Biofilm Formation on Clinically Noninfected Penile Prostheses

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Purpose: Biofilms are matrix enclosed bacterial populations that adhere to each other and/or to surfaces of implanted medical devices. Biofilm formation has consistently been demonstrated in association with infected penile prosthetic material. Clinically noninfected patients undergoing revision for mechanical malfunction have a surprisingly high rate of positive intraoperative cultures. After revision replacement prostheses have a higher rate of postoperative infection than first time implants. We characterized biofilm formation on penile prostheses in clinically noninfected patients undergoing revision surgery.

Materials and Methods: Ten patients undergoing revision or removal of inflatable penile prosthetic devices due to mechanical malfunction were included. Specimens from the corporeal cylinders, scrotal pump and reservoir were analyzed. Bacterial biofilm coverage was detected and characterized using confocal scanning laser microscopy.

Results: Bacterial biofilm formation associated with multiple microorganisms was demonstrated on 8 of 10 prostheses. Biofilms consisted of gram-positive rods, cocci and fungal elements.

Conclusions: The degree of biofilm formation on these prosthetic devices suggests that most patients have bacterial coverage on the implant. Host mechanisms to control infection may lead to a homeostatic balance that enables biofilms to exist on the surface of the prosthesis without generating clinical infection. A critical threshold of biofilm extent may exist beyond which clinical infection may occur. These results justify further evaluation of biofilms and penile prosthesis infections. Furthermore, the findings help to explain why strategies such as mini salvage procedures to eliminate subclinical biofilms may decrease the postoperative infection risk in patients undergoing repair or replacement of penile prostheses.

Key Words: penis, prostheses and implants, biofilms, infection

In nature bacteria and microorganisms exist primarily by attaching to and growing on a range of living and inanimate surfaces. These adherent bacteria grow in colonies called biofilms, which Costerton et al broadly defined as "a matrix enclosed microbial population adherent to each other and/or surfaces or interfaces."[1] In medicine biofilms have a major impact on temporary and permanent devices placed in the human body. Implanted prosthetic devices are at increased risk for biofilm colonization because they lack the protective mechanisms of healthy tissue surfaces. Generally bacterial contamination in the vicinity of the device leads to rapid biofilm formation.[2-4] Biofilms have been shown to form on central venous catheters, prosthetic heart valves, artificial hip prostheses and intrauterine devices. Relevant to urology, biofilms have been found on urethral catheters, ureteral and prostatic stents, artificial urinary sphincters and penile prostheses.[5-8]

The biofilm growth mode offers protection against host defenses and many antimicrobial agents, such as antibiotics and biocides, which they can withstand at concentrations 1,000 to 1,500 times higher than the concentrations that kill freely floating (planktonic) bacteria of the same species.[9] Therefore, biofilms are extremely difficult to prevent and even more difficult to eradicate after they have formed, creating a high probability for persistent infection.[10,11]

Bacteria in a biofilm are phenotypically different from planktonic bacteria and they can alter their local environment, further distinguishing them from planktonic cells. Biofilm bacteria can maintain slow growth rates and remain quiescent for long periods. These factors can pose problems for laboratory bacterial culture procedures, which were developed to identify planktonic bacteria. Clinical culture techniques are often unable to detect bacteria present in a biofilm, which can make the detection of biofilms in an implanted device a diagnostic dilemma.[1] Licht et al reported that 43% of penile prostheses and 36% of artificial urinary sphincters cultured organisms at revision.[12] More recently Henry et al presented data that showed a 70% culture positive rate in clinically noninfected penile prostheses.[13] Moreover, Henry et al observed that doing a washout of implant

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Microscopy gram stain results in 3-piece inflatable penile prostheses

Implant no.	Implant Type	Bacteria	Biofilm	Biofilm Site	Bacteria
1	Alpha 1®	No	No	—	
2	Alpha 1®	Yes	Yes	Cylinder	Gram-neg rods, gram-pos cocci, yeast
3	700 CX™	No	No	—	
4	Alpha 1®	Yes	Yes	Cylinder, pump, reservoir	Gram-neg rods, gram-pos cocci
5	700 CX™	Yes	Yes	Cylinder, pump, reservoir	Gram-neg rods, multiple species of gram-pos cocci, yeast
6	700 CX™	Yes	Yes	Cylinder, pump, reservoir	Gram-neg rods, gram-pos cocci, yeast
7	Alpha 1®	Yes	Yes	Cylinder, reservoir	Gram-neg rods, gram-pos cocci, yeast
8	Alpha 1®	Yes	No	Bacteria only on all 3 components	Single cells of gram-pos cocci on all 3 components
9	Alpha 1®	Yes	Yes	Pump, reservoir	Gram-neg rods, gram-pos cocci, yeast
10	700 CX™	Yes	Yes	Pump, reservoir	Gram-neg rods, gram-pos cocci, yeast

spaces at revision surgery lowered infection rates in clinically noninfected cases.[14] It is believed that revision washout removes the biofilm from the implant spaces, thereby decreasing the bacterial load at reimplantation. Probably the act of mechanically removing the bacteria is more important than the specific antibiotic protocol used at the time of revision washout. However, to our knowledge there is no published study of whether biofilm truly exists on the implants. Therefore, we characterized the extent of biofilm formation on clinically noninfected penile prostheses.

MATERIALS AND METHODS

Ten patients with a penile prosthesis underwent revision or explantation for mechanical failure between February 2003 and September 2003 at Duke University Medical Center, as performed by a single surgeon (CFD). All 10 patients had a 3-piece inflatable penile prosthesis with no clinical evidence of infection preoperatively. None of the patients had pain associated with the prostheses and all were more than 2 years out from implantation.

After surgical removal of the entire device pieces of biomaterial, each approximately 1 cm long, were excised from the pump, cylinder and reservoir of each prosthesis. If there was visible evidence of biofilm, strips were excised from the affected area. Excised segments were immediately placed into ethanol to prevent further bacterial growth or contamination. They were sent for further analysis to the Center for Biofilm Engineering, Montana State University.

Upon receipt at the Center for Biofilm Engineering each sample was cut with a sterile razor into 3 subsections. The first section was stained with Gram stain, similar to Gram staining of histological samples. Samples were initially rinsed in deionized water and then stained following standardized Gram protocols, in which the samples were subsequently washed with crystal violet for 2 minutes, in iodine for 3 minutes, in decolorization solution for 30 seconds and in safranin for 1 minute using a Gram stain kit (Fisher Scientific, Hampton, New Hampshire).[15,16]

The second sample section was stained with Giemsa stain by first dehydrating it in 100% ethanol. The section was then washed in methanol, followed by a 5-minute stain in Wright stain, pH 6.8. Subsequently sterile deionized water was added to the Wright stain and the section was let to stand for an additional 5 minutes. The samples was then soaked in Giemsa stain for 45 minutes, rinsed with sterile deionized water and again dehydrated in a series of 70%, 95% and 100% ethanol.

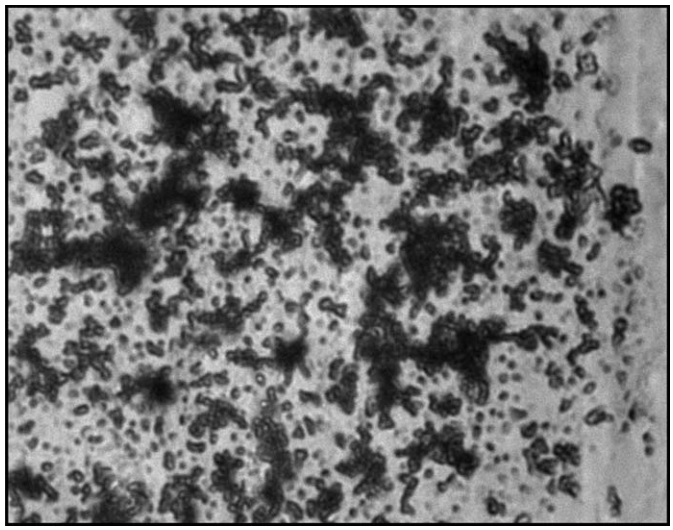
The third section of the prosthesis was treated for fluorescence in situ hybridization. However, due to the fluorescent nature of these devices it was not possible to obtain meaningful data from these sections.

Following staining samples were viewed under an Eclipse E800 epifluorescence microscope (Nikon, Melville, New York). Image capture was performed using WinView/32 software (ViewPoint Software, London, United Kingdom) and a CCD-782-Y camera (Princeton Instruments, Trenton, New Jersey). Images were then analyzed to determine the presence, type and morphology of biofilm.

RESULTS

All 10 patients had no evidence of clinical infection at the time of removal for mechanical failure. All 10 implants were 3-piece inflatable penile prostheses, of which 6 were an Alpha 1® and 4 were a 700 CX™. None of the 10 implants removed had an antibiotic coat, that is none had an InhibiZone™ or Titan™ coating.

Bacteria were found on 8 of the 10 (80%) prostheses (see table). Seven of the 10 prostheses had biofilm present (see figure). One prosthesis had biofilm only on the cylinder, 2 had biofilm only on the pump and reservoir, 1 had biofilm only on the cylinder and reservoir, and 3 had biofilm on all components. A single prosthesis had single bacteria cells present on the cylinder, reservoir and pump but no biofilm formation was present. On 2 prostheses no organisms of any type were found.



Biofilm development of coccus bacteria from pump. Reduced from ×400.

DISCUSSION

Penile prostheses are an accepted and efficacious treatment for erectile dysfunction, yielding remarkably high satisfaction rates. Improvements in mechanical reliability have markedly decreased the need for revision for mechanical failure in the last several decades. In fact, the devices are thought to be more often revised because of patient specific factors, such as infection and medical problems, rather than because of mechanical failure.[13]

Most authorities believe that genitourinary prosthetic infection is caused by contamination of the implant at surgery and bacteria on the implant inevitably causes infection.[6] Studies show that preoperative nasal swab cultures of certain Staphylococcus species significantly correlated with the development of postoperative surgical site wound infections in general surgical cases.[17] Hematogenous late infections can occur but they are thought to be rare.[18] After adherence to the implant many Staphylococcus species produce a protective mucin coat or biofilm.[4-8] Bacteria present in the biofilm may survive at a lowered metabolic rate chronically without the patient realizing that bacteria are present in the implant spaces. Occasionally bacteria are released from the biofilm in planktonic fashion and they may cause symptoms.[10] Antibiotics or the defense mechanisms of the body can kill these planktonic bacteria. However, those organisms in the biofilm are protected and they cannot be eradicated except by removal of the implant and lavage of the implant spaces.

In 1996 Brant et al reported salvage success with clinical infections.[19] Their method, which has been successfully repeated by others, involves removal of the infected device, sequential lavage of antiseptic solutions to sterilize the implant space and immediate reimplantation of a sterile replacement device. The new implant is placed only after the complete implant has been removed and the entire capsular space is thoroughly irrigated. We believe that the success of this technique for eradicating infection is predicated on the thorough removal of bacteria and biofilm and not on the specific antibiotic protocol used for lavage. Perhaps the increased infection rate in revision prosthetic surgery is due to the activation of preexisting biofilms.

Multiple studies in the medical literature have indicated an increased risk of infection when repeat operations (revisions) are performed on genitourinary prostheses.[12,13,20] This increased incidence of infection associated with reoperation has been postulated to result from decreased host resistance factors, impaired antibiotic penetration of the area because of the capsule surrounding the components and the decreased wound healing related to scar formation. The organism most often found responsible for infection at reoperation is Staphylococcus epidermidis,[12] which has been shown to be able to form a biofilm matrix.[8] This bacterium is also the most common cause of infection during the original implantation, accounting for 35% to 80% of all positive cultures.[12,20]

Previously it had been assumed that uninfected implants were surrounded by a sterile environment and the presence of bacteria equated to infection. However, there are data in the literature indicating that this may not be true. The Cleveland Clinic group reported in 1995 that 40% of uninfected penile prostheses and 36% of artificial urinary sphincters had low colony counts of bacteria at the time of mechanical revision.[12] More recently the multicenter study of Henry et al showed that 54 of 77 patients (70%) were culture positive for bacteria at reoperation for a clinically uninfected penile prosthesis.[13] Although a small sample was investigated, in the current study confocal scanning laser microscopy revealed that 8 of 10 patients (80%) had biofilm matrix on the implant at reoperation.

The limitations of scanning microscopy are such that only small areas of the studied small strips of implant material were visualized. Another limiting factor in this study is that bacteria were not cultured at the time of prosthesis removal, just material sent for scanning microscopy. We believe that more through visualization of the surface of these materials would show that essentially all implants have some biofilm matrix formation at revision surgery.

Using scanning microscopy to our knowledge we report for the first time that biofilm exists on implants at revision surgery for noninfectious reasons. Before this study we only had culture data available. Not surprisingly scanning microscopy demonstrated a higher rate of bacterial presence than did the culture data. Only with larger, controlled studies can we be sure of the rates of culture positivity and the bacterial presence, and we suggest that these studies should be done. With the recent innovation of antibiotic coated inflatable penile prostheses aimed at decreasing bacterial adherence and colonization it would be interesting to see if the antibiotic coating yields a lower degree of bacterial presence during noninfectious revision surgery. Moreover, prosthetic urologists must think of new ways to decrease biofilm production beyond our currently used methods.

It appears that the majority of clinically uninfected penile prostheses have organisms growing in the implant space at reoperation. Some aspect of revision surgery may stimulate the bacteria to become clinically active and symptomatic to the patient, resulting in a higher revision infection rate compared with primary implantation infection rates. Early results show that removing the entire prosthesis and washing out the implant space with an irrigation protocol with complete replacement of the original prosthesis with an antibiotic coated penile prosthesis appears to decrease the infection rate of clinically uninfected penile prostheses at revision surgery.[14] However, replacement with an antibiotic coated prosthesis without revision washout did not decrease revision infection rates.[13]

To our knowledge this study only shows for the first time that bacterial biofilm exists on most inflatable penile prostheses at revision surgery done for noninfectious reasons. Tissue cultures of the capsule surrounding the implant before and after revision washout may show a decrease in the presence of positive cultures after washout. We propose a future study looking at the bacterial presence on the capsule.

CONCLUSIONS

The degree of biofilm formation on these clinically noninfected prosthetic devices suggests that most patients have bacterial coverage of the implant. Host mechanisms to control infection may lead to a homeostatic balance that enables biofilms to exist on the surface of the prostheses without clinical infection. A critical threshold of biofilm extent may exist beyond which clinical infection may occur. Furthermore, strategies such as revision washout that are aimed at

eliminating subclinical biofilms in patients with revision might decrease the postoperative infection risk in those undergoing repair or replacement due to penile prosthetic mechanical malfunctions.

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