THE ROLE OF BIOFILM FORMATION IN PERCUTANEOUS KIRSCHNER-WIRE FIXATION OF RADIAL FRACTURES

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This study examines the formation of bacterial biofilms on percutaneous wires used for fracture fixation. Twelve control (clinically uninfected) wires and ten infected wires were collected and examined using broth culture and scanning electron microscopy. Three of the 12 control wires grew *Staphylococcus* spp. with very low bacterial counts in their percutaneous portions. In the clinically infected wires, six wires in four subjects had positive cultures in their percutaneous portions and four of these also had positive cultures in their deep portions with much higher bacterial counts than the controls. In two patients (four wires) treated with antibiotics, cultures were negative except for the percutaneous portion of one wire. Scanning electron microscopy did not reveal bacterial biofilm formation, but biological deposit without bacteria was noted on most wires. During the 6 weeks of fracture fixation, some bacterial colonization of wires occurred, but bacteria did not form biofilms which may increase bacterial resistance to systemic antibiotics, cause implant loosening and act as a source of late infection.

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INTRODUCTION

Bacteria naturally attach to living or inert surfaces to form biofilms. Biofilms consist of microcolonies of bacteria bound together in a carbohydrate matrix, with a complex system of channels allowing delivery of nutrients, clearance of waste and communication via extracellular signalling molecules (Costerton et al., 1999; Potera, 1996; Shapiro, 1998). Bacteria within biofilms differ from their planktonic counterparts both morphologically and metabolically. They are more resistant to antibiotics, the host immune response and physical cleansing agents, which makes eradication difficult (Costerton et al., 1999). Biofilm formation on medical implants has been investigated both clinically and experimentally for a range of devices including prosthetic implants and catheters (An and Friedman, 1998; Gristina et al., 1991; Khardori and Yassien, 1995; Liñares et al., 1985; Reid et al., 1992; Rupp et al., 1999). The normally nonpathogenic *Staphylococcus epidermidis* accounts for at least half of orthopaedic device infections, highlighting the importance of normal skin flora in infection (Gristina, 1994).

Kirschner (K)-wires are widely used for percutaneous fracture fixation. In clinical practice, they can become inflamed with subsequent development of frank sepsis in some cases. More commonly, K-wires may loosen during the course of treatment. These clinical signs may be consistent with bacterial colonization of wires. In order to determine whether bacterial colonization and biofilm formation occur on percutaneous K-wires, we have conducted a prospective clinical study of wires collected from patients with distal radial fractures treated by the Department of Hand Surgery and Peripheral Nerve Surgery at Royal North Shore Hospital.

METHODS

Patients having percutaneous K-wire fixation of distal radial fractures were monitored for signs of infection or irritation during the 6-week postoperative period until wire removal. Clinical infection was quantified using the Oppenheim wound score (Table 1). Twelve clinically uninfected (Oppenheim score 0 or 1) wires from six patients (Table 2) and ten clinically infected (Oppenheim score >1) wires from six patients (Table 3) were collected for the study. Two patients in the clinically infected group had received antibiotic treatment prescribed by a local doctor prior to wire removal. A swab of pus around infected pin sites was collected when appropriate and was analysed using routine microbiological techniques.

After removal, each wire was cut into three sections (Fig 1) using sterile instruments and each section was stored in sterile normal saline and sent for analysis.

Microbiology

Each wire section was placed into glass MacConkey bottles containing 15 ml of tryptone soy broth (TSB). After vigorous shaking, sample bottles were ultrasonicated in a soniclean ultrasonic bath (JMR, Australia). The sections were then shaken again and 100 µl of the broth was then plated onto blood agar plates (oxoid) and spread for quantitative analysis. These plates were incubated overnight aerobically and for 48 hours

Table 1—Oppenheim wound score

Grade	*Description*	*Treatment*
0	No discharge, wound dry and clean	
1	Slight discharge, redness around pin	Local pin care
2	Redness and tenderness in soft tissues with or without discharge of pus	Local pin care and oral antibiotics
3	As above + failure to improve with local care and oral antibiotics	Infected pin removed and oral antibiotics
4	Severe soft tissue involvement affecting more than one pin	Infected pins removed and oral antibiotics
5	As above + bone involvement visible on X-ray	Pins removed and curettage of bone
6	A sequestrum has formed within the bone and a persistent sinus has developed	Further surgery required to eradicate the problem

Table 2—Results of biofilm testing of control wires

			Microbiology results		
Wire	*Wound score*	*SEM*	*Percutaneous*	*Near cortex*	*Far cortex*
A1	0	No biofilm	*S. epidermidis*	neg	neg
B1	1	No biofilm	neg	neg	neg
B2	1	No biofilm	neg	neg	neg
C1	1	No biofilm	*S. epidermidis*	neg	neg
C2	1	No biofilm	neg	neg	neg
C3	1	No biofilm	*S. capitis*	neg	neg
D1	0	No biofilm	neg	neg	neg
D2	0	No biofilm	neg	neg	neg
E1	0	No biofilm	neg	neg	neg
E2	0	No biofilm	neg	neg	neg
F1	0	A few cocci end portion, deposit mid portion	neg	neg	neg
F2	0	A few cocci mid portion	neg	neg	neg

Wire designation refers patient (letter) and wire number. *S.* = *Staphylococcus*. neg = negative (no growth).

Table 3—Results of biofilm testing of clinically infected wires

			Microbiology results		
Wire	*Wound score*	*SEM*	*Percutaneous*	*Near cortex*	*Far cortex*
G1	N/A	No biofilm	*S. epi* and *aureus*	*S. epi* and *aureus*	*S. aureus*
H1	3	Deposit with bacteria, skin portion	*S. epi*	neg	neg
H2	3	No biofilm	*S. epi*	neg	neg
I1	3	N/A	*Diphteroid* and *Bacillus*	*Diphteroid* and *Bacillus*	*Diphteroid* and *Bacillus*
I2	3	N/A	*Diphteroid* and *Bacillus*	*Diphteroid* and *Bacillus*	*Diphteroid*
J1	2	No biofilm	*S. aureus*	*S. aureus*	*S. aureus*
K1*	4	A few cocci	neg	neg	neg
K2*	4	A few cocci	neg	neg	neg
L1*	4	A few cocci, skin portion	*S. epi* and *enterococci*	neg	neg
L2*	4	A few cocci, skin portion	neg	neg	neg

Wire designation refers patient (letter) and wire number.
* = patient receiving antibiotic therapy prior to wire removal; N/A = not available; *S.* = *Staphylococcus*; *epi* = *epidermidis*; neg = negative (no growth).

anaerobically at 37°C. Bacteria were identified by standard microbiological methods.

Another 1 ml of sonicated and shaken TSB was removed and inoculated into cooked meat media (oxoid) for anaerobic growth and incubated at 37°C for 7 days. The remainder of the TSB was incubated aerobically in nutrient broth under the same conditions. Cultures were examined daily for 7 days. If growth occurred organisms were subcultured and identified using standard microbiological methods. *Staphylococcus* species were identified using the Staph Ident Kit (bioMerieux).

All precautions were taken to avoid contamination of samples and all the work was carried out in a Biological Safety Cabinet Class II, with sterile instruments and cultures of proven sterility.

Scanning electron microscopy

Samples of approximately 1 cm length were fixed in 3% glutaraldehyde containing 0.05% Rutheium Red (Sigma) in phosphate buffer for a minimum of 1 hour.

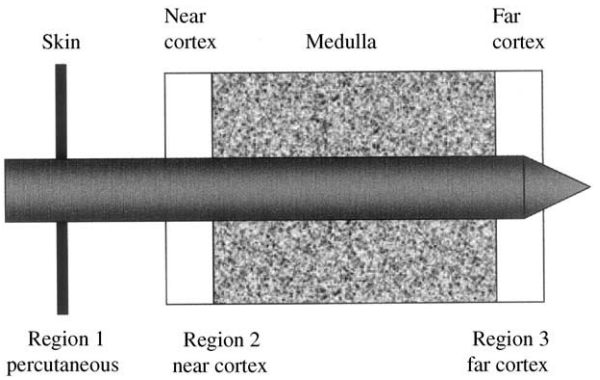

Fig 1 Wire sections.

Fixed specimens were washed, dehydrated and dried with a Critical Point Drier (Philips). Sections were scanned by a XL30 (Philips) Scanning Electron Microscope at spot size 3, accelerating voltage 10 KV. Backscattered electron mode was also utilized to observe organic deposit on wires.

RESULTS

Microbiology

Only three of 12 wires which were clinically not infected had a positive culture for the percutaneous portion and no organisms were found on the deeper portions of any of these wires (Table 2). The number of organisms was too low for quantitation.

The four patients with infected wires who had not been treated with antibiotics had positive cultures for the percutaneous portion of the wire (Table 3). Bacterial quantitation yielded $>1.0 \times 10^4$ CFU/section prior to overnight incubation. The deeper portions of the wire returned positive cultures in three of these four patients (Table 4), with variable numbers of CFUs found.

In the two patients receiving antibiotic treatment prior to wire removal, only one of four wires had a positive culture in the percutaneous portion, with the same organisms present in the skin swab (Table 4).

Scanning electron microscopy

Biological deposit devoid of bacteria was noted on most wire sections. Bacterial biofilms were not observed on any samples. Occasional cocci were noted, mainly in the percutaneous portions of infected wires. Only two wires from a single patient which were not infected had visible cocci, despite negative bacterial culture. Unfortunately, scanning electron microscopy was not available for the patient with the highest bacterial concentrations along the wire.

Table 4—Microbiology of wound swabs around infected wires

Patient	*Microbiology results*
I	mainly *Diphteroid*, a few *Bacillus*
K*	no growth
L^	moderate *Staphylococcus epidermidis*, scanty *Enterococci*

*treated with Keflex beginning 7 days before pin removal at 6 weeks. ^treated with IV Flucloxacillin for 2 days prior to pin removal at 11 days.

DISCUSSION

The bacterial counts correlated well with signs of clinical infection and skin flora appeared to be the source of K-wire infection based upon both the bacterial species and the higher bacterial counts on the percutaneous portion of the wires. Infected wires were uncommon and early treatment with systemic antibiotic therapy for suspected sepsis made recovery of organisms difficult.

Although isolated bacterial cells were found along a number of wires, in no instance did they form a bacterial biofilm. It is likely that the 6-week postoperative period of K-wire fixation for radial fractures did not provide sufficient time for the formation of a bacterial biofilm. K-wire infections become more common if the wires are left in situ for longer than 10 weeks (Botte, 1992). Gristina (Gristina et al., 1985) has suggested that bacteria and host tissue cells ''race for the surface'' of an implant. If host tissue wins there is less surface for bacterial adhesion, thus reducing the risk of late infection of the implant. If bacteria win the race, clinical sepsis or biofilm formation may result. A biofilm can be present without early clinical evidence of infection, but may cause late infection or loosening of an implant.

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