Bacterial Contamination of a Marking Pen in Anterior Cruciate Ligament Reconstruction

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Background: A sterile surgical marking pen is commonly used during anterior cruciate ligament reconstruction (ACLR) to outline the proposed skin incision and then to mark the graft during preparation. Once in contact with the skin, the pen is a potential source of bacterial transmission and subsequent infections after ACLR.

Purpose/Hypothesis: The purpose of this study was to assess whether the skin marking pen is a fomite for contamination during arthroscopic ACLR. We hypothesized that there would be a difference in the rate of culture-positive pens between control pens and the study pens used to delineate the proposed skin incision.

Study Design: Controlled laboratory study.

Methods: Twenty surgical marking pens were collected prospectively from patients undergoing ACLR over a 12-month period. All patients underwent standard preoperative sterile preparation and draping procedures. Proposed incisions were marked with a new sterile pen, and the pen tip was immediately sent for a 5-day inoculation in broth and agar. Negative controls (unopened new pen) and positive controls (used to mark the skin incisions preoperatively) were also cultured. Additionally, blank culture dishes were observed during the growth process. All pens were removed from the surgical field before incision, and new marking pens were used when needed during the procedure.

Results: Three of the 20 study pens (15%) demonstrated positive growth. All 3 pens grew species of *Staphylococcus*. None of the negative controls demonstrated growth, 6 of the 12 positive controls showed growth, and none of the blank dishes exhibited growth.

Conclusion: This study found a 15% rate of surgical marking pen contamination by *Staphylococcus* during ACLR. It is recommended that the skin marking pen not be used for any further steps of the surgical case and be discarded once used.

Clinical Relevance: Infections after ACLR are rare but may result in significant morbidity, and all measures to reduce them should be pursued. Surgeons performing ACLR should dispose of the surgical marking pen after skin marking and before intraoperative use such as graft markup.

Keywords: ACL reconstruction; contamination; infection; SSI; marking pen

Anterior cruciate ligament reconstruction (ACLR) is one of the most commonly performed procedures by orthopaedic surgeons.[14] A postoperative infection, despite occurring in less than 1% of cases, causes significant morbidity, including repeat surgery, graft failure, arthrofibrosis, and potentially irreversible changes to the articular cartilage.[8,17] Contamination of the ACLR graft is possible; however, there is no current literature with regard to the rate of bacterial inoculation during graft preparation for ACLR. Multiple potential sources of contamination exist, including gloves, gowns, and knife blades, as demonstrated by Davis et al.[3] To date, the marking pen as a source of contamination has not been studied. Nakayama et al[10] demonstrated a 6% incidence of positive cultures, predominantly *Staphylococcus epidermidis*, obtained intraoperatively from the skin adjacent to the incision in ACLR. Based on these results, it is possible that a surgical pen could transfer *S epidermidis* and other skin flora from the colonized skin to the graft.

Marking pens are commonly used to mark skin incisions (Figure 1A), not discarded, and then used to mark graft preparation sites (Figure 1B). They are a potential source of graft contamination. The purpose of this study was to assess whether the sterile skin marking pen is a vector for contamination during ACLR. It was hypothesized that there would be a difference in the rate of culture-positive pens between control pens and the study pens used to delineate the proposed skin incision.
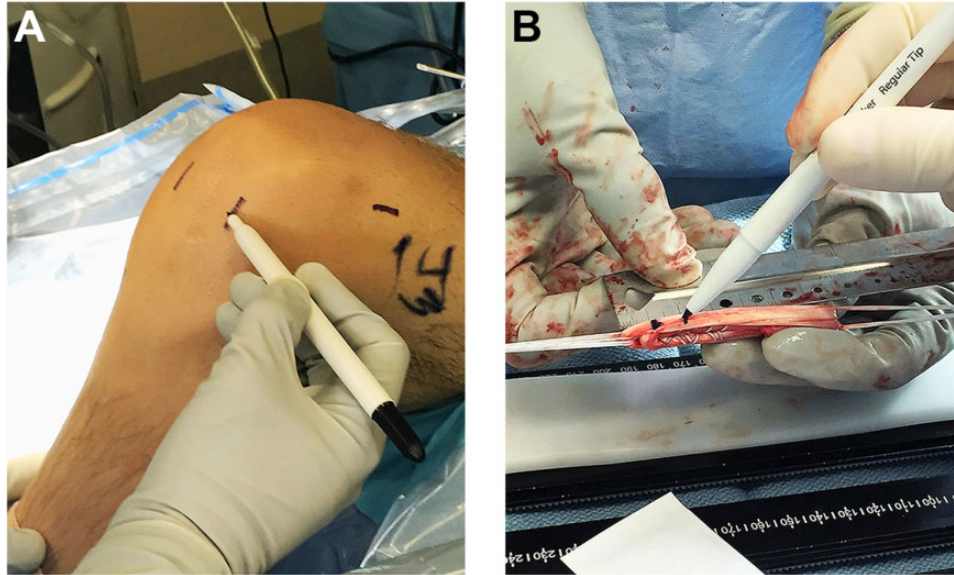
Figure 1. (A) Patient's left knee, supine, demonstrating the use of a sterile marking pen on the skin for the proposed incisions and (B) marking of the hamstring autograft.

METHODS

Twenty alcohol-based solvent surgical marking pens (Devon Surgical Skin Marker, Regular Tip; Covidien) were collected prospectively from patients undergoing ACLR between March 2013 and March 2014. Ten consecutive patients were chosen initially. After initial analysis of culture results, it was felt that an additional 10 consecutive patients were needed to add power to the study. The inclusion criteria for the study were patients older than 18 years undergoing arthroscopic ACLR and no previous knee surgery.

A single sports medicine fellowship–trained orthopaedic surgeon (J.A.M.) performed outpatient arthroscopic ACLR at one academic outpatient surgery center with the use of a bone–patellar tendon–bone (BPTB) or hamstring autograft. All patients received intravenous cefazolin or clindamycin within 30 minutes of the incision. Before surgical site and incision marking, the patient's skin was cleaned with 2% chlorhexidine gluconate cloths (Sage Products) in the preoperative area, per standard hospital policy. DuraPrep Surgical Solution (3M) was then applied to the skin during preoperative preparation and was allowed to dry for 3 minutes before sterile draping. After draping,

the patella and patellar tendon were outlined and arthroscopic portals marked with the sterile surgical marking pen. The mean length of total incisions marked was 8 cm. The pen tip was then immediately placed into a sterile culture and sent to a laboratory for a 5-day inoculation in broth and agar. All pens were removed from the surgical field before incision, and new marking pens were used when needed, per the senior author's (J.A.M.) standard operative technique.

Positive controls were obtained by similarly marking the skin before application of the DuraPrep Surgical Solution using sterile gloves and instruments. One positive control was obtained per day rather than for each operative case. Negative controls consisting of new unopened pens handled directly in the laboratory were also cultured. Additionally, blank culture dishes were observed during the growth process to confirm that growth did not occur during the incubation period. Results were reported as either growth or no growth. Positive cultures were further classified using standard laboratory tests.

This study was submitted and reviewed by our institutional review board and was determined to be exempt from further review under 45 Code of Federal Regulations (CFR) 46.102(d), as this work was judged to be a quality assurance

TABLE 1
Study Pen Culture Results From 20 Consecutive
ACLR Patients[a]

Study Pen	Growth (+/–)	Organism
1	+	*Staphylococcus*
2	+	*Staphylococcus*
3	–	
4	–	
5	–	
6	–	
7	–	
8	–	
9	–	
10	–	
11	–	
12	–	
13	–	
14	–	
15	–	
16	+	*Staphylococcus, Bacillus*
17	–	
18	–	
19	–	
20	–	

[a]The study pen cultures were inoculated for 5 days in broth and agar. Three of the 20 (15%) cultures were positive for a *Staphylococcus* species. ACLR, anterior cruciate ligament reconstruction.

TABLE 2
Positive Control Culture Results of 12 Pens[a]

Positive Control	Growth (+/–)	Organism
1	+	*Staphylococcus*
2	–	
3	+	*Staphylococcus, Bacillus*
4	+	*Aspergillus*
5	+	*Staphylococcus*
6	–	
7	+	*Staphylococcus*
8	–	
9	+	
10	+	
11	+	
12	+	*Staphylococcus, Bacillus*

[a]Positive controls were obtained by marking the skin before application of the sterile preparation solution (DuraPrep Surgical Solution; 3M) using sterile gloves and instruments. Six of the 12 (50%) cultures were positive.

0.0014), study pens versus positive controls ($P = .05$; power = 0.50), study pens and positive controls versus negative controls ($P = .16$; power = 0.49), or positive controls versus negative controls ($P = .04$; power = 0.61). Power reached exactly 80% for positive controls versus blank dishes.

DISCUSSION

The most important finding of this study was that 3 of 20 (15%) marking pens used on skin after application of a sterile preparation solution were found to be contaminated, specifically with *Staphylococcus* species. There was a difference in the rate of culture-positive pens between the control pens and study pens, confirming the study hypothesis; thus, surgical marking pens should be considered a potential source of contamination. Although not the primary aim of this study, there was a statistically significant difference in the inoculation rate of study pens versus positive controls and positive controls versus negative controls.

Causes of postoperative septic arthritis after ACLR more frequently include *S epidermidis* and *S aureus* but can also consist of less common organisms such as *Peptostreptococcus, Pseudomonas, Escherichia coli, Enterobacter aerogenes*, and *Propionibacteriaceae*.[2,5-7,9,13,15,16,18] A study by Davis et al[3] found that 63 of 100 joint replacement procedures had some sort of field contamination by *S epidermidis*, as found on sucker tips, light handles, skin blades, gloves, gowns, and needles. Infections after arthroscopic surgery have been similarly linked to *S epidermidis* operative field contamination that is introduced into the joint through mechanisms such as preoperative razor shaving and use of contaminated inflow cannulas, graft boards, and suture clamps.[1,13,15] Studies have shown other risk factors for the development of postoperative septic arthritis after ACLR to include intra-articular corticosteroid joint injection, previous knee surgery, and meniscal repair.[1,6,9]

activity. Further, cultures were not obtained from patients but from the marking pen itself. The senior author's current clinical practice is to dispose of the pen after skin marking in all surgical procedures. Therefore, this study represents the authors' current clinical practice, and because the cultures were obtained after removal of the pen from the field, no risk of contamination was present. All ethical standards of maintaining patient confidentiality have been employed, including those in accordance with the United States Health Insurance Portability and Accountability Act (HIPAA). A typical sterile surgical marking pen costs US$0.53 in our hospital system.

RESULTS

Twenty patients met the study inclusion criteria, with 12 positive controls, 8 negative controls, and 9 blank dishes, for a total of 49 cultures. Three of the 20 study pens (15%) demonstrated positive growth. All 3 contaminated pens grew species of *Staphylococcus*, and 1 study pen also grew a species of *Bacillus* (Table 1). Six of the 12 (50%) positive controls showed growth, while none of the 8 negative controls or blank dishes exhibited growth. Organisms found in the positive control and study groups were predominantly *Staphylococcus* but also included *Bacillus* and *Aspergillus* (Table 2). No patients clinically developed a postoperative infection.

A post hoc power analysis was performed, assuming an alpha value of 0.05. This study failed to reach 80% power for study pens versus negative controls ($P = .53$; power =

The mechanism of surgical site inoculation with microorganisms, including *S epidermidis*, during ACLR is not always traceable, however. In one study of 1231 patients undergoing ACLR with 6 postoperative infections (0.5%), neither surgical instruments nor the technique could be identified as the source of the infection.[2] Another study of 1615 ACLR cases revealed that 8 of 11 postoperative septic arthritis infections were caused by *S epidermidis* from an unknown source, possibly from contamination during or after surgery.[6]

The recent literature has attempted to resolve the uncertainty surrounding the unknown mechanisms and rate of contamination during ACLR by looking at the autograft preparation technique. A 2008 study by Hantes et al[4] of 60 patients undergoing ACLR examined the autograft preparation process (both BPTB and hamstring tendon) at 3 different time intervals and found a graft contamination rate of 12%. *S epidermidis* contaminants were first identified in each of the 3 different time intervals, but no reason was given for why a contaminant would first appear at a different time interval in graft preparation. This study did not find any statistically significant difference between autograft type and contamination rate. However, a more recent investigation demonstrated an 8 times higher infection rate with the use of hamstring autografts as compared with BPTB autografts.[8] One potential explanation is that the typical practice is to mark the hamstring grafts but not patellar tendon grafts. The graft itself may be a source of contamination before and/or after marking it with a sterile pen. Some authors have advocated soaking the grafts in antibiotics (ie, vancomycin sponges), which have demonstrated decreased infection rates.[11,12]

To our knowledge, no previous study has evaluated the surgical marking pen as a possible fomite for intraoperative graft bacterial transmission during ACLR. During ACLR, the standard practice is for the surgeon to mark the prepared surgical site with a sterile surgical pen. This surgical pen is then used during the preparation process to mark the ACLR graft. Results of a 2012 study by Nakayama et al[10] support the suggestion that a surgical pen used on prepared skin may transfer microorganisms into the joint during ACLR. Their study of 50 patients undergoing ACLR revealed that 46% had preoperative bacterial colonization of the skin. After the skin was prepared intraoperatively, 6% still had positive skin colonization near the incision site, and 2% had positive graft colonization. The majority of this colonization (93%) was *S epidermidis*. Based on these results, it is possible that a surgical pen could have transferred *S epidermidis* from the colonized skin to the graft. Our study likely represents the best-case scenario, as the pens were processed immediately as opposed to letting them incubate and sit out exposed during the procedure.

Different types of sterile surgical marking pens have been analyzed for their capacity to resist bacterial colonization. As early as 2006, a laboratory study cultured skin markers of different solvent bases (eg, alcohol vs water) that had been contaminated with methicillin-resistant *S aureus* (MRSA); one marker type continued to produce positive MRSA cultures for up to 3 weeks after inoculation.[19] Alcohol- and ethanol-based markers seem to manifest rapid bactericidal action, usually within minutes. In our study, all pens were standardized and used an alcohol-based solvent.

Limitations

This study is not without its limitations. Cross-contamination from sterile gloves or instruments may have led to false-positive results in the study pens; however, no growth was observed for the negative controls or the blank dishes. Unexpectedly, 6 of the positive controls showed no growth. This is likely because of the use of Sage cloths preoperatively, which may have affected the test samples and may have led to underestimation of the contamination rates. ACLRs only, and not all knee arthroscopic procedures, were included. In addition to skin marking, ACLR involves markup of the graft during the procedure. Including only ACLR and not other procedures emphasized this further markup of the graft and helped to simplify the methodology of the study design. Last, the sample size used was small and underpowered. However, any evidence of contamination is concerning, especially considering the cost-effective and low-risk alternative of using a new sterile pen intraoperatively.

CONCLUSION

After obtaining cultures of marking pens used to mark the sterile field, we found that the marking pens had a 15% rate of contamination. Infections are rare but may result in significant morbidity, and all measures to reduce these should be pursued. Surgeons performing ACLR should consider disposal of the surgical marking pen after skin marking and before intraoperative use such as graft markup. We recommend that surgeons use a fresh marking pen when marking the graft is required. This requires minimal cost and effort.

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