SURGICAL INFECTIONS
Volume 10, Number 5, 2009

DOI: 10.1089/sur.2008.062

Chronic Surgical Site Infection Due to Suture-Associated Polymicrobial Biofilm

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Abstract

Background: Surgical site infection (SSI) is a common surgical complication; culture-negative SSI presents a particular problem in management.
Methods: Examination of explanted foreign bodies (sutures) using confocal laser scanning microscopy (CLSM) and fluorescent in situ hybridization (FISH) after surgical exploration of a chronic culture-negative SSI.
Results: Confocal microscopy (CM) demonstrated bacilli and cocci attached to the surface of the explanted sutures in a mixed biofilm. Florescent in situ hybridization confirmed that Staphylococci were components of the mixed biofilm. Removal of the foreign bodies (sutures) resolved the chronic infection.
Conclusion: Chronic SSI can arise from underlying bacterial biofilms, which can invest implanted foreign bodies and associated soft tissue surfaces.

Surgical site infection (SSI) is a common postoperative complication and has been estimated to occur in up to 5% of all procedures [1]. The cost associated with management of SSI varies with the location and nature of the infection but in the aggregate is substantial, accounting for 0.5% of the annual hospital budget according to one report [2]. Independent risk factors for SSI include abdominal surgery, contaminated or "dirty" procedures, and more than three diagnoses at the time of discharge from the hospital. Patient-related characteristics raising the risk for SSI include older age, obesity, smoking, and diabetes mellitus [1].

Surgical site infections can be classified into three overarching categories: Superficial incisional; deep incisional; and organ/space, or intracavitary [3]. Superficial SSIs typically require only opening and drainage, whereas deep incisional SSIs will usually necessitate more extensive surgical debridement and often adjuvant antibiotic therapy. Intracavitary SSI will also often require formal surgical intervention.

A particularly challenging form of SSI is that which presents as "culture-negative." It has been suggested that a number of factors, including prior antibiotic therapy, slow-growing or fastidious microorganisms (e.g., mycobacteria), and the presence of normally harmless bacteria that are dismissed as clinically insignificant, may cause culture negativity in SSI [4].

We report a case in which a patient undergoing gastric bypass surgery first suffered an intraabdominal SSI that required operative drainage and then developed a chronic draining sinus tract (a second, separate SSI) that persisted for 14 months despite multiple courses of antibiotics and local care. This chronically draining wound, interpreted as culture-negative on several occasions, ultimately required surgical exploration, with the removal from the abdominal wall of implanted foreign bodies (sutures). Examination of these sutures with confocal laser scanning microscopy (CLSM) and fluorescent in situ hybridization (FISH) revealed the presence of a polymicrobial bacterial biofilm that was probably responsible for the chronic infection.

Case Report

A 36-year-old female with morbid obesity underwent open Roux-en-Y gastric bypass surgery in September 2004. On postoperative day 10 she required urgent laparotomy for drainage of an intra-abdominal fluid collection, although no clear source for the infection within the abdomen was identified. Intra-operative cultures were positive for *Staphylococcal aureus, Streptococcus viridans*, *Enterococcus spp*., and *Candida albicans*. The patient was treated adjunctively with intravenous imipenem-cilastatin and fluconazole.

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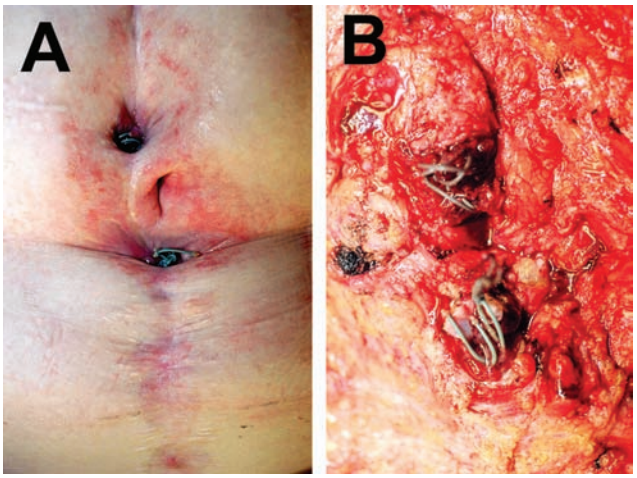

FIG. 1. Intraoperative views of the draining sinuses. (**A**) Green suture material was visible in the depth of the sinuses after curetting of the sinus tracks; the surrounding limited erythema is consistent with a localized infectious focus. (**B**) Suture material resident in the abdominal wall after removal of overlying skin and fat. (Color image is available online at www.liebertpub.com/sur)

Her course was thereafter uneventful, and she was discharged.

Two months later the patient noted yellowish serous drainage from the most inferior aspect of her incision, near her umbilicus, without accompanying pain or fever. Routine bacterial culture was negative, and she was empirically treated with trimethoprim-sulfamethoxazole, then with two courses of cefaclor, with only transient improvement in the drainage.

Seven months after her surgery she developed two separate draining sites near the umbilicus. Twelve months after surgery bacterial culture of wound fluid revealed only *Corynebacterium* species and was interpreted clinically as negative; fungal culture was negative. Nonetheless, she was treated empirically with oral clindamycin, without effect.

Sixteen months after her surgery, she underwent surgical exploration of the wound sites because of suspicion of retained foreign body (with concomitant abdominal panniculectomy.) An intra-operative examination revealed large knotted sutures at the bases of her draining sinuses (Fig. 1); more complete surgical dissection revealed multiple further such large knotted polyester permanent, multifilament sutures at the level of the abdominal wall musculofascial closure, each surrounded with dense granulation tissue or amidst cloudy fluid. These infected suture tracks all connected with one another subcutaneously. Intraoperative cultures at the final operation revealed *Corynebacterium*, *Streptococcus milleri*, and coagulase-negative *Staphylococcus*, sensitive to trimethoprim-sulfamethoxazole and clindamycin.

Twelve foreign bodies (sutures), with their surrounding accumulations of granulation or scar tissue or fluid, were removed. The patient has subsequently been free of drainage or other symptoms for almost two years.

Confocal Microscopy for Detection of Biofilms

In addition to standard microbiological culture, suture material and associated reactive tissue recovered from the patient at the time of her ultimate surgery were placed in Hanks Balanced Salt Solution (HBSS) without phenol red (Invitrogen, Carlsbad, CA) and placed on wet ice directly after removal. Specimens of sutures and tissue investing the sutures were rinsed with HBSS, blotted on sterile tissue paper to remove excess water from one side, and mounted on the bottom of a 35-mm petri plate on vacuum grease by gently pressing into the grease, avoiding contact with the central region to be imaged. Some of the specimens were stained for viability assessment using a BacLight Live/Dead kit (Molecular Probes, Eugene, OR) by drop-pipetting approximately 50 mcL to wet the desired viewing area and incubated in the dark at room temperature for 30 min. The basis for the assays in the kit is that all bacteria readily take up the "live" green fluorescent stain Syto9, but only cells with damaged membranes take up the "dead" red fluorescent stain propidium iodide. A wetted sheet of tissue paper was placed in the plate with the lid on to maintain a hydrated environment during staining. After incubation the plate was flooded with HBSS to immerse the specimen completely. The specimen was imaged using confocal microscopy (CM) using a Leica DM RXE upright microscope attached to a TCS SP2 AOBS confocal system (Leica Microsystems, Exton, PA) using a 63X long-working-distance water-immersion objective. Live (green) and dead (red) bacteria were imaged using the 488-nm and 594-nm lasers and appropriate detector settings [5]. The sutures were imaged using natural autofluorescence of the polyester.

In addition to viability staining, we used florescent in situ hybridization (FISH) to specifically identify Staphylococcal biofilms present on tissue surrounding the sutures. Tissue specimens were mounted in 35-mm petri plates as described. The tissue was fixed immediately in 4% paraformaldehyde (PFA, Electron Microscopy Sciences, Hatfield, PA) in 3X phosphate-buffered saline (PBS) for 12 h at 4°C to preserve structure and permeabilize the bacteria to allow uptake of the FISH probe [6]. The tissue was washed three times with PBS, the buffer was removed, and the tissue was submerged in 50% PBS 50% ethanol solution and stored at −20°C. To further permeabilize the bacteria, specimens were treated with 10 mg/mL lysozyme (Sigma-Aldrich, St Louis, MO) in 0.1M Tris hydrochloric acid (Sigma-Aldrich) to pH 8.0 and 0.05M disodium ethylenediamine tetraacetate (Sigma-Aldrich) for 3 h at 37°C and washed with ultrapure water. The sample was dehydrated in a graded series of ethanol (50%, 80%, and 100%) for 3 min at each concentration. Hybridization was performed using the *Staphylococcus* genus–specific 16S ribosomal probe: "sta" 5′- (TCCTCCATATC TCTGCGC) – 3′ [6] labeled with the green fluorescent probe Cy3 (Integrated DNA Technologies Inc, Coralville, IO). Propidium iodide (Molecular Probes) was used to counterstain all bacteria and the nuclei of host cells red. To visualize the cytoplasm of the host cells, we stained F-actin in the tissue with phalloidin (Invitrogen) conjugated with Alexa488 (shown in blue). To permeabilize the host cells we incubated the specimen in 0.1% Triton (Sigma-Aldrich) for 3 min. The tissues were imaged fully submerged in HBSS using CM with a 63X water-immersion objective. A positive identification of staphylococcal organisms was made on the basis of cocci that were stained with both the staphylococcal FISH probe and propidium iodide; these cells appeared yellow.

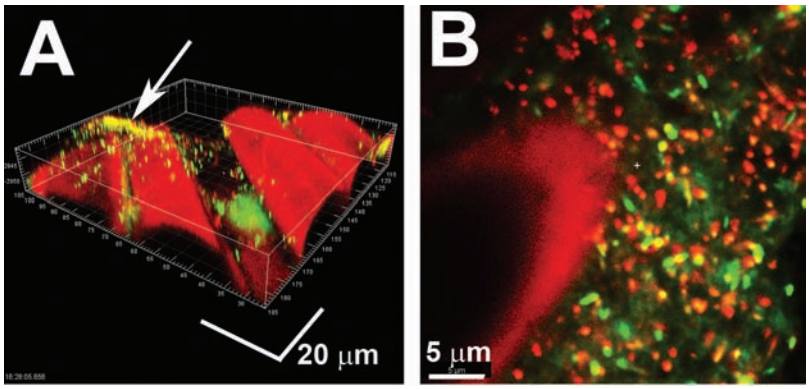

FIG. 2. Confocal microscopic examination of explanted suture material. (**A**) Viability staining showing a biofilm (arrow) attached to the suture braids (autofluorescing red). Green and yellow bacteria indicate viable cells at the wound site. (**B**) Higher magnification of the crevice region between the braids of the suture showed that the biofilm was polymicrobial, consisting of bacilli and cocci. (Color image is available online at www.liebertpub.com/sur)

Viability staining revealed patches of dense polymicrobial biofilms containing live and dead bacilli and cocci directly on the surface and between the braids of the sutures (Fig. 2A, B). Many of the bacteria were stained with both the live (green) and dead (red) stains and appeared yellow. This type of "intermediate" staining has been noted in other studies and using flow cytometry analysis and has been interpreted to represent bacteria that are alive and metabolically active but with incipient membrane damage [7]. The presence of many dividing bacilli and dividing cocci suggested some active growth of biofilm bacteria. Some of the bacilli had a "club-like" morphology, consistent with corynebacteria. The biofilms were heterogeneously distributed on the sutures and were denser in some locations than others. Some areas of the sutures were devoid of any evidence of biofilms. There appeared to be a greater concentration of biofilm in the crevices between the individual braids of the sutures. Florescent hybridization confirmed clusters of *Staphylococcus* bacteria attached to the soft tissue investing the suture material (Fig. 3A, B).

Discussion

Using CM to examine fully hydrated specimens, we have demonstrated that viable biofilms were associated with infected sutures and associated reactive soft tissue in this patient. Unlike scanning electron microscopy, which has been more commonly used for clinical specimens, CM has the advantage that specimens can be examined while fully hydrated, and staining techniques such as LIVE/DEAD and FISH can assess viability and distinguish specific target bacterial species. Furthermore, because we examined the specimens while they were fully submerged using a water-immersion objective to improve resolution, we provide clear evidence that the bacteria were firmly attached to the suture and soft-tissue, with three-dimensional bacterial clusters protruding from the surfaces.

We base our conclusion that this chronic SSI had an underlying biofilm etiology using the criteria proposed by Parsek and Singh [8] for use as a clinical diagnostic guide;

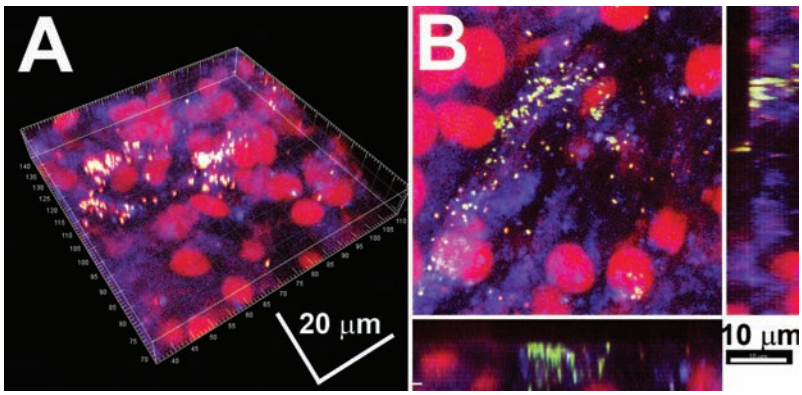

FIG. 3. Fluorescent in situ hybridization shows Staphylococcal cells (yellow) in a patchy biofilm attached to the reactive soft tissue that enveloped the infected suture. The nuclei of the host cells were stained red with propidium iodide, and f-actin in the cytoplasm was stained blue with phalloidin. (**A**) Isometric view showing small biofilm cell clusters and single cells attached to the tissue. (**B**) Plan view of the biofilm in a different area of tissue. The XZ and YZ sagittal sections through the host tissue and biofilm are shown below and to the right of the plan view, respectively. (Color image is available online at www.liebertpub.com/sur)

"(*a*) The infecting bacteria were adherent to some substratum or are surface associated; (*b*) direct examination of infected tissue shows bacteria living in cell clusters, or microcolonies, encased in an extracellular matrix. The matrix may often be composed of bacterial and host components; (*c*) the infection is generally confined to a particular location. Although dissemination may occur, it is a secondary phenomenon; (*d*) the infection is difficult or impossible to eradicate with antibiotics despite the fact that the responsible organisms are susceptible to killing in the planktonic state." The fourth criterion was demonstrated in the patient's history; the patient had been receiving multiple courses of antibiotics, yet the infection persisted. Negative bacterial cultures in the patient's course were also consistent with the biofilm paradigm, because biofilm bacteria can be difficult to culture [9]. It is believed that the difficulty in obtaining positive culture is related to sampling the wrong locations (e.g., the associated fluids rather than surfaces) and the notion that bacteria within the biofilm enter a "dormant" state due to local nutrient depletion within the biofilm [10]. Starvation-induced "dormancy" has been shown to make bacteria in staphylococcal biofilm clusters significantly less susceptible to oxacillin [11].

Biofilms have been documented to occur in association with a wide variety of implanted materials, such as central venous catheters, urinary catheters, heart valves, and orthopedic joint prostheses and internal fixation devices [10,12–14], and in vitro studies demonstrate that biofilms readily form on nonabsorbable surgical sutures and meshes [15,16]. However, suture material as the focus of biofilm infection in clinical specimens has been only rarely described, and most of these cases involved ocular infections [17,18]. We found one study that identified biofilms on percutaneous sutures from the skin closure of healed wounds using electron microscopy [19]. To our knowledge the present case report is the first to directly demonstrate a suture-associated biofilm as the cause of persistent infection after abdominal wall repair. The SSI rate associated with open gastric bypass surgery has been reported to be as high as 9.2% in a study of 399 patients [20], although further details were not provided. We suspect that the high incidence of infection might in part be biofilm-related; in particular, another reason for culture negativity in SSIs may be the presence of bacteria in a biofilm configuration.

The causative organisms ultimately identified in this chronic infection appear to be unrelated to those found in the patient's initial intra-abdominal collection, probably deriving instead from skin flora but becoming pathogenic once established on the suture surfaces. Nonabsorbable sutures are as much a permanent implant as any of the larger implanted medical devices that are now recognized as being susceptible to biofilm infections and should be kept in mind as a source of potential infectious complications. In addition, the reactive soft tissue that forms around a biofilm-affected foreign body can itself become a site for biofilm attachment and should be removed meticulously. This patient's clinical presentation is a quintessential example of a chronic infection of biofilm etiology; a protracted period of symptoms confined to a specific anatomical site, refractory to conventional antibiotic therapy, and resolution only when the underlying foreign body substratum was removed.

Acknowledgments

We wish to thank Ms. Mary O'Toole for her expert help in the preparation of this manuscript. Funding from Allegheny-Singer Research Institute, NIH NIDCR DE014780(SK), NIH DC04173 (GDE).

Author Disclosure Statement

No author has any commercial association that might create a conflict of interest in connection with this manuscript.

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