BASIC RESEARCH



Bacteria Adhere Less to Barbed Monofilament Than Braided Sutures in a Contaminated Wound Model

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Abstract

Background Previous studies have found fewer clinical infections in wounds closed with monofilament suture compared with braided suture. Recently, barbed monofilament sutures have shown improved strength and increased timesavings over interrupted braided sutures. However, the adherence of bacteria to barbed monofilament sutures and other commonly used suture materials is unclear.

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Questions/Purposes We therefore determined: (1) the adherence of bacteria to five suture types including a barbed monofilament suture; (2) the ability to culture bacteria after gentle washing of each suture type; and (3) the pattern of bacterial adherence.

Methods We created an experimental contaminated wound model using planktonic methicillin-resistant Staphylococcus aureus (MRSA). Five types of commonly used suture material were used: VicrylTM, VicrylTM Plus, PDSTM, PDSTM Plus, and QuillTM. To determine adherence, we determined the number of bacteria removed from the suture by sequential washes. Sutures were plated to determine bacterial growth. Sutures were examined under confocal microscopy to determine adherence patterns.

Results The barbed monofilament suture showed the least bacterial adherence of any suture material tested. Inoculated monofilament and barbed monofilament sutures placed on agar plates had less bacterial growth than braided suture, whereas antibacterial monofilament and braided sutures showed no growth. Confocal microscopy showed more adherence to braided suture than to the barbed monofilament or monofilament sutures.

Conclusions Barbed monofilament suture showed similar bacterial adherence properties to standard monofilament suture.

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Clinical Relevance Our findings suggest barbed monofilament suture can be substituted for monofilament suture, at the surgeon's discretion, without fear of increased risk of infection.

Introduction

Although the closure of clean surgical wounds can be accomplished successfully using a wide variety of suture materials, closure of contaminated wounds such as with an infected total joint arthroplasty magnifies the properties of the individual suture types and their association with bacterial infection. Previous studies showed there were less infections in contaminated wounds closed with monofilament suture compared with braided suture [1, 10, 20]. Several studies have shown bacteria adhere more tightly to braided suture [2, 10, 12, 13, 25, 27] and that braided suture leads to more infections as compared with monofilament suture [1, 9, 10, 14, 28].

Recently, a barbed bidirectional monofilament suture, QuillTM (Angiotech Pharmaceuticals, Vancouver, BC, Canada), has allowed for knotless closure of wounds [18]. The barbs allow the suture to distribute tension across the length of a wound without the need for knots, whereas the bidirectionality of barbed monofilament suture with needles on both ends makes it possible for two surgeons to work simultaneously to close the wound [5, 18, 21, 26]. Studies using barbed monofilament suture have shown decreased operative time, decreased costs from operating room charges and anesthesia fees [26], and wound strength and tissue reaction scores comparable to those of monofilament suture tied with knots [17, 19]. Some have suggested that knotting the suture exaggerates the physical characteristics of the monofilament and multifilament sutures causing inflammatory reactions [3]. Therefore, a barbed suture that does not require knots may be less likely to provoke inflammatory reactions. However, the bacterial properties of barbed monofilament suture, such as bacterial adherence and correlation with postoperative wound infection, have not been defined in comparison to standard monofilament and braided sutures.

Using an in vitro model of contaminated soft tissues we determined (1) the adherence of bacteria to five suture types including a barbed monofilament suture; (2) the ability to culture bacteria after gentle washing of each suture type; and (3) the pattern of bacterial adherence.

Materials and Methods

An experimental model was created to simulate the passage of suture through contaminated soft tissues while closing a

wound. A bacterial broth consisting of brain heart infusion broth (BHI) inoculated with planktonic methicillin-resistant Staphylococcus aureus (MRSA) was created and two concentrations were developed: 5×10^{10} CFU/mL (high concentration broth) and 1×10^6 CFU/mL (physiologic concentration broth). The bacterial load required for infection in healthy hosts is reportedly 1×10^5 to $1 \times$ 10⁶ CFU/mL [6, 11]. Sutures were taken from sterile. unexpired packages and cut into 2-cm strands. The sutures then were incubated in either the high concentration broth or physiologic concentration broth for 5 minutes, then removed and placed in a normal saline vortex wash for 15 seconds. The sutures were placed in the normal saline vortex zero, one, two, three, or four times and the number of vortex washes recorded (Fig. 1). The normal saline from the vortex washes then was examined and the CFU/mL recorded. After the vortex washes, the sutures were placed on agar plates and growth observed after 24 hours. A suture of each type underwent no vortex washes and then was examined under confocal microscopy after Syto® 9 (Invitrogen, Grand Island, NY, USA) staining. Five types of suture material were compared in this study: (1) braided polyglactin 910 (VicrylTM; Ethicon Inc, Somerville, NJ, USA); (2) monofilament polydioxanone (PDSTM II; Ethicon Inc); (3) braided polyglactin 910 with Irgacare MPTM (VicrylTM Plus Antibacterial; Ethicon Inc); (4) monofilament polydioxanone with Irgacare MP (PDS Plus Antibacterial; Ethicon Inc); and (5) barbed monofilament knotless tissue-closure device (Quill). The antibacterial coating on Vicryl Plus and PDS Plus is triclosan, an antimicrobial agent that achieves its effects by blocking an essential enzyme in bacterial fatty acid synthesis [8].

To avoid carryover of bacteria on the forceps between washes, the forceps were flamed with ethanol between each wash. The vortex washes of the high bacteria concentration (5 \times 10¹⁰ CFU/mL) consisted of each suture strand being placed in 2 mL of sterile 0.9% saline in a 15-mL FalconTM tube (BD Biosciences, San Jose, CA, USA) and vortex washed for 10 seconds to remove bacteria that did not tightly adhere to the suture. For the physiologic bacteria concentration experiments (1 \times 10⁶ CFU/mL), each suture strand was placed in 1 mL of sterile 0.9% saline in a 15-mL FalconTM tube and vortex washed for 10 seconds.

To determine the number of CFU/mL in the vortex wash solutions, 500-μL spread plates of the saline solution were incubated overnight. These plates were examined for bacterial growth. The data are reported as CFU/mL recovered at each wash and total CFU/mL recovered. To detect bacteria remaining on the suture after the vortex washes, sutures were placed on mannitol agar plates and incubated overnight at 37°C. Bacterial growth was detected in two ways. Formation of opaque colonies around the suture and the formation of a yellow halo produced by the color



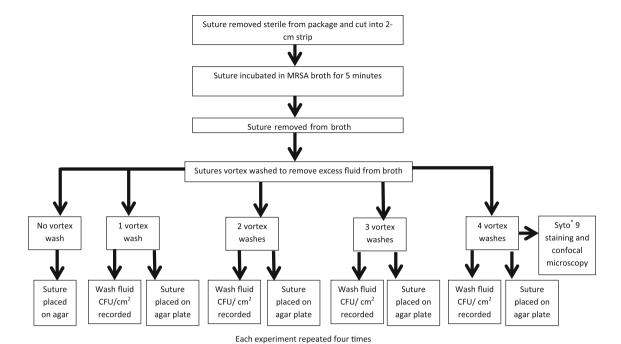


Fig. 1 A diagram of the experimental design is shown. Each suture was placed in MRSA broth for 5 minutes. The sutures were removed and underwent a vortex wash in normal saline for 15 seconds. The wash solution then was examined and CFU/mL recorded. The suture

was placed on an agar plate and cultured overnight. Sutures from the four vortex wash groups underwent Syto $^{(8)}$ 9 DNA staining and were examined under confocal microscopy to determine adherence patterns (CFU = colony forming units).

change in a pH indicator on production of acidic end products of mannitol fermentation by the bacteria. Laser scanning confocal microscopy and Syto 9 DNA staining were used to observe the adherence. Stained sutures were imaged using a Leica Sp5 scanning confocal microscope (Leica Microsystems Inc, Buffalo Grove, IL, USA). Syto 9 was excited at 488 nm and emission was detected from 496 to 534 nm. After a 5-minute incubation in the MRSA solution (approximately 5×10^{10} CFU/mL) and four washes in 3 mL of sterile 0.9% saline, suture strands were stained with fluorescent DNA stain Syto[®] 9. With this fluorescent green stain, bacteria could be seen on the suture using a laser scanning confocal microscope. The culture of bacteria from the suture and use of confocal microscopy were performed to determine if tight adherence to the suture resulted in less bacteria washed off the suture. If this were the case, then we would expect there to be increased growth from these sutures and more bacterial adherence on confocal microscopy.

An a priori power calculation was not performed for this pilot model. We determined the differences in the number of CFU/mL between the different suture types. The number of CFU/mL was recorded for each trial and the average number of CFU/mL calculated for each suture type. An independent weighted ANOVA was performed on the data from each trial. Tukey's test was used to determine if a difference existed between the individual suture types. The amount of bacteria cultured from each suture was viewed

qualitatively, observing the relative amount of growth on the agar plates, rather than quantitatively. Statistical analysis was performed using SPSS 20.0 Software (IBM, Armonk, NY, USA).

Results

QuillTM showed the least amount of bacterial adherence (48,000 CFU/cm²) of the five sutures tested (Table 1). Vieryl (213,000 CFU/cm²) and Vieryl Plus (299,000 CFU/ cm²) had the most bacterial adherence (Fig. 2). At high bacteria concentrations (5 \times 10¹⁰ CFU/mL), Quill showed less bacterial adherence than Vicryl and Vicryl Plus (p = 0.05 and 0.04, respectively). The bacterial adherence to PDS plus (81,000 CFU/mL/suture) was less than Vicryl* Plus (Table 2), p = 0.02. There were no statistical differences between other suture types regarding bacterial adherence at high bacterial concentrations. At physiologic bacteria concentrations $(5 \times 10^6 \text{ CFU/mL})$, (12 CFU/cm²) again showed the lowest amount of bacterial adherence (Fig. 3) when compared with Vicryl (47 CFU/ cm²) and PDS (21 CFU/cm²), p = 0.5 and p = 0.9, respectively.

Photographs of the suture material on agar plates (Fig. 4) qualitatively showed substantial growth of bacteria around the Vicryl sutures (shown as yellow), even after four washes. The PDS* and Quill sutures showed visibly less growth.



Table 1. Number of CFU/cm² recovered from sutures after washing by suture type and initial bacterial concentration

Type of suture	Supraphysiologic bacterial concentration (CFU/cm ²)	Physiologic bacterial concentration (CFU/cm ²)
Vicryl TM (braided)	213,000	47
PDS TM (monofilament)	101,000	21
Quill TM (barbed)	48,000	12
Vicryl TM Plus (braided)	299,000	
PDS TM Plus (monofilament)	81,000	

CFU = colony-forming units.

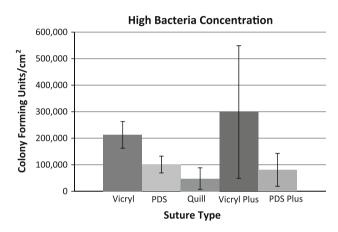


Fig. 2 The number of recovered bacteria from each vortex wash were added together for a total number of bacteria recovered from the suture by four washes. The mean number of bacteria for each suture is displayed. The error bars show two standard deviations (95% confidence intervals).

Table 2. ANOVA followed by Tukey's test to determine significance between individual groups

Sutures	p value
Vicryl* versus PDS*	0.3
Vicryl* versus Quill TM	0.05
Vicryl* versus Vicryl* Plus	0.8
Vicryl* versus PDS* Plus	0.2
PDS* versus Quill TM	0.8
PDS TM versus Vicryl TM Plus	0.1
PDS TM versus PDS TM Plus	0.8
Quill TM versus Vicryl TM Plus	0.04
Quill TM versus PDS TM Plus	0.7
Vicryl TM Plus versus PDS TM Plus	0.02

The antibacterial braided and monofilament sutures qualitatively showed no growth, even after no washes.

By confocal microscopy a qualitatively greater amount of bacteria adhered to Vicryl* as compared with PDS* (Fig. 5). A brighter green signal was seen in the grooves

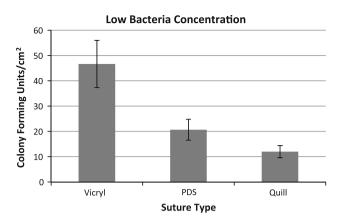


Fig. 3 The number of colony forming units (CFU) initially associating with the sutures after using physiologic bacterial concentrations is shown. The bacteria decreased below the lower limit of detection (2 CFU/suture) after one wash. The error bars indicate two standard deviations (95% confidence intervals).

throughout the length of the Vicryl suture, whereas few bacteria were seen throughout the length of the PDS suture. Confocal microscopy of the Quill suture showed adherence patterns similar to those of the PDS suture.

Discussion

Previous research has shown the superiority of monofilament sutures in contaminated wounds [1-3, 10, 13, 14, 20, 25]. Barbed monofilament suture is being used more frequently as a result of its efficiency, cost savings, and improved or comparable wound closure outcomes [5, 7, 15-17, 19, 21, 23, 24, 26, 28]. The use of barbed monofilament suture for closure in a TKA has shown superiority in tightness of closure and failure analysis [16, 24]. However, the barbed nature of this monofilament suture has the theoretical concern of the barbs acting as a place for bacteria to hide, resulting in higher infection rates if contaminated wounds are closed with barbed monofilament suture. We therefore determined (1) the adherence of bacteria to each of five suture types including a barbed monofilament suture; (2) the ability to culture bacteria after gentle washing of each suture type; and (3) the pattern of bacterial adherence.

There are several limitations of this study. First, these in vitro data may not translate directly to the in vivo setting. Although we have attempted to simulate incubation of the sutures with bacteria, this scenario would not necessarily be replicated in vivo. Second, this study does not control for numerous factors that would be present in vivo, such as the properties of specific bacteria, presence of gross tissue infection or biofilm formation at the site, an active immune system, and the flow of fluids over the sutures. Third, we chose to use MRSA for our study as it is the most



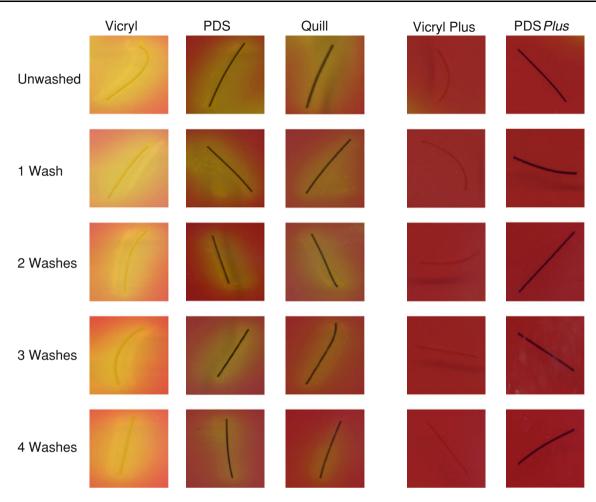


Fig. 4 Sutures were incubated for 5 minutes in approximately 5×10^{10} CFU of MRSA. After a rinse vortex wash to remove broth, sutures were vortex washed zero, one, two, three, or four times. The

sutures then were plated on mannitol salt agar and incubated overnight at 37°C. Data shown are representative of three independent experiments. The yellow around the suture indicates bacterial growth.

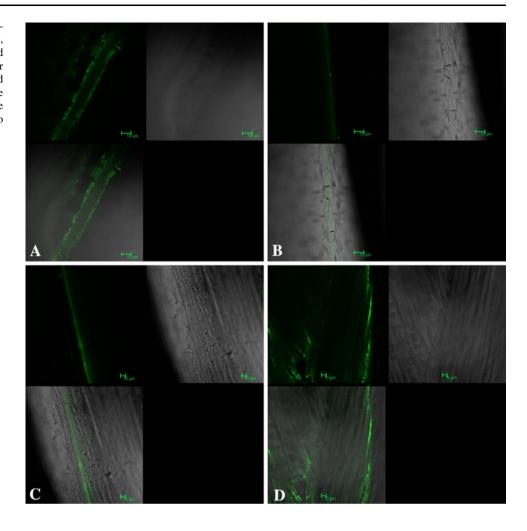
common isolate at our institution (66% of all Staphylococcus cultures). One study suggested coagulase negative Staphylococcus aureus to be the most common bacteria isolated from wounds [22]. Different bacteria could have different adherence properties and could affect our findings. Further studies comparing the adherence patterns of different bacteria are needed. Fourth, we chose to use planktonic bacteria rather than a biofilm. Bacteria in a biofilm, a common finding in infected total joint arthroplasties, likely would show increased adherence and that could alter our findings.

Our current study echoes those of Masini et al. [13]. They used 10⁸ CFU/mL of Staphylococcus aureus engineered to emit photons, allowing them to quantify the amount of bacteria adhered to each suture using imaging software. They found a greater amount of bacteria adhered to Vicryl than monofilament sutures (Monocryl and Prolene). In contrast to our study, Masini et al. observed less bacterial adherence to Vicryl Plus than to Vicryl sutures. It is possible

that prolonged exposure to the antibacterial properties of the Vicryl* Plus (24-hour incubation) killed the bacteria before it was able to emit photons to be detected by their imaging software, whereas, in our study, the bacteria were washed off the suture in 5 minutes and the number of bacteria were recorded from the wash. Based on the findings of their study and ours, it is unclear whether the increased adherence was the result of the braided nature of Vicryl or the suture material, given that silk suture did not adhere a different amount of bacteria compared with monofilament sutures. Our study adds to the literature as Masini et al. did not examine the most commonly used absorbable monofilament suture for deep layer closure [13].

Edmiston et al. [4], using scanning electron microscopy, compared bacterial adherence with antibiotic-(triclosan) coated suture (Vicryl Plus) and traditional absorbable braided suture (Vicryl) using three different bacterial broths: MRSA, Staphylococcus epidermidis, and Escherichia coli. They exposed the suture materials to the





bacterial broths for either 5 seconds or 2 minutes, gently washed the suture with normal saline, and then incubated the suture for 24 hours. They observed fewer bacteria adhered to the sutures coated with triclosan compared with those not coated. Although fewer bacteria appeared to adhere to the antibiotic suture by scanning electron microscopy after 24 hours of incubation, our findings suggest that this is a function of the bactericidal activity of the antibacterial coating, rather than a function of adherence. We found qualitatively equivalent adherence of bacteria to Vicryl and Vicryl Plus sutures.

Barbed monofilament suture appears to have comparable performance to monofilament suture in a contaminated wound model. The barbed monofilament suture, Quill, showed less bacterial adherence than Vicryl and Vicryl Plus, both absorbable braided sutures. Antibacterial-coated sutures effectively prevented bacterial growth, although adherence to the suture was unchanged compared with nonantibacterial-coated sutures. Although in vivo models will be needed to confirm use of this in vitro model in the clinical setting, our observations suggest barbed monofilament suture might be substituted for monofilament suture,

at the surgeon's discretion, without fear of increased risk of infection.

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