphate buffer and placed in 2% phosphate-buffered osmium, followed by three rinses in distilled water. Specimens were then serially dehydrated in ethanol and subjected to critical-point drying with carbon

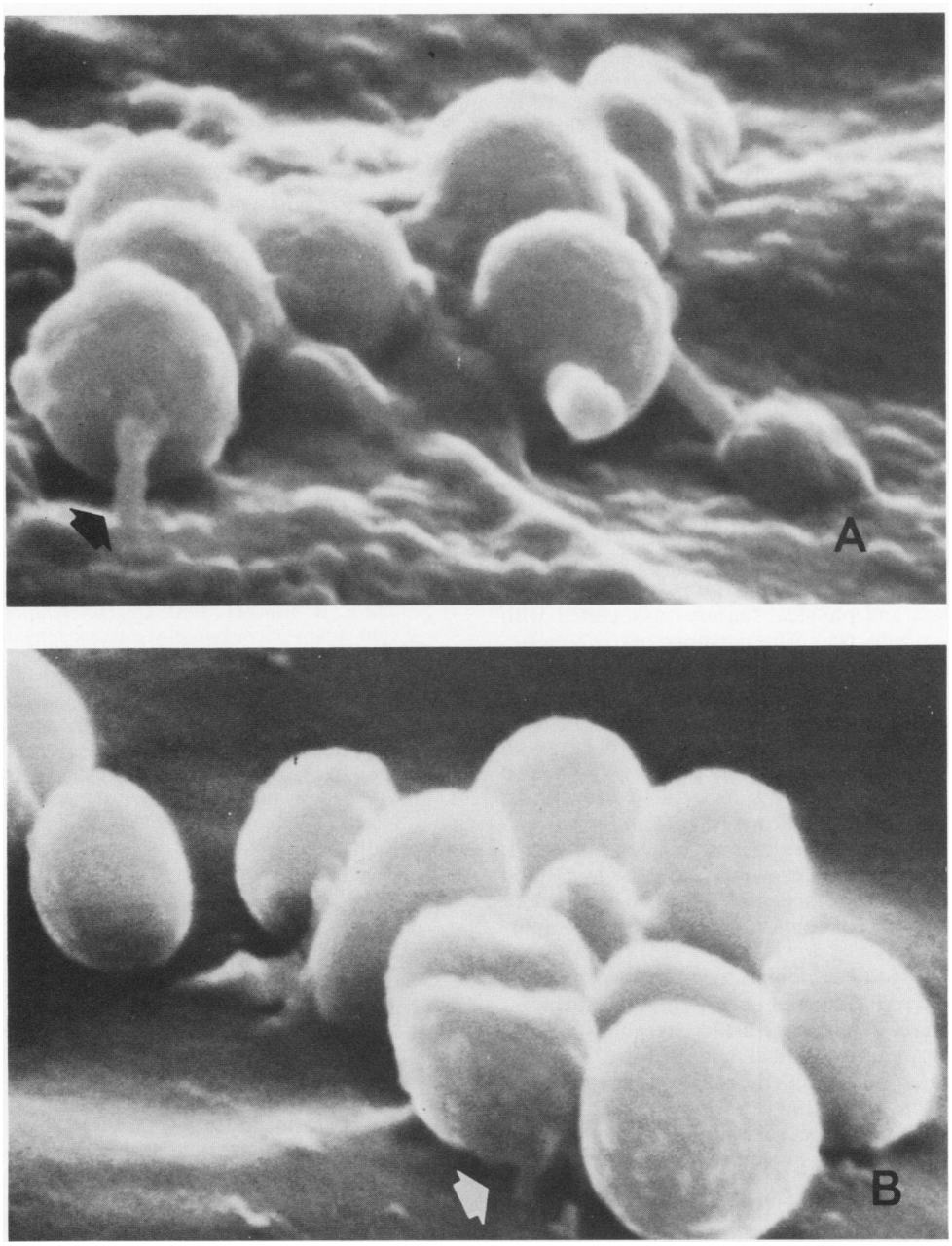

FIG. 4. SEM of coagulase-negative staphylococci on the surface of clinically infected (A) and in vitro prepared (B) PVC catheters: foot processes (arrows) (magnification, ×20,000).

dioxide in a Pelco Critical Point Drier (Ted Pella, Inc., Justin, Calif.), mounted on metal stubs, and coated with gold-palladium. Each specimen was viewed in an AMR model 1000 SEM (Advanced Metallurgical Research, Bedford, Mass.) at magnifications of ×50 to ×20,000. Photographs of representative findings were obtained.

RESULTS

This study was designed to assess morphological features of coagulase-negative staphylococci adherent to PVC catheters by SEM, with clinically infected specimens and specimens produced in the in vitro model system described above.

Sterile unused PVC catheters. The surfaces of the two sterile PVC catheters were characterized by multiple gentle linear irregularities and some particulate debris (Fig. 1).

Clinically infected catheters. SEM of all four clinical specimens demonstrated diffuse amorphous material covering the catheter external surface. Bacteria appeared to be attached to the surface by several different means, including lodgement in surface irregularities (Fig. 2A), a slime layer surrounding the bacteria (Fig. 3A) in two specimens, and apparent "foot" processes (Fig. 4A) in three specimens. The latter foot processes are ca. 0.5 by 0.1 μm in size and appear to project from the bacterial cell wall to the catheter surface in single or multiple linear configurations.

In vitro catheter specimens. SEM of four in vitro speci-

mens demonstrated no background surface coating but did show attachment of cocci to the catheter surface by apparent lodgement in surface irregularities (Fig. 2B), a slime layer surrounding the cocci (Fig. 3B), and foot processes (Fig. 4B). These foot processes were observed on three separate specimens.

DISCUSSION

The mechanisms by which colonization of intravascular catheters with bacteria occurs have yet to be defined. Several studies have examined factors which may relate to this phenomenon. Locci et al. (11) have observed intrinsic irregularities on the surfaces of unused catheters by SEM. Amorphous deposited substances or glycocalyx have been noted surrounding bacterial colonies on the surface of catheters in vitro (5) and covering the surfaces of bacteria in nature (7), therefore suggesting that bacteria may elaborate or attract substances that solidify their attachment to inert surfaces. Recent studies suggest that these deposited substances are exopolysaccharides which can be produced by organisms such as coagulase-negative staphylococci (4, 5) and *Pseudomonas aeruginosa* (2), strains of which have identifiable chemical and physical features associated with exopolysaccharide production. The presence of exopolysaccharide surrounding bacteria in clinical specimens has been observed to be associated with symptomatic infections involving intravascular catheters (3, 4, 12) and peritoneal dialysis catheters (16), suggesting that the ability of bacterial strains to elaborate such substances may positively influence the development of foreign-body associated infections. Leake et al. (10) have also shown that *Staphylococcus aureus* and *P. aeruginosa* adhere to and grow on polyethylene and stainless steel surfaces when incubated in blind chemotaxis chambers. Other factors such as the presence of trace metals (1) and hydrophobicity (19) may also be important in such interactions.

The findings in our study demonstrate several morphological correlates of bacterial attachment to catheter surfaces. Lodgement of bacteria in catheter surface irregularities was observed to occur in both clinical and in vitro settings. However, the gentle nature of the surface irregularities probably does not fully account for trapping bacteria on catheter surfaces. Furthermore, it was observed that both slime layers and foot processes were present in most specimens, surrounding coagulase-negative staphylococci adherent to clinical and in vitro PVC catheter specimens. The amount of slime layer observed surrounding coagulase-negative staphylococci appeared to increase with increased incubation time for in vitro specimens. This observation is consistent with previous reports by Peters et al. (18) and suggests some ongoing interaction between catheter and bacterial surfaces beyond that of an initial attachment stage. This finding is of interest as it has been postulated that bacterial attachment to catheter surfaces in patients may depend in part on coating of the catheter with host substances such as plasma proteins and platelets (21). Our observations suggest that bacterium-catheter interactions may occur independent of such host substances, as the attachment processes in vitro were identical to those seen in clinical specimens. The exact nature of these attachment processes and their genesis remains unclear, but we have observed that the attachment of coagulase-negative staphylococci to PVC catheters in vitro can be inhibited by the addition of D-mannosamine (9). Whether such observations will lead to further clues in elucidating the interactions of bacteria, catheters, and host substances remains to be shown, but a complete understanding of the pathogenesis of intravascular catheter-associated infections is necessary before interruption of these processes can be effected clinically.

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