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Title: Bacterial deoxyribonucleic acid (DNA) is often present in failed revision anterior cruciate ligament (ACL) reconstructions

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Abstract: Purpose: To determine whether bacterial DNA will be detectable via polymerase chain reaction (PCR) in torn graft tissue at time of revision anterior cruciate ligament reconstruction (ACLR).

Methods: A total of 31 consecutive revision ACLR cases from one center were recruited from 2014-2016. No patients had clinical signs of infection on presentation. Torn graft tissue was obtained in revision cases and subjected to clinical culture and PCR analysis with a universal bacterial primer. Fluorescent microscopy was utilized to confirm presence of a biofilm. Negative controls samples were obtained of water open to air on the field and excess primary ACLR graft tissue as well as torn native ligament to evaluate for PCR positivity due to environmental contamination.

Results: Clinical cultures were positive (coagulase negative staphylococcus) in one revision case (3%, 1/31). Bacterial DNA was detectable in most revision ACLR cases 27/31 (87.0%) and there was a low rate of PCR positivity in negative control samples of water open to air (0%, 0/3), excess primary ACLR graft tissue after passage (20%, 1/5) or native torn ligament (20%, 1/5). Bacterial biofilm presence on failed graft tissue as well as monofilament suture was visually confirmed with fluorescent microscopy.

Conclusions: Bacterial DNA is frequently present in failed ACLR grafts, with high rates of DNA detection by PCR but low culture positivity.

Level of Evidence: Level IV, case series

# Bacterial deoxyribonucleic acid (DNA) is often present in failed revision anterior cruciate ligament (ACL) reconstructions

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**Short title:** Bacterial DNA in failed ACLR

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**Abstract**

**Purpose**: ~~We hypothesize that~~To determine whether bacterial DNA will be detectable via polymerase chain reaction (PCR) in torn graft tissue at time of revision anterior cruciate ligament reconstruction (ACLR) ~~at higher rates than in primary ACLR graft tissue~~.

**Methods**: A total of 31 consecutive revision ACLR cases ~~and 5 primary ACLR controls~~ from one center were ~~included~~~~were~~ recruited from 2014-2016. No patients had clinical signs of infection on presentation. Torn graft tissue was obtained in revision cases and ~~and torn native ligament as well as excess hamstring autograft was obtained in primary ACLR controls. Samples were~~ subjected to clinical culture and ~~,~~ PCR analysis with a universal bacterial primer. ~~, and f~~Fluorescent microscopy was utilized to confirm presence of a biofilm. Negative controls samples were obtained of water open to air on the field and excess primary ACLR graft tissue as well as torn native ligament to evaluate for PCR positivity due to environmental contamination.

**Results**: Clinical cultures were positive (coagulase negative staphylococcus) in one revision case (3%, 1/31). Bacterial DNA was detectable in most revision ACLR cases 27/31 (87.0%) and there was a low rate of PCR positivity in negative control samples of water open to air (0%, 0/3), excess primary ACLR graft tissue after passage (20%, 1/5) or native torn ligament (20%, 1/5). ~~and less commonly 1/5 (20%) in primary ACL autograft controls (p=0.002, Chi-square test); staphylococcal~~ Bacterial biofilm presence on failed graft tissue as well as monofilament suture was visually confirmed with fluorescent microscopy. ~~A trend toward higher bacterial DNA concentrations was observed with prior autograft (median 19 ng/sample range 0-101) vs. allograft (median 13 ng/sample range 0-21; p=0.13, Wilcoxon rank sum).~~

**Conclusions**: ~~Staphylococcal~~ Bacterial DNA is frequently present~~biofilms are present on~~ in failed ACLR grafts, with high rates of DNA detection by PCR but low culture positivity. ~~There is likely bacterial colonization of many failed ACLR grafts, though the causal relationship between graft colonization and failure remains unclear.~~

**Level of Evidence:** Level ~~III~~IV, ~~therapeutic study~~case series

**Key words:** bacterial biofilm; revision ACL reconstruction; failed ACLR

## Introduction

Clinically significant postoperative infection related to anterior cruciate ligament reconstruction (ACLR) is a rare occurrence, with two recent studies showing rates between 0.49% and 1.7%.[1, 2] A large case series by Gobbi et al. reported an incidence of post-operative infection of 0.37% after 1850 ACLRs, and an accompanying literature review of 16 studies reporting on 35,795 ACLRs had a pooled mean infection rate of 0.68% (range 0.14-2.6%).[3]

-Staphylococcus species are by far the most common causative organisms.[1] Septic arthritis following ACLR commonly presents with a fever and modest local signs of infection as well significantly elevated inflammatory markers, particularly synovial fluid white blood cell count.[1] Judd et al. report that clinically evident intra-articular infections after ACLR presented with elevated serum ESR (mean 67) CRP (mean 14) and markedly elevated synovial fluid WBC counts (mean 52,000).[4]

Failure following primary ACLR remains a significant problem. A 2011 systematic review of level 1 studies reported rates of primary ACLR failure of 7.2% for patellar tendon autografts and 15.8% for hamstring tendon autografts.[5] Despite extensive study of graft failure, the mechanism of graft failure remains poorly understood. Traumatic re-injury, infection, and technical error have all been suggested to play a role.[6] Recent work by Hiller et al suggests there may be bacterial colonization of these ruptured grafts in the absence of clinical symptoms.[7] Colonization of the ACL reconstruction graft or materials used for fixation such as suture, interference screws and other fixation devices with low virulence bacteria could cause graft tissue attenuation without overt clinical symptoms and predispose patients to ACL graft failure.

While clinically apparent infection post-ACLR is rare, subclinical bacterial colonization of orthopaedic graft material have shown colonization rates as high as 23%,[8] In

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other procedural fields, biofilms are noted to be frequently present in certain culture-negative soft tissue lesions; Bjarnsholt et al. demonstrate biofilms on 7/8 of culture negative long-lasting nodules after soft tissue filler injections.[9] Colonization and biofilm formation with low virulence organisms such as *P. acnes* is now a well-recognized entity in the shoulder literature. Millet et al. published a case series of patients with *P. acnes* post-operative shoulder infections with presented no clinical signs of infection other than pain.[10] Hou et al. performed a case-control study of patients presenting for revision arthroplasty with positive versus negative *P. acnes* cultures, and the culture positive group was more likely to have glenoid sided loosening and a soft tissue membrane between the humeral component and endosteum.[11] In 2015, Hiller et al. demonstrated presence of bacteria in failed ACL grafts and found significantly different species specific markers when comparing failed ACLR grafts to control ACLs removed during arthroplasty.[7]

Polymerase chain reaction (PCR) is a highly sensitive method for detecting bacterial DNA present in very low concentrations and detecting species that cannot be reliably cultured in a clinical laboratory. ~~When investigating low virulence bacteria or bacteria that reside within a biofilm, this~~ This is a useful screening method for detecting bacterial DNA in settings that would otherwise likely be culture-negative, such as low virulence bacteria, bacteria with fastidious growth requirements, and bacteria that are quiescent within a biofilm. The purpose of this study is to determine whether bacterial DNA will be detectable via polymerase chain reaction (PCR) in torn graft tissue at time of revision anterior cruciate ligament reconstruction (ACLR). We hypothesize that bacterial DNA will be detectable via PCR in torn graft tissue at time of revision ACL reconstruction at higher rates than in primary ACL reconstruction graft tissue.

**Methods**

~~*Recruitment*~~*Sample size estimation and recruitment*

Institutional review board approval was obtained prior to patient enrollment. There ~~is~~are no *a priori* data available to determine the prevalence of bacterial DNA in failed ACL reconstructions as detected by conventional PCR methods. The only prior report of bacterial assessment by molecular methods was by, Hiller et al. who detected bacteria in 8/10 samples via a hybrid PCR-mass spectroscopy assay[7]. The sensitivity of bacterial PCR assays can vary depending on the specimen being analysed[12] as well as the PCR protocol that is utilized including the specific primers and number of cycles applied. Ryu et al. report a rate of PCR positivity of 78% from sonicate fluid but only 16% from tissue from the same cohort of infected total knee arthroplasty patients.[12] Assuming our rate of detection could potentially be low with our specific PCR protocol when applied to samples from patients in the absence of obvious clinical infection symptoms, we estimated that 28 revision cases would be required to detect a 20% prevalence with a 15% margin of error and alpha =0.05.

A total of 31 consecutive revision ACL reconstructions ~~and 5 primary ACL reconstruction controls (all hamstring autograft)~~ from one center from 2014-2016 were included (Table 1). Inclusion criteria included presentation with a ruptured ACL graft necessitating revision reconstruction. There were no age requirements for study participation. Exclusion criteria included any prior history of deep or intraarticular knee infection. All patients within the study period met criteria for inclusion and consented to participate. ~~Institutional review board approval was obtained prior to patient enrollment.~~ Among revision cases, ~~23~~ 25 (~~64~~81%) had an autograft and ~~, 5~~ 6 (~~14~~19%) had an allograft~~, and 3 (9.3%) had an unknown graft placed~~ during the prior ACL reconstruction (Table 1). ~~Among revision cases, t~~The time from the previous reconstruction to graft failure was a median of 5.4 years (range, 105 days-20.6 years). All patients were treated by one of three sports-medicine fellowship trained surgeons within a single academic medical center practice. No patients had

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clinical signs of infection as demonstrated by clinical exam. Additionally, no included patients had elevated serum inflammatory markers. Synovial aspirate was not routinely performed on first time revisions, but patients presenting for a second or third revision ACL reconstruction or those who had ever had a history of post-operative knee infection did undergo pre-operative aspiration and were all found to have normal synovial fluid white blood cell counts.

*Sample procurement and clinical testing*

For revision cases, tissue biopsies of the from the tendon graft were obtained from the femoral tunnel, intraarticular segment, and tibial tunnel. A set of instruments previously unused during the procedure were utilized for sample procurement. A portion of the sample from each of the three sites (tibial tunnel, intra-articular, and femoral tunnel) were sent to the clinical microbiology lab for routine aerobic, anaerobic, and fungal cultures as well as gram stain. The remaining portion was kept for later PCR analysis.

*Selection of negative control samples*

Prior studies have suggested that environmental contamination in the operating room can frequently result in positive bacterial PCR of air samples in what was otherwise considered sterile cases.[13] We also believe there is a potential for contamination from skin flora during tissue handling or passage of instruments through arthroscopic portals. Several sources of control specimens were therefore selected to evaluate the rate of positivity due to environmental contamination for our specific PCR assay in conditions typically seen throughout an ACL reconstruction procedure. We obtained a portion of ~~The~~ intra-articular torn native ligament ~~-as~~ as well as excess hamstring tendon graft after passage and tibial tunnel interference screw placement from 5 primary ACL reconstructions; we selected these

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samples from primary ACLR cases to evaluate for positive results from skin flora after soft tissue handling or instrument passage through portals while also eliminating the possibility of cross-contamination from the torn graft in revision ACLR cases. Finally, samples of sterile water left open to air throughout revision cases (n=3) were tested to evaluate for positive results due to air contaminants.

~~was obtained immediately following the initial diagnostic arthroscopy.~~ ~~As PCR is very sensitive, control specimens were used to confirm that PCR positivity of failed ACLR grafts is not simply due to operating room contamination or ubiquitous presence of small amounts of bacteria during ACL reconstructions in general. For primary ACL reconstruction controls, a portion of the torn native ACL as well as excess tendon graft were removed and transferred to a specimen container with instruments previously unused during the procedure. The graft was fixed within the tibial tunnel with a bioabsorbable interference screw; the excess graft outside of the tibial interference screw was collected after graft passage and fixation.~~ ~~The intra-articular torn native ligament was obtained immediately following the initial diagnostic arthroscopy.~~ ~~Clinical cultures were not obtained on primary ACL reconstructions.~~

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*PCR analysis and microscopy*

A PCR analysis was performed with a universal bacterial primer (16S rRNA gene) on all tissue samples using a previously described protocol~~l~~ by an experienced laboratory researcher.~~.~~[14] The forward primer 27F 5′-AGAGTTTGATCMTGGCTCAG-3′ as described by Lane et al.[15] and 907R reverse primer 5′-CCGTCAATTCMTTTRAGTTT-3′ as described by Muyzer et al.[16] were used. The PCR was performed in 25-µL reactions containing 50 nmol each of two primers and 23 µL of PCR supermix (Invitrogen). The reactions received 1-µL of DNA preparation as template. The PCR was performed with 30 cycles of 94°C for 30 s, 55°C

for 30 s and 72°C for 1 min followed by a hold sequence at 4°C. Aliquots taken from reactions at the final cycle were electrophoresed on 1.2% agarose gel at 20 volts/cm for 30 min and stained with 1 µg/mL ethidium bromide, added to the agarose.

All specimens were subjected to DNA purification using MoBio Laboratories Inc. PowerSoil® DNA Isolation Kit. To facilitate DNA extraction, the samples were pulverized per the kit recommendations. We were unable to obtain a dry weight of the graft tissue to standardize the amount of tissue utilized. ~~Additionally, the physical characteristics of the torn graft tissue itself added substantial variability to the degree to which the sample could be pulverized by the PCR kit-recommended method~~. Therefore, reporting ~~of the amount of~~ bacterial DNA detected via PCR is limited to qualitative reporting (present versus absent) rather than quantitative reporting ( ~~limited to semi-quantitative reporting of nanograms per sample (rather than~~ nanograms bacterial DNA per unit weight of tissue graft).

To confirm the presence of bacteria in a biofilm state, a revision ACLR tissue specimen was subjected to dual fluorescent staining with SYTO59 for nucleic acids and WGA-alexa488 for extracellular polysaccharide. Imaging was obtained for both soft tissue as well as recovered inert material (suture and fixation devices) and interpreted by a researcher with expertise in orthopaedic biofilms.

~~*Statistical analysis*~~

~~Data was analyzed using a standard statistical software program (STATA 12.1, StataCorp, College Station, TX). Differences between demographic groups with respect to bacterial colonization rates were determined by student's t-test or Fisher's exact test. Non-parametric testing (Wilcoxon rank-sum) was used as appropriate for variables with non-normal distribution. The associations between bacterial colonization and number of revisions,~~

~~and time to failure of the previous reconstruction were assessed via ANOVA or Pearson correlation.~~

**Results**

*Presence of bacterial DNA*

Bacterial DNA was detectable in torn graft tissue in most revision ACL cases ~~27/31~~ (87.0%, n=27/31). There was a low rate of positive PCR results among control specimens, indicating a low rate of positivity due to environmental contamination from air or skin flora. A total of 0% (0/3) of sterile water samples left open to air throughout revision cases and 20% of native ligament (1/5) and excess tendon graft samples (1/5) from primary ACLR cases were PCR positive; both positive native ligament and graft control samples were from the same patient. ~~and less commonly 1/5 (20%, both hamstring graft and native ligament specimens from the same patient were positive) in primary ACL controls (p=0.002, Fisher's exact test) (Table 2).~~

*Bacterial culture and fluorescent microscopy*

One revision patient (3%, 1/31) had positive aerobic bacterial cultures (coagulase negative staphylococcal species) and was treated with IV antibiotics per the recommendations of an infectious disease specialist. This patient did not have antecedent clinical signs or symptoms of infection.

Microscopy confirmed the presence of bacterial biofilms on failed ACLR grafts. ~~Staphylococcal~~ Bacterial biofilms were identified on both soft tissue portions of the specimens (Figure 1) as well as monofilament suture material (Figure 2).

~~*Association between bacterial DNA load, graft type, and time to failure*~~

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~~Median bacterial DNA concentration in torn grafts at time of revision ACL was low at 18 ng/sample (range 0-101) (Table 2) with a trend toward higher concentrations found among revision patients with prior autograft (median 19 ng/sample range 0-101) vs. allograft (median 13 ng/sample range 0-21) used at time of the previous ACL reconstruction (p=0.13, Wilcoxon rank sum). There was no association between bacterial DNA concentration and time to failure (p=0.75, R-square=0.00) or number of prior ACL reconstructions (p=0.63, R-square=0.01).~~

**Discussion**

The results of the current study confirm the hypothesis that bacterial colonization is often present on torn ACLR graft tissue and less commonly present on torn native ligament or primary ACLR hamstring tendon autograft. The lack of antecedent clinical symptoms, delayed time to failure (median 5.4 years) and low clinical culture positivity rate indicate that these bacteria are primarily present in a biofilm state. The degree to which biofilm formation contributes to graft attenuation or loss of fixation following ACL reconstruction is unclear.

Polymerase chain reaction (PCR) is a useful technology for investigating bacterial colonization or infection in orthopaedic surgeries, though caution must be taken when comparing results between analyses as substantial variability is introduced by the sample collection and preparation methods as well as technical aspects of the PCR assay itself. As demonstrated by Ryu et al., PCR positivity rates can vary widely depending on the source specimen even in cases of confirmed infection; in their study of infected knee arthroplasties, tissue samples had low PCR sensitivity but sonicated fluid had high sensitivity[12]. The specific protocol utilized in our study appears to be useful in the setting of evaluating soft tissue bacterial colonization, as we had a high positivity rate of case samples but a low positivity rate from environmental contamination of negative controls. Our high PCR positivity rate of

ruptured ACLR graft tissue (87%) is in contrast to Ryu et al. who report a low tissue PCR positivity rate (16%) from infected knee arthroplasties; this discrepancy may be in large part due to may be due to differences in sample preparation. We pulverised our tissue samples which likely greatly enhanced DNA extraction, whereas the study by Ryu et al. did not employ any method of tissue mechanical treatment to facilitate DNA extraction.

Non-adherent, planktonic (free-floating) bacteria tissue are the primary culprit in acute infections due to production of a large amount of virulence factors and resulting clinically significant infectious symptoms (Figure 3).[17] Patients with deep infections after ACLR present with symptoms early after surgery and are usually culture positive[1], consistent with infection due to planktonic bacteria.[17] However, there is evidence that bacteria can switch phenotype early-on from a planktonic state to a biofilm state, [17] which we believe may occur in an unknown percentage of ACLR's (Figure 3).~~[17]~~ If this occurs, bacteria in a biofilm state have far less interaction with surrounding host tissue and may remain undetected or years;~~.~~[18] thiThe presence of bacterial biofilms within the surgical site is highly underestimated in orthopaedics due to reliance on clinical cultures as a primary diagnostic tool.[19] Several studies of culture-negative surgeries for fracture nonunion or revision arthroplasty have shown high rates of bacterial DNA as well as direct visualization of bacteria with microscopy.[20-22]

The results of the current study as well as Hiller et al.[7] indicate bacteria are frequently present in failed ACLR graft tissue in a biofilm state (Figure 3). It does not provide evidence for a causative link between bacterial colonization and graft failure after ACL reconstruction, though it does provide sufficient data to support further experiments on the topic. By establishing the presence of bacteria, there is justification for further work regarding characterization of any potential biofilms (where do they occur?) and DNA sequencing to determine whether the bacterial "community" affects clinical behavior. Hiller et al. report that

the bacterial species present in torn ACLR grafts are distinct from those found in ACL tissue at time of total knee arthroplasty,[7] though the effect of these distinct communities on the surrounding tissue has yet to be determined. Further research is needed to determine the degree to which biofilms on ACL grafts affect clinically relevant parameters such as failure rates.

There is some evidence of increased incidence of post-operative infection after ACL reconstruction performed with autograft vs. allograft. Katz et al report an incidence of clinically significant post-operative infection after ACL reconstruction of 1.2% after use of autograft and 0.6% after use of allograft.[23] Maletis et al. further distinguished between types of autograft and found that hamstring autograft has an incidence of infection of 0.61% versus 0.07% for bone patella tendon bone (BPTB) autograft or 0.27% for allograft.[24] In our study, we did observe a trend toward higher bacterial concentrations among cases in which autograft was utilized during the prior ACL reconstruction. Though not directly proven, the reduction of infection rates by Vertullo et al. after pre-soaking hamstring autograft in vancomycin solution[25] suggests that higher infection rates with hamstring autograft may in part be due to higher bacterial loads on the graft at time of fixation.

Interestingly, we found evidence of a bacterial ~~a staphylococcal~~ biofilm on monofilament suture material (Figure 1) (Figure 2). Biofilms have been associated with sutures from clinical specimens previously in an infected total knee revision[14] as well as in surgical site infections in hernia repair.[26] However, these were all braided sutures. In vitro studies suggest that braided sutures are more prone to biofilm colonization than monofilament sutures[27, 28]; however, our data suggest that monofilament sutures should not be discounted as a possible nidus for an infecting biofilm.

## Limitations

There were several limitations to the current study. Due to variations in torn graft tissue structural properties and the inability to obtain an accurate dry weight of the sample, substantial variability was introduced in the initial tissue steps required for bacterial DNA extraction. This did not affect our ability to detect whether bacterial DNA was present though it did limit our ability to assess concentration of bacterial DNA ~~(it was reported semi-quantitatively as nanograms per sample in this study rather than nanograms per unit volume)~~. Our choice of control samples ~~(primary ACL reconstruction cases)~~ effectively controlled for environmental contamination in the operating room as a source of bacterial DNA; the ~~one positive control sample (1/5, 20%) is~~control samples that did have positive PCR results were likely due to contaminating bacterial DNA from surgical instruments and fluids as well as patient skin. However, ~~use of a primary ACLR hamstring autograft control does not~~we could not control for the possibility of ubiquitous bacterial colonization of sites of previous soft tissue transosseous grafts about the knee. Therefore, w~~W~~e cannot determine with the current study design whether bacterial DNA is equally as prevalent on intact ACL reconstruction grafts.

## Conclusions

~~Staphylococcal b~~ Bacterial DNA is frequently present in failed ACLR grafts, with high rates of DNA detection by PCR but low culture positivity. ~~iofilms are present on failed ACLR grafts, with high rates of DNA detection by PCR but low culture positivity. There is likely bacterial colonization of many failed ACLR grafts, though the causal relationship between graft colonization and failure remains unclear.~~

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**Table 1.** Clinical data

| | Revision cases (n=31) |
|---|---|
| **Age** | Mean 28.2 years SD 11.9 |
| **Gender** | |
| **Male** | 18 (58%) ~~Male~~ |
| **Female** | 13 (42%) ~~Female~~ |
| **Number of prior ACL reconstructions** | |
| **Failed primary ACLR** | ~~Failed primary ACLR:~~ 24 (77%) |
| **Failed 1st revision ACLR** | ~~Failed 1st revision ACLR:~~ 6 (19%) |
| **Failed 2nd revision ACLR** | ~~Failed 2nd revision ACLR:~~ 1 (3%) |
| **Graft used during previous ACLR** | |
| **Autograft** | Autograft: ~~23~~ 25 (81~~74~~%) |
| **Allograft** | Allograft: ~~5~~ 6 (19~~16~~%) |
| | ~~Unknown: 3 (10%)~~ |
| **Time to failure** | Median 5.4 years |
| | Range 105 days-20.6 years |
| **Mechanism of failure** | |
| **Acute contact reinjury** | 3 (10%) |
| **Acute non-contact reinjury** | 23 (74%) |
| **Chronic insufficiency with no specific recalled injury** | 5 (16%) |

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**Table 2.** PCR and clinical culture results

| Patient number | Detectable bacterial DNA | Positive culture growth |
|---|---|---|
| **Revision ACL patients** | 27/31 (87%) Yes<br>4 /31(13%) No | 1/31 (3%) |
| **Failed primary ACLR** | 21/24 (88%) | 0/24 (0%) |
| **1st revision ACLR** | 5/6 (83%) | 1/6 (17%) |
| **2nd revision ACLR** | 1/1 (100%) | 0/1 (0%) |
| **Allograft during prior ACLR** | 5/6 (83%) | 1/6 (17%) |
| **Autograft during prior ACLR** | 22/25 (88%) | 0/25 (0%) |
| **Acute contact re-injury** | 2/3 (67%) | 0/3 (0%) |
| **Acute non-contact reinjury** | 20/23 (87%) | 1/23 (4%) |
| **Chronic insufficiency with no specific recalled injury** | 5/5 (100%) | 0/5 (0%) |
| **Failure within 15 months of ACLR** | 8/10 (80%) | 0/10 (0%) |
| **Failure 16 months-5 years after ACLR** | 10/11 (91%) | 1/11 (9%) |
| **Failure 6 or more years after ~~aclr~~ACLR** | 8/9 (89%) | 0/9 (0%) |
| ~~**Primary ACL controls**~~ | ~~1 (20%) Yes~~<br>~~4 (80%) No~~ | ~~N/A (no cultures on controls)~~ |

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**Figure Legend**

**Figure 1.** Fluorescent staining of a torn graft at time of revision ACLR. The red stain (SYTO59) represents nucleic acids and the green stain (WGA-alexa488) is for extracellular matrix. Scale bars are provided. **Left:** A portion of tendon graft (labeled host cells) and adjacent clusters of bacteria (white arrows). **Right:** A cluster of bacteria within a biofilm. The green polysaccharide around the red bacterial nucleic acid is a hallmark feature of a ~~staphylococcal~~ bacterial biofilm.

**Figure 2.** Fluorescent staining of monofilament suture with adherent bacterial biofilm (white arrows) recovered from the femoral tunnel at time of revision ACLR (scale bar provided). The red stain (SYTO59) represents nucleic acids and the green stain (WGA-alexa488) is for extracellular polysaccharide. The green polysaccharide around the red bacterial nucleic acid is a hallmark feature of a ~~staphylococcal~~ bacterial biofilm.

**Figure 3.** Conceptual diagram of the role of environmental bacterial contamination in ACL reconstructions. Deep infection following ACLR is rare (around 1%) and is caused by planktonic bacteria with early, clinically significant symptoms. An unknown proportion of ACLR grafts are colonized with environmental bacteria that switch early on to a biofilm phenotype~~ and do not cause clinical symptoms~~. The relationship between biofilm formation and ACLR failure rates is currently unknown.

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**Figure 1**

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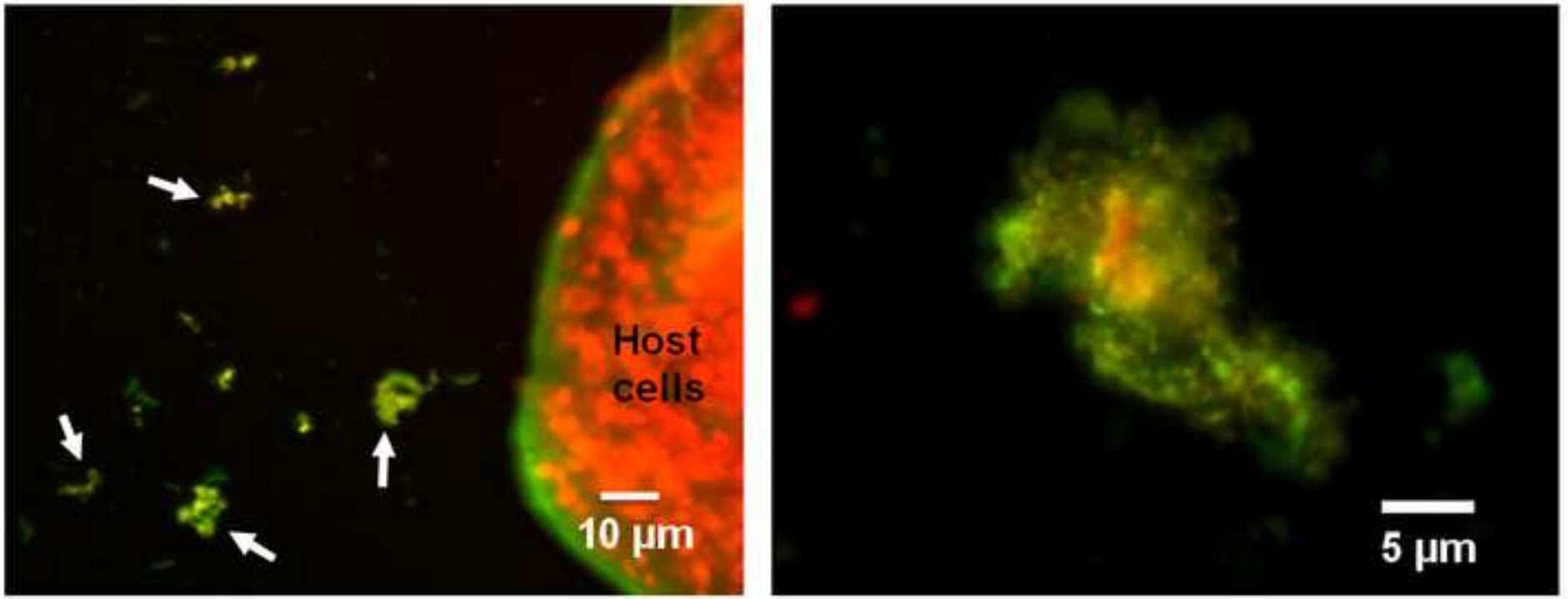

**Figure 2**

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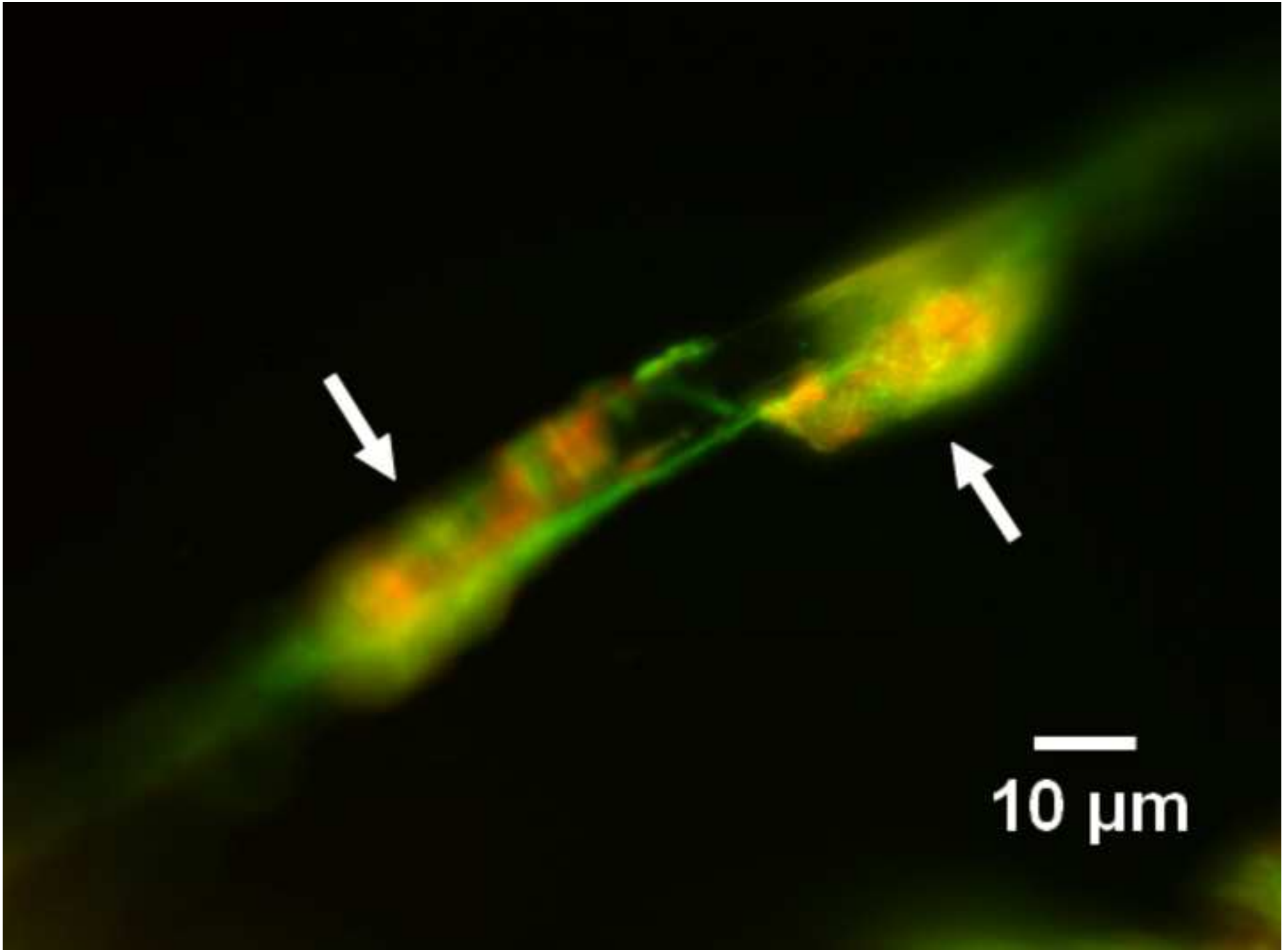

Figure 3
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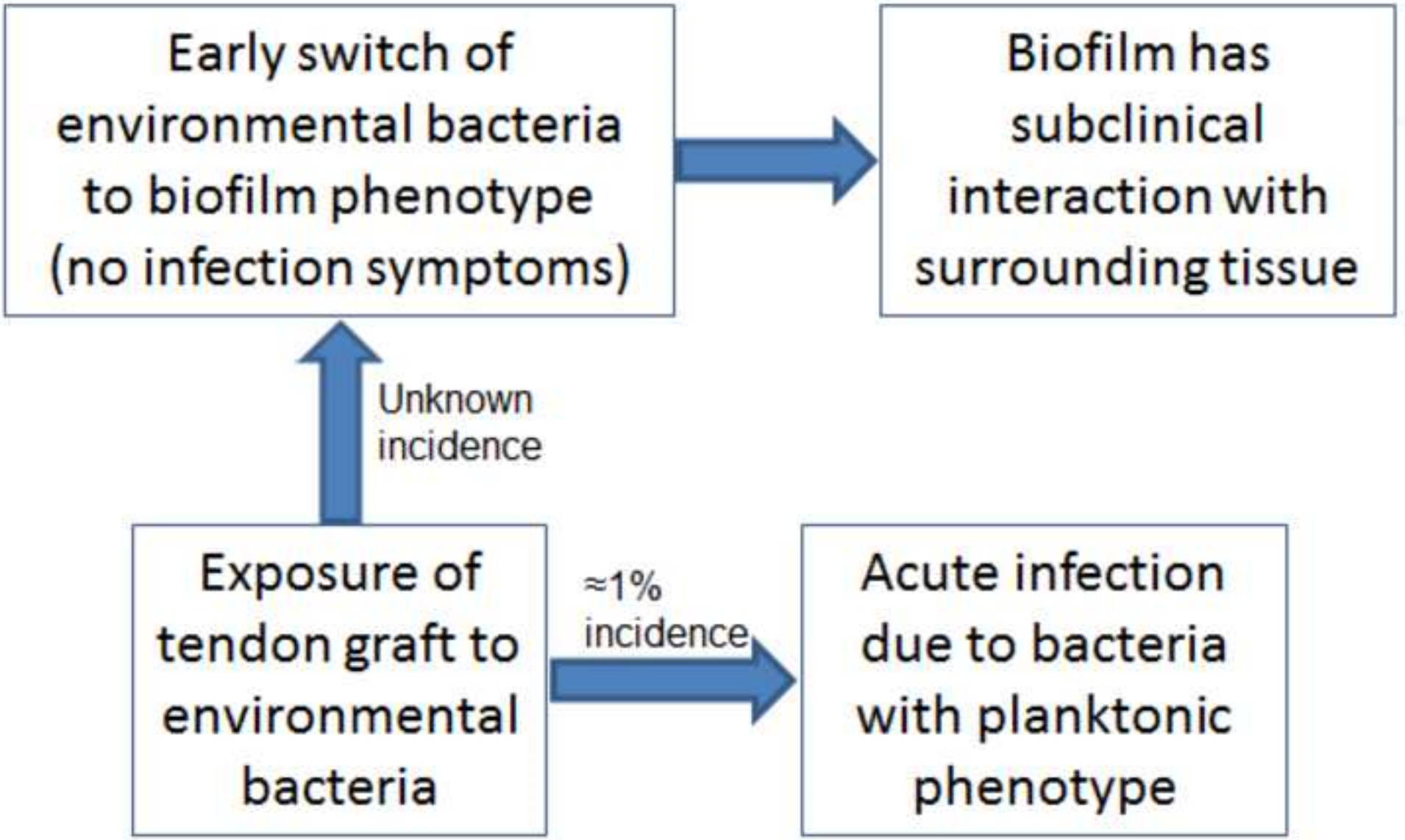

***3. ICMJE Disclosure Form**

**Click here to download 3. ICMJE Disclosure Form: Arthroscopy COIs.pdf**

# ICMJE Form for Disclosure of Potential Conflicts of Interest

## Section 1. Identifying Information

1. Given Name ____Alex_____ 2. Surname ____DiBartola_____
3. Are you the corresponding author? Yes ___ No_X_
4. Effective Date _____6/26/17_____
5. Manuscript Title Bacterial deoxyribonucleic acid (DNA) is often present in failed revision anterior cruciate ligament (ACL) reconstructions

## Section 2. The Work Under Consideration for Publication

Did you or your institution at any time receive payment or services from a third party for any aspect of the submitted work (including but not limited to grants, data monitoring board, study design, manuscript preparation, statistical analysis, etc…)?

Complete each item by typing an X in answer yes or not and completing the information requested if an answer is Yes. If you have more than one relationship, add lines.

1. Grant

_X _No ___Yes, money paid to you _ _Yes, money paid to institution* Name of entity_ __ Comments†___

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1. Given Name ____David_____ 2. Surname ____Flanigan_____
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1. Given Name ____Christopher_____ 2. Surname ____Kaeding_____
3. Are you the corresponding author? Yes ___ No_X_
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1. Given Name ____Robert_____ 2. Surname ____Magnussen_____
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13. Other (err on the side of full disclosure)

_X_No ___Yes, money paid to you ___Yes, money paid to institution* Name of entity___ Comments___

** For example, if you report a consultancy above there is no need to report travel related to that consultancy on this line.

## Section 4. Other relationships

Are there other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work?

_X_No other relationships/conditions/circumstances that present a potential conflict of interest

___Yes, the following relationships/conditions/circumstances are present (explain below):

>>>>>>>>>>><<<<<<<<<<<

**The International Committee of Medical Journal Editors**

The ICMJE Disclosure of Potential Conflicts of Interest Form was adopted by *Arthroscopy: The Journal of Arthroscopic and Related Surgery* along with 17 other leading orthopaedic journals at the 2011 annual meeting of the American Academy of Orthopaedic Surgeons.

# ICMJE Form for Disclosure of Potential Conflicts of Interest

## Section 1. Identifying Information

1. Given Name ____Paul_____ 2. Surname ____Stoodley_____
3. Are you the corresponding author? Yes ___ No_X_
4. Effective Date _____6/26/17_____
5. Manuscript Title Bacterial deoxyribonucleic acid (DNA) is often present in failed revision anterior cruciate ligament (ACL) reconstructions

## Section 2. The Work Under Consideration for Publication

Did you or your institution at any time receive payment or services from a third party for any aspect of the submitted work (including but not limited to grants, data monitoring board, study design, manuscript preparation, statistical analysis, etc…)?

Complete each item by typing an X in answer yes or not and completing the information requested if an answer is Yes. If you have more than one relationship, add lines.

1. Grant

_X _No ___Yes, money paid to you _ _Yes, money paid to institution* Name of entity_ __ Comments†___

2. Consulting fee or honorarium

_X_No ___Yes, money paid to you ___Yes, money paid to institution* Name of entity___ Comments†___

3. Support for travel to meetings for the study or other purposes

_X_No ___Yes, money paid to you ___Yes, money paid to institution* Name of entity___ Comments†___

4. Fees for participation in review activities such as data monitoring boards, statistical analysis, end-point committees, and the like

_X_No ___Yes, money paid to you ___Yes, money paid to institution* Name of entity___ Comments†___

5. Payment for writing or reviewing the manuscript

_X_No ___Yes, money paid to you ___Yes, money paid to institution* Name of entity___ Comments†___

6. Provision of writing assistance, medicines, equipment, or administrative support

_X_No ___Yes, money paid to you ___Yes, money paid to institution* Name of entity___ Comments†___

7. Other

_X_No ___Yes, money paid to you ___Yes, money paid to institution* Name of entity___ Comments†___

* This means money that your institution received for your efforts on this study.
† Use this section to provide any needed explanation.

## Section 3. Relevant financial activities outside the submitted work

1. Board membership

_X_No ___Yes, money paid to you ___Yes, money paid to institution* Name of entity___ Comments___

2. Consultancy

_X _No ___Yes, money paid to you ___Yes, money paid to institution* Name of entity __ Comments___

3. Employment

_X_No ___Yes, money paid to you ___Yes, money paid to institution* Name of entity___ Comments___

4. Expert testimony

_X_No ___Yes, money paid to you ___Yes, money paid to institution* Name of entity___ Comments___

5. Grants/grants pending

_X_No ___Yes, money paid to you ___Yes, money paid to institution* Name of entity___ Comments___

6. Payment for lectures including service on speakers bureaus

_X_No ___Yes, money paid to you ___Yes, money paid to institution* Name of entity___ Comments___

7. Payment for manuscript preparation

_X_No ___Yes, money paid to you ___Yes, money paid to institution* Name of entity___ Comments___

8. Patents (planned, pending or issued)

_X_No ___Yes, money paid to you ___Yes, money paid to institution* Name of entity___ Comments___

9. Royalties

_X_No ___Yes, money paid to you ___Yes, money paid to institution* Name of entity___ Comments___

10. Payment for development of educational presentations

_X_No ___Yes, money paid to you ___Yes, money paid to institution* Name of entity___ Comments___

11. Stock/stock options

_X_No ___Yes, money paid to you ___Yes, money paid to institution* Name of entity___ Comments___

12. Travel/accommodations/ meeting expenses unrelated to activities listed**

_X_No ___Yes, money paid to you ___Yes, money paid to institution* Name of entity___ Comments___

13. Other (err on the side of full disclosure)

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