Bacterial adhesion to cerebrospinal fluid shunts

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Bacterial adherence to cerebrospinal fluid (CSF) shunts was analyzed in vivo and in vitro. Scanning electron micrographs (SEM's) of catheters removed from pediatric patients with shunts infected by Staphylococcus aureus or Klebsiella pneumoniae revealed numerous bacterial cells and microcolonies, leukocytes, and erythrocytes attached to the CSF catheters' inner walls, as well as the existence of surface irregularities, such as fissures, rugosities, and holes. Permeability analyses and SEM's demonstrated that catheters develop physical alterations over the period of implantation. Different bacterial strains presented a different in vitro adherence to CSF shunts, suggesting that this attachment may be affected by specific properties of the outer structures of each strain.

The attachment of microbial pathogens to CSF shunts seems to contribute to the persistence of bacterial cells within a catheter and the onset of recurrent shunt infection. This study demonstrated that some bacteria can remain attached within shunts in vitro despite a CSF flow at rates up to 200 times higher than those normally demonstrated in vivo. Furthermore, surface irregularities found throughout this study may help to anchor and hide bacterial microcolonies. Based on these findings, it seems advisable to remove an infected shunt and to replace it with a new one after proper antimicrobial therapy, in order to prevent recurrent infections.

**KEY WORDS**  • bacterial adhesion  • cerebrospinal fluid  • shunt  • hydrocephalus

A present, the most widely used and successful treatment of hydrocephalus involves shunting excess cerebrospinal fluid (CSF) into an extracranial body compartment. Ventriculoperitoneal (VP) shunts are most commonly used; they generally involve less serious complications, and surgical procedures are faster and easier than alternative routes. Complications of VP shunts include mechanical malfunctions (such as disconnection, breaking, and plugging), occlusion of the abdominal tip, migration of the shunt, and perforation of the viscera. Infection constitutes a major problem associated with ventricular shunt procedures, the management of which is still a controversial issue. Some authors recommend removal of the infected shunt in conjunction with antimicrobial therapy and implantation of a new shunt after the infection has been cleared. Other authors suggest sterilization of the CSF shunt with the device in place; if shunt malfunction occurs, shunt replacement should be performed at the time of revision surgery. O'Brien, et al. recommended that the decision as to whether to remove an infected shunt should be based on a protocol that varies according to the type of shunt infection involved in each case. They divided shunt infections into two major groups: those external and those internal to the shunt. The latter are further subdivided into Gram-positive and Gram-negative internal infections.

Colonization of implanted medical devices seems to occur more commonly with bacterial strains able to adhere to smooth surfaces. The adhesion of microorganisms to various medical implants has been reported, and bacterial adhesion has been associated with the persistence of pathogens in infections of indwelling foreign bodies. The analysis of bacterial adhesion to CSF shunts and bacterial persistence in these systems should contribute to the understanding of the pathogenesis and therapy of shunt infections.

We have previously analyzed VP shunts by scanning electron microscopy (SEM), and have demonstrated the existence of topographical irregularities present on the internal surface of both used and unused catheters.
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These observations suggest that irregularities might help to anchor and hide bacterial cells within the shunt. Moreover, we have recently reported the in vitro adherence of some strains of *Klebsiella pneumoniae* to VP catheters. In this paper we further analyze the in vitro and in vivo bacterial adherence to CSF shunts. The results presented here support the conclusions of those who suggest the removal of the entire shunt as the safer procedure in the therapy of infected ventricular shunts.

**Materials and Methods**

*Scanning Electron Microscopy Procedures*

The components of 15 different VP shunts with valves (manufactured by Holter, Hakim, and Medix-Carrea) were obtained in the course of revision surgery prompted by shunt malfunction and infection in pediatric patients. The period of implantation ranged from 6 months to 5 years. Several unused systems were also included in this study. Immediately after operation, specimens were rinsed twice in saline solution, usually cut in 1-cm pieces, split longitudinally, and fixed, washed, and dried as previously described. After drying, the material was placed in a vacuum evaporator and coated with carbon and gold: palladium (60:40). Finally, the samples were examined under a scanning electron microscope.

*Catheter Permeability Analyses*

The ventricular catheters under study were cut into 10-cm segments. With both ends elevated, each catheter section was carefully filled with 100 μl of aqueous solution of tritiated (3H)-uridine (50 μCi/ml) by means of a tuberculin syringe, and then placed in a small glass vial containing 3 ml of distilled H₂O. Both ends of each catheter fragment were sealed with clay and left outside the vial. After 1, 3, 5, and 23 hours of incubation at room temperature, 0.2-ml aliquots of the aqueous solution were withdrawn from the outer compartment, mixed with 10 ml of Liquiscint, and evaluated for radioactivity using a liquid scintillation counter. Counting efficiency was 55%. To evaluate the passage of viable bacterial cells across the tubing walls, a 10-cm piece of two separate ventricular catheters (unused and used) was washed with saline solution and sterilized by autoclaving. After sterilization, all the following procedures were conducted within a biological safety cabinet under a sterile laminar flow to prevent external contamination. With both ends elevated, each section of catheter was filled with a fresh culture of a nosocomial strain of *Staphylococcus aureus*, isolated from an infected CSF, and grown in nutritive broth. Each piece was then placed in a glass vial containing sterile nutritive broth. Both ends of the catheter piece were kept outside the vial, covered with sterile gauze and aluminum foil. Finally, the upper half of the vial was also covered with sterile aluminum foil and the vial was transferred to an incubator maintained at 37°C. Samples from the external nutritive broth were withdrawn daily and cultured for the presence of micro-organisms. The viability of *S. aureus* cells inside the catheters was also checked and found to be stable during the entire experiment.

*Bacterial Adherence Strength Assays*

Three different nosocomial multiresistant *K. pneumoniae* strains were used: 1) *K. pneumoniae* strain KD08, isolated from an abscess of a patient with a lower respiratory infection; 2) *K. pneumoniae* strain KMD01, isolated from the infected CSF of a hydrocephalic child with an implanted VP shunt (this strain has been shown to carry two cryptic plasmids of 2.5 and 85 megadaltons, respectively, and a multiple resistance plasmid of 50 megadaltons); and 3) *K. pneumoniae* strain KMD02, isolated 1 month later from the infected CSF of the same hydrocephalic child. This strain had the same antimicrobial susceptibility pattern as strain KMD01, but the plasmid pattern was slightly altered. A 100-megadalton plasmid was observed in this strain instead of the 85-megadalton plasmid observed in strain KMD01. Each strain was grown in adherence medium containing 1.2 M NaCl, 0.012 M CaCl₂, 0.04% bovine serum albumin, and 0.08% glucose (pH 7.0), a composition aimed to resemble that of natural CSF, supplemented with 40 μg/ml ampicillin and 20 μg/ml gentamicin. After overnight growth in an incubator maintained at 37°C, bacterial cell concentration was adjusted to 1 × 10⁸ viable cells/ml with adherence medium: 50 μl of this suspension was inoculated into a 4-cm section of unused ventricular catheter. After incubation for 30 minutes at room temperature inside a sterile chamber, one end of the catheter was connected to a syringe driven either by hand or by a peristaltic pump in order to generate various calibrated flows of adherence medium. The other end of the catheter segment was suspended over a series of sterile tubes placed on a fraction collector, and 20 consecutive 10-ml samples flowing out of the silicone tube were collected. To determine the number of viable colony-forming units, 0.1-ml serial aliquots of bacterial suspensions diluted 10:1 in liquid medium were spread onto nutritive agar plates. After overnight incubation at 37°C, the number of colonies was counted. After washing was completed, the catheter segment was split longitudinally under sterile conditions and the presence of viable bacterial cells attached to the inner surface of the catheter was evaluated by culture of the two halves of the tubing in nutritive broth. Bacterial strains were confirmed by standard identification procedures, and antibiotic sensitivities were tested by disc diffusion.

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* Tritiated (3H)-uridine solution obtained from New England Nuclear, Boston, Massachusetts.
† Liquiscint solution obtained from National Diagnostics, Somerville, New Jersey.

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Results

Scanning Electron Microscopy of Shunts from Infected Patients

The inner surface of VP shunts removed from patients with infected CSF was investigated by SEM. Catheters from patients infected with *S. aureus*, *Staphylococcus epidermidis*, or *K. pneumoniae* were selected for this study due to the frequency and severity of infections related to these pathogens in this hospital.

The SEM analysis of these systems revealed the presence of bacterial cells, leukocytes, erythrocytes, and fibrin-like amorphous material attached to more than 90% of the samples examined. However, the amount of biological material adhering to the walls of the catheter showed a wide variation. Figure 1A and B show *K. pneumoniae* cells adhering to the inner surface of a valve from a used VP shunt. Several sections of the

![Image of SEM images showing bacterial cells adhering to the inner surface of a valve from a used VP shunt.](image-url)
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Ventricular and peritoneal catheters of this shunt were examined as well, and all presented well-defined bacterial cells and colonies; however, the largest density of colonization by *K. pneumoniae* cells was found in the valve of this system. At high magnification, a typical erythrocyte can be seen (Fig. 1B), demonstrating that the sample preparation and SEM procedures employed did not introduce significant changes in the materials under observation. Samples of two ventricular catheters removed from different infected patients are illustrated in Fig. 1C and D, showing chains of attached *K. pneumoniae* cells, which suggests that these bacterial cells were actively dividing while adherent to the inner surface of the catheter wall. Figure 1B and D also show fissures and rugosities along the inner surface of the valve and ventricular catheter, respectively. These surface alterations are characteristic of shunts that have been implanted for 1 year or longer.17

Figure 2A is a composite of two scanning electron micrographs showing a colony of *S. aureus* on the inner surface of a ventricular catheter obtained from a removed shunt. The colony is clearly encased in a bacterial film or slime (presumably constituted by extracellular polysaccharide material36) with an erythrocyte anchored to it. Another colony of *S. aureus* is seen hidden in a cavity about 10 μm in diameter, and again the cells are covered by a slimy material (Fig. 2B). Figure 2C shows the inner surface of an unused ventricular catheter after being incubated *in vitro* for 30 minutes with a culture of an *S. aureus* strain isolated from infected CSF from the preceding case. The two *S. aureus* cells attached to the catheter seem to have developed some projections of cementing material that presumably contribute to their adherence. With a longer incubation time, more cells and colonies were observed,

![Figure 2A](image)

![Figure 2B](image)

![Figure 2C](image)

**Fig. 2.** Scanning electron micrographs of *Staphylococcus aureus* cells attached to cerebrospinal fluid shunts. A: Composite view of a colony of *S. aureus* that had developed on the inner surface of an infected ventricular catheter. × 7660. B: View of a colony of *S. aureus* partially covered by a slimy material and hidden inside a hole in the inner surface of the catheter shown in A. × 10,670. C: Higher magnification of two *S. aureus* cells *in vitro* attached to the surface of an unused catheter after 30 minutes of incubation. × 15,500.
as well as increasing amounts of the slimy material around them.

**Permeability of Ventriculoperitoneal Catheters**

Unused valve systems from different commercial sources have been shown by SEM to contain irregularities such as fissures, depressions, faulty polymerization, and cavities. Similar observations have been made on unused intravenous catheters by Locci, et al. Over the implantation period, the inner surface of implanted catheters develops an increasing number of irregularities, such as wrinkles, fissures, and irregular holes. In addition, the presence of microcolonies of bacterial cells inside these holes was frequently observed during this study (Fig. 2B); however, no data about the depth of these holes were available.

To test the permeability of these catheters, passage through the catheter wall of a radiolabeled nucleotide (3H-uridine) with a molecular diameter of less than 1 nm was evaluated. Two different ventricular catheters (one from a new shunt system and the other from a shunt implanted for 2 years) were included in this analysis. The 3H-uridine passed slowly through the catheter walls into the external solution via a typical diffusion function, the rate of diffusion being lower for the unused than for the removed catheter (Fig. 3). Similar results were observed with other pairs of used and unused catheter pieces. These results support previous data demonstrating that catheters develop physical alterations during implantation.

A similar approach was used to evaluate the possibility that bacterial cells could migrate through the catheter walls via the above-mentioned holes and other irregularities. For this purpose, two different catheters (one used and one unused) were filled with a fresh culture of S. aureus and submerged under sterile conditions within vials containing nutritive broth (see Materials and Methods). In none of the catheter preparations analyzed did micro-organisms appear in the outer compartment, even after 7 days of incubation at 37°C. Additional pairs of used and unused catheters were tested as before with the same results, suggesting that the 10- to 20-μm holes revealed on the inner surface of some VP catheters by SEM were not deep or wide enough to allow the passage of bacterial cells across the catheter walls to the external surface of the tubing.

**Strength of Bacterial Adherence and CSF Flow**

Since bacterial cells cannot migrate through the catheter walls via the irregularities usually observed by SEM, as shown above, the presence of attached bacterial cells and subsequent colonization of a shunt may be attributed to micro-organisms that gain access into the lumen of the catheter either by descending from the ventricles or by ascending from the peritoneal cavity. Bayston and Spitz have demonstrated this last alternative to be unlikely, even in the presence of a shunt with a refluxing valve. In either case, bacterial cells able to adhere to the inner surface of the catheter would have an increased chance to persist and colonize a shunt despite the "clearance" effect of the CSF flowing down along the system. Information regarding the strength with which different bacterial strains are attached to the catheters in spite of a descending flow of CSF should contribute to the understanding of shunt colonization. For this study, capability of attachment of three different nosocomial strains of *K. pneumoniae* to ventricular catheters *in vitro* under a flow of artificial CSF (see Materials and Methods) was evaluated. An equal number of viable cells of each strain was inoculated inside similar segments of ventricular catheter, the preparation was incubated for 30 minutes at room temperature, the tube was washed out with artificial CSF at a flow rate of 200 ml/min, and the washing fluid was collected and cultured. Figure 4 shows the progressive release of attached bacterial cells with an increasing number of washes. The *K. pneumoniae* strain KD08, which originated from a patient without an infected shunt, showed the lowest adherence capacity. After the fifth wash, both the fluid and the washed catheter were negative after culture. Both strains of *K. pneumoniae* isolated from infected CSF obtained from the valve of an implanted shunt (strains KMD01 and KMD02, see Materials and Methods) demonstrated a higher adherence capacity. The *K. pneumoniae* strain KMD01 produced 15 consecutive positive fractions, yet with a decreasing count. The *K. pneumoniae* strain KMD02 presented the highest adherence strength, all 20 washes and the remaining catheter giving positive cultures. The results shown in Fig. 4 suggest that each *K. pneumoniae* strain under
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**Figure 4.** Graph showing the rate at which *Klebsiella pneumoniae* cells attached to ventricular catheters in vitro were released by a flow of artificial cerebrospinal fluid (CSF). Three segments of ventricular catheter were inoculated with *K. pneumoniae* strain KD08 (squares), strain KMD01 (circles), or strain KMD02 (triangles), respectively. The flow rate of CSF was 200 ml/min, and the wash volume was 10 ml. The amount of bacteria released in each wash is expressed as a percentage of the total colony-forming unit recovered throughout the whole washing procedure.

Analysis had a characteristic adherence capacity, and that this property may contribute to the particular pathogenicity of each strain. With this experimental design it was also possible to characterize the adherence capacities of other *K. pneumoniae* strains, and of *Escherichia coli* and *S. aureus* strains.

**Discussion**

Bacterial adhesion to epithelial cell surfaces plays an important role in human pathogenic processes such as infections of the oropharynx and of the urinary tract. Similarly, bacterial adherence to smooth surfaces of inert prosthetic devices is a critical factor in colonization of medical prostheses such as vascular catheters, or intrauterine contraceptive devices, and urinary catheters. Results presented in this paper confirm and extend previous observations that bacterial cells can attach to CSF shunts as well. Early work by Bayston and Penny suggested that the production of a mucoid substance by some *S. epidermidis* strains may play a role in colonization of CSF shunts. In addition, Barrett has recently shown that some strains of *S. aureus* were able to attach in vitro to a suspension of crushed CSF-shunt silicone polymer. The SEM studies presented in this paper allowed visualization of *S. aureus* and *K. pneumoniae* cells attached in vivo and in vitro to CSF catheters. The adherence of bacteria to these catheters seems to follow a dynamic time-dependent process, where single cells are able to adhere firmly, divide, and form microcolonies on the surfaces of the shunt. In addition, the existence of surface irregularities along these tubes was revealed by SEM, suggesting a relationship between the topographical distribution of attached bacteria and these irregularities.

Flow of CSF through an implanted shunt has been estimated to fluctuate between 0.02 and 0.78 ml/min in a 24-hour period. Our results show that viable *K. pneumoniae* cells attached to the inner surface of shunt tubes in vitro during a 30-minute preincubation time, can be found still adherent even after CSF has passed through the tube at flow rates up to 200 times higher than those normally demonstrated in vivo. These results demonstrate the colossal adherence capacity to silicon tubes inherent in some strains of *K. pneumoniae*. The finding that various bacterial strains analyzed here possessed a different capability of adherence to CSF shunts suggests that this kind of attachment may be mediated by specific properties of the outer structures of each particular strain. Moreover, we have recently presented evidence that the adherence capacity of some *K. pneumoniae* strains to CSF shunts and other smooth surfaces is genetically correlated with the presence of some deoxyribonucleic acid (DNA) extrachromosomal elements. The experimental approach shown in Fig. 4 has since been useful in characterizing a number of different bacterial strains of *S. aureus* and *S. epidermidis* according to their adherence strength. Similarly, Christensen, et al., have recently shown that coagulase-negative staphylococcal strains associated with CSF shunt infections had a higher in vitro adherence capacity to plastic plates than had saprophytic strains that were used for comparison. The methodology presented in this paper, added to a list of other experimental procedures intended to evaluate cell adherence, should contribute to epidemiological studies of bacterial strains involved in shunt infections, as well as to the analysis of those factors involved in the bacterial adhesion to CSF shunts.

The attachment of microbial pathogens to CSF shunts seems to be a decisive factor contributing to the persistence of bacterial cells within a catheter and the onset of recurrent shunt infection. The SEM studies presented here demonstrated that some of the bacterial cells and microcolonies found attached to the surface of CSF shunts were encased within a layer of slimy material. Bacterial microcolonies attached to smooth surfaces are generally surrounded by a rather heterogeneous glycocalyx (composed mainly of a mixture of polysaccharides and proteins) that gives them effective protection against a number of antibacterial agents such as chemicals, antibodies, and phagocytic leukocytes. Johnson, et al., suggested that *S. epidermidis* slime may interact and interfere with human neutrophils and may contribute to the persistence of this organism on
the surfaces of foreign bodies in the vascular system or central nervous system. In addition, Franson, et al., have recently reported that coagulase-negative Staphylococci adherent to intravascular catheters are particularly capable of in vitro survival under adverse nutritional conditions during extended periods of time. They suggested that this phenomenon may be related to the genesis of occult prosthetic-associated infections. Similarly, Marrie, et al., demonstrated the persistence of Staphylococcus microcolonies attached to an implanted endocardial pacemaker even after several massive courses of antibiotic therapy without removal of the prosthesis. Furthermore, surface irregularities found throughout this study with higher frequency in shunts that had been implanted longer may help to anchor and hide bacterial microcolonies, and to favor pathogen persistence. Our results also demonstrated that some attached bacteria can persist successfully in shunts despite the continuous flow of CSF through the catheters. It seems to be advisable therefore to remove an infected shunt, and to replace it with a new one after the patient has received proper antimicrobial therapy, in order to prevent recurrent infections.

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