

Microbiology of Explanted Suture Segments from Infected and Noninfected Surgical Patients

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Sutures under selective host/environmental factors can potentiate postoperative surgical site infection (SSI). The present investigation characterized microbial recovery and biofilm formation from explanted absorbable (AB) and nonabsorbable (NAB) sutures from infected and noninfected sites. AB and NAB sutures were harvested from noninfected (70.9%) and infected (29.1%) sites in 158 patients. At explantation, devices were sonicated and processed for qualitative/quantitative bacteriology; selective sutures were processed for scanning electron microscopy (SEM). Bacteria were recovered from 85 (53.8%) explanted sites; 39 sites were noninfected, and 46 were infected. Suture recovery ranged from 11.1 to 574.6 days postinsertion. A significant difference in mean microbial recovery between noninfected (1.2 isolates) and infected (2.7 isolates) devices (P < 0.05) was noted. Staphylococcus epidermidis, Staphylococcus aureus, coagulase-negative staphylococci (CNS), Peptostreptococcus spp., Bacteroides fragilis, Escherichia coli, Enterococcus spp., Pseudomonas aeruginosa, and Serratia spp. were recovered from infected devices, while commensal skin flora was recovered from noninfected devices. No significant difference in quantitative microbial recovery between infected monofilament and multifilament sutures was noted. Biofilm was present in 100% and 66.6% of infected and noninfected devices, respectively (P < 0.042). We conclude that both monofilament and braided sutures provide a hospitable surface for microbial adherence: (i) a significant difference in microbial recovery from infected and noninfected sutures was noted, (ii) infected sutures harbored a mixed flora, including multidrug-resistant health care-associated pathogens, and (iii) a significant difference in the presence or absence of a biofilm in infected versus noninfected explanted devices was noted. Further studies to document the benefit of focused risk reduction strategies to minimize suture contamination and biofilm formation postimplantation are warranted.

'he classical studies conducted by Varma et al., Elek and Cohen, and Raju et al. documented the microbial burden required to produce an infection in a clean surgical wound (1-3). These studies further characterized the role of suture material as a foreign body, functioning as a nidus for infection in the presence of wound contamination. Recent reports by Kathju and colleagues suggest that contamination of surgical sutures at the time of implantation by biofilm-forming organisms leads to recalcitrant infection, necessitating eventual removal of the infection device (4, 5). While closure technologies such as surgical sutures have not always been viewed in the same light as other implantable biomedical devices, the surface characteristics of these devices make them a susceptible substrate for bacterial adherence and/or contamination. Intrinsically, microbial contamination of the wound bed results in delayed wound healing, since the presence of bacteria in the wound at closure alters the local environment of the wound, lowering the oxygen tension within the wound and depressing fibroblast proliferation (6). A heavily contaminated wound (with \geq 5.0 log₁₀ CFU) may present acutely with incisional (wound) dehiscence. When the wound microbial burden is $\log (<2.0 \log_{10})$ CFU), the infection may present as a late-onset or chronic process that is nonresponsive to traditional therapeutic strategies (5, 7).

While the incidence of surgical site infections associated with contaminated surgical sutures is presently unknown, data from other device-related infections suggest that these inert surfaces provide a hospitable niche for bacterial growth and proliferation. Many of these infections involve organisms capable of producing a luxurious biofilm, allowing microbial persistence even in the presence of appropriate antimicrobial therapy or a competent host immune response. There have been limited studies investigating the microbial recovery from sutures explanted from noninfected or infected clinical specimens. Those that have been conducted have involved fewer than 10 patients and were limited to a selective surgical patient population (4, 5). In the present study, absorbable and nonabsorbable infected/noninfected suturing devices were explanted from 158 surgical patients representing a broad patient population and were evaluated for aerobic/anaerobic microbial recovery and biofilm formation.

MATERIALS AND METHODS

Following review and approval by the institutional review board (IRB), multiple nonabsorbable and absorbable suture segments were collected at random from 158 patients (112 noninfected and 46 infected surgical cases). Explanted sutures were recovered from 5 separate surgical services (orthopedic, plastic, vascular, bariatric, and colorectal). Suture segments were obtained upon patient return to the clinic for suture removal or during reoperation. Designation of noninfected versus infected was determined by clinical presentation and criteria defined by the National Healthcare Safety Network (NHSN) of the Centers for Disease Control and Prevention (CDC) (8). All suture segments were collected aseptically

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Surgical service	No. of sutures										
	Material	a						Clinical			
	AB			N-AB		Explanted site		presentation ^b			
	PG	PL	РО	N	PP	Skin	Fascia/organ space	NI	Ι		
Orthopedic				59		59		57	2		
Plastic	3			6	11	6	14	13	7		
Vascular	4	6	8	5	11		34	18	16		
Bariatric/colorectal	7	12	5	6	15		45	24	21		
Total	14	18	13	76	37	65	93	112	46		

TABLE 1 Demographics of explanted absorbable and nonabsorbable braided and monofilament surgical sutures

^a AB, absorbable; PG, polyglactin; PO, poliglecaprone; PL, polydioxanone; N-AB, nonabsorbable; N, nylon; PP, polypropylene.

^b NI, not infected; I, infected (superficial incisional, 11; deep incisional/organ space, 35).

and then transported to the Surgical Microbiology Research Laboratory, where care was taken to remove any gross tissues attached to suture segments, and washed gently twice in phosphate-buffered saline (PBS). To resolve microbial populations from the surface of suture segments, following measurement individual sutures were sonicated in 1 ml PBS at 20 kHz for 30 s, serially diluted in PBS, plated to Trypticase soy agar (TSA), and incubated for 48 hours at 35°C. Aerobic and facultative microbial isolates (Gram positive and Gram negative) were characterized using standard methodology (9). Suture segments recovered from abdominal fascia or devitalized tissues were processed for anaerobic bacteriology and incubated within an anaerobic chamber. Microbial recovery was expressed as log₁₀ CFU per cm suture segments (15 from noninfected cases and 15 from infected cases) were selected at random and processed for scanning electron microscopy (SEM) as previously described (7).

RESULTS

Suture segments were explanted from 158 surgical patients from 5 surgical services. Table 1 documents the surgical services, types of closure device, explant sites, and clinical indications (infected or noninfected). The majority of sutures collected were monofilament devices (144; 91.1%), while 14 (8.9%) multifilament (braided) sutures were explanted from fascia. Nylon (nonabsorbable, monofilament) was the most common suture collected during the study (n = 76), and the majority of these devices (n = 65)were obtained from skin closure sites. Suture segments were obtained from fascia or organ space surgical sites in 93 patients (58.9%). Nonabsorbable, monofilament sutures (polypropylene and nylon) comprised 71.5% (n = 113) of all explanted devices. A total of 39 (24.7%) suture segments were culture positive but from sites designated noninfected (skin), while 46 (29.1%) suture segments were collected from sites identified as infected (skin, fascia, or organ site). No organisms were recovered from 73 (42.6%) suture segments from sites designated noninfected (skin, fascia, or organ space). The mean suture explant times (Fig. 1) ranged from 11.1 days (nylon monofilament sutures from skin) to 574.6 days (polypropylene monofilament sutures from fascia). Microbial recovery from infected and noninfected, culture-positive suture segments is reported in Table 2. A total of 46 separate bacterial isolates were recovered from 39 culture-positive, noninfected orthopedic (foot, n = 38) and plastic (face and breast, n = 8) surgery skin surface suture explants. The mean microbial recovery from noninfected explanted sutures was 1.2 isolates per device. Corynebacterium spp. were the most common isolate from noninfected cases, followed by Bacillus spp., Staphylococcus epidermidis, Micro*coccus* spp., and coagulase-negative staphylococci (CNS). The majority of these isolates, representing normal skin commensals, were recovered from nylon (monofilament) skin closure devices.

A total of 127 isolates were recovered from 46 infected surgical cases, including orthopedic (n = 2), plastic (n = 7), vascular (n = 7)16), bariatric (n = 4), and colorectal (n = 17) surgical cases. The microbial recovery was predominantly Gram positive in infected orthopedic, plastic, and bariatric surgical cases. Gram-positive bacteria were recovered from 50% of infected vascular graft cases, while the remaining cases yielded a polymicrobial flora involving both facultative and anaerobic populations. A facultative/anaerobic polymicrobial flora was recovered from greater than 75% (13/ 17) of infected colorectal surgical cases, while 4 cases yielded a single bacterial (Gram-negative) isolate. The mean microbial recovery from infected explanted sutures was 2.7 isolates per infected device, and Staphylococcus epidermidis (35), Staphylococcus aureus (16; 9 methicillin-resistant S. aureus [MRSA]), Peptostreptococcus spp. (13), Bacteroides fragilis (11), E. coli (10), Enterococcus spp. $(\overline{7})$, Pseudomonas aeruginosa (6), Serratia marcescens (6), and coagulase-negative staphylococci (5) represented the predominant flora recovered from infected skin, fascia, and organ space sites (Table 2).

All suture segments (noninfected and infected) were acquired in a random fashion, and the percent infected cases and mean microbial recovery per suture type are reported in Fig. 2. Mean

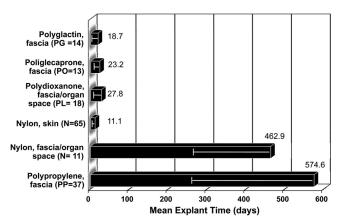


FIG 1 Mean explants time of absorbable and nonabsorbable suturing devices. The absorbable devices were polyglactin (PG), poliglecaprone (PO), polydioxanone (PL); the nonabsorbable devices were nylon and polypropylene (PP).

	No. of isolates recovered from culture-positive patients ^{<i>a</i>}										
	Noninfected ($n = 39$)					Infected $(n = 46)$					
Organism(s)	PG	РО	PL	Ν	PP	PG	РО	PL	Ν	PP	
Bacillus spp.				7	1						
Corynebacterium spp.				12							
Micrococcus spp.				6							
Kocuria varians				1							
S. epidermidis				7		8	7	5	4	11	
CNS ^b				5		1		2	2		
S. aureus ^c				1		2	1	4	3	6	
Enterococcus spp.						1		2	1	3	
Acinetobacter spp.										1	
Escherichia coli						3	2	2		3	
Escherichia vulneris				1						1	
Morganella morganii						1					
Klebsiella pneumoniae							2	2		1	
Pseudomonas aeruginosa				1				1	2	3	
Serratia marcescens								1	2		
Weeksella virosa				1							
Neisseria spp.									1		
Gemella morbillorum				1			5	2			
Peptostreptococcus spp.						6		2	1	5	
Aspergillus spp.				1							
Bacteroides fragilis						1	3	2	3	2	
Clostridium spp.						1		2		1	
Microsporum spp.				1							
Total				45	1	24	20	27	19	37	

TABLE 2 Aerobic and anaerobic microbial recovery from infected and noninfected patient suture segments

^{*a*} Absorbable devices: PG, polyglactin; PO, poliglecaprone; PL, polydioxanone. Nonabsorbable devices: N, nylon; PP, polypropylene.

^b CNS, coagulase-negative staphylococci other than S. epidermidis.

^c MRSA, 52.9% (9/17).

microbial recovery (\log_{10} CFU/cm suture segment [length]) ranged from 5.1 in polypropylene (nonabsorbable, monofilament) to 6.9 in polyglactin (absorbable) multifilament suturing devices. It is interesting to note that while mean quantitative recovery was highest in infected polyglactin (multifilament) sutures, there was no significant difference in mean quantitative recovery

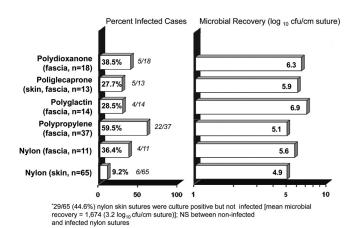


FIG 2 Percent suture recovery from explanted absorbable and nonabsorbable infected suturing devices. Note that 29/65 (44.6%) nylon skin sutures were culture positive but not infected. The mean microbial recovery was 1,674 (3.2 \log_{10} CFU/cm suture segment). There was no significant difference in microbial recovery between monofilament and multifilament suturing devices.

between multifilament and monofilament infected suturing devices. The 39 noninfected (culture-positive) skin sutures yielded a mean quantitative recovery of 3.2 log₁₀ CFU/cm suture segment. More than twice as many nonabsorbable sutures (32; 69.6%) as absorbable devices (14; 30.4%) were recovered from infected cases. Polypropylene (22) and nylon (4) nonabsorbable, monofilament sutures were explanted from 26 deep incisional infections involving selected prosthetic material (Dacron, polytetrafluoro-ethylene [PTFE], and polyester). All of these device-related infections were characterized as late onset, i.e., >6 months postinsertion. An SEM examination of infected and noninfected explanted devices revealed a biofilm on the surface of 66.6% (10/15) of the culture-positive noninfected suture segments (Fig. 3A), while a biofilm was observed on 100% of infected (deep incisional and organ space infections) suture segments (Fig. 3B) (P < 0.042).

DISCUSSION

Upon implantation in the host, surgical sutures function as a foreign body within the surgical wound, sequestering microbial contamination and under selective host conditions serving as a potential nidus for infection (10-12). In the present study, suture segments were recovered from a total of 158 surgical patients; 46 (29.1%) were recovered from documented infected cases. An additional 39 suture segments (24.7%) were harvested from noninfected patients but were culture positive with commensal skin bacterial populations, including *Staphylococcus epidermidis* and coagulase-negative staphylococci. A bacterial biofilm was ob-

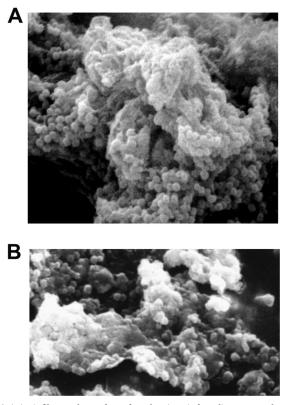


FIG 3 (A) Biofilm on the surface of a nylon (noninfected) suture explanted at 10 days postinsertion, documenting coccus-like structures and commensal coagulase-negative staphylococci. Magnification, ×12,350. (B) Coccus-like structures enmeshed in biofilm on the surface of a monofilament (polypropylene) suturing device associated with abdominal mesh explanted at 397 days postinsertion. Cultures revealed MRSA plus anaerobic Gram-negative rods. Magnification, ×11,750.

served on 100% of infected suturing devices, the majority of which were from device-related infections, while only 2/3 of the noninfected culture-positive sutures examined by SEM harbored a surface biofilm (P < 0.042). These findings are complementary to previous in vitro studies which suggest that bacterial adherence to surgical sutures is associated with the formation of a luxurious bacterial biofilm (13, 14). A significant difference in biofilm formation in the randomly sampled suture segments (noninfected versus infected) was noted. The reason for this difference at first glance is less than intuitive, since previous work conducted in our laboratory has suggested that biofilm-forming staphylococci commonly inhabit the surface of the skin in selective hospitalized patients (15). The failure to detect a biofilm in 1/3 of the sampled skin suturing devices may be due in part to device processing or sentinel wound defense factors commonly operative in the normal host. It is well documented that the first 48 h following skin closure is a period of intense granulocytic cell activity within the wound bed. The presence of a suture actually intensifies this process, which can typically be documented by observing redness along the intact suture line. Ironically, the presence of a foreign body (suture) left within the wound can also exacerbate infection in the presence of wound contamination, since it lowers the inoculum burden required for infection in a clean surgical wound (1-3). Further studies to reconcile this observed difference in biofilm formation in suture segments obtained from noninfected versus infected surgical wounds are warranted.

It is interesting to note that under *in situ* conditions, no quantitative difference between mean microbial recovery from explanted monofilament or multifilament suturing devices was noted. Traditional dogma has suggested that the surface conformation of a multifilament suture will harbor (entrap) a larger number of bacterial cells than monofilament devices. The present study suggests that once implanted in the surgical wound, all suture surfaces, regardless of structural configuration, provide a hospitable environment for microbial adherence.

An unexpected observation, however, documented in infected cases was the recovery of multiple microbial populations on suture segments from deep incisional or organ space infections. Several of these infections involved infected prosthetic devices (Dacron, PTFE vascular grafts, and/or polyester mesh) exhibiting a polymicrobial flora that was not observed in culture-positive noninfected skin closure devices. In selected infected cases involving both absorbable and nonabsorbable devices, aerobic and anaerobic isolates were recovered in culture (Table 2). Peptostreptococcus spp. and Bacteroides fragilis were recovered from infected sites involving the peritoneal cavity. In several patients, multidrug-resistant Gram-positive and Gram-negative isolates were recovered from both acute and late-onset device-related infections. These infections can also have systemic consequences; two separate patients had positive blood cultures that yielded the same clonal variant of MRSA that was recovered from infected explanted polypropylene and poliglecaprone sutures.

An exopolysaccharide (biofilm) matrix was observed on nylon sutures explanted at 10 days postclosure (Fig. 3A) and on explanted infected polypropylene devices at >300 days postclosure (Fig. 3B) associated with inert prosthetic materials. Biofilm formation from noninfected (skin) explanted sutures was associated with recovery of Staphylococcus epidermidis or commensal coagulase-negative staphylococcal skin colonizers. The microbial burden on these culture-positive noninfected explanted sutures was <10⁴ CFU/cm suture segment. Biofilms associated with infected explanted suturing devices were observed in both deep incisional and organ space infections. In the majority of these infected cases, the microbial burden exceeded $>10^5$ CFU/cm suture segment. In three separate cases involving infected mesh segments, the primary device had been removed but recurrent disease required exploration and removal of retained suture segments. All three of these suture segments (polypropylene) exhibited a polymicrobial microbial flora comprised of Gram-positive (2 MRSA and 1 MSSA) and Gram-negative aerobic (E. coli), and anaerobic (Peptostreptococcus and Bacteroides spp.) bacteria enmeshed in a luxurious biofilm. It would appear that no specific quantitative threshold is required for biofilm formation on an inert prosthetic surface, nor does the presence of a biofilm necessarily always convey an infectious potential. Whether or not this process (biofilm formation) potentiates a surgical site infection is dependent upon localized host risk factors which render the tissue (and implanted suturing device) susceptible to invasive disease. Under planktonic conditions, most microbial populations will exhibit susceptibility to selective antimicrobial agents. However, when the same cells are encased within an extracellular (biofilm), matrix they exhibit a recalcitrance to surgical prophylaxis and/or antimicrobial therapy (7, 16).

Reducing the risk of surgical site infections in at-risk patient

populations requires a multifaceted approach that includes appropriate skin antisepsis, antimicrobial prophylaxis, knowledge of comorbid risk factors, and other adjunctive (evidence-based) interventional strategies (17, 18). Aggressive efforts to prevent prosthetic device contamination at the time of insertion is a hallmark of the orthopedic, cardiothoracic, and vascular surgical services. Previous studies conducted in our facility have documented that intraoperative wound contamination can occur through nasopharyngeal shedding or microperforation of surgical gloves, allowing hand flora to migrate across the compromised glove surface into the wound (19, 20). Preventing microbial adherence and biofilm formation on the surface of a multifilament or monofilament suturing device would appear to be a beneficial risk reduction strategy. As an example, several investigators have documented a reduction in bacterial adherence (Gram positive and Gram negative) to the surfaces of multifilament and/or monofilament closure devices which are coated with the biocide triclosan (21-23).

In situ studies of explanted surgical sutures strongly suggests that following implantation within contaminated surgical wounds, microbial populations will adhere tenaciously to either monofilament or multifilament suturing devices. In selective patient populations, this adherence component may well play a significant role in the development of a postoperative surgical site infection. The present investigation has revealed that both multifilament and monofilament sutures provide a hospitable surface for microbial contamination, harboring a broad range of microbial populations in both infected and noninfected patient populations. A limitation of this current investigation involves the sensitivity of traditional culture methodology to recover selective bacterial populations, especially on the surfaces of biofilm-laden suture specimens. It is possible that microbial populations were present in the 73 culture-negative suture samples but that traditional culturing strategies lacked the ability to resolve these occult bacterial populations. Further studies using confocal laser scanning microscopy or molecular technology such as fluorescent in situ hybridization (FISH) or 16S PCR (sequence analysis) are warranted to fully elucidate the genetic diversity that likely exists within the microbial biofilm of explanted suturing devices. These advanced technologies would enhance our ability to detect discrete microbial populations, thereby increasing the mechanistic understanding of in situ (perioperative) suture contamination as an antecedent process to wound infection.

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