


Mapping bacterial biofilm on explanted orthopedic hardware: An analysis of 14 consecutive cases

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Hardware implanted during primary total joint arthroplasty carries a serious risk for periprosthetic joint infection (PJI). The formation of bacterial biofilms, which are highly tolerant of antibiotics and host immunity, is recognized as being a major barrier to treatment. It is not known whether some components and their surface features are more prone to biofilm than others. This study attempted to map biofilm on different components and features of orthopedic hardware recovered during revision. Implant surface culture (ISC) was used on 53 components from 14 hip and knee revisions. ISC achieves a thin agar coating over components, followed by incubation and observation for colony outgrowth over 9 days. Recovered organisms were identified by selective culture and 16S rRNA sequencing. Outcomes were compared with clinical culturing and PJI diagnosis based on 2013 Musculoskeletal Infection Society criteria. ISC paralleled clinical culturing with a sensitivity of 100% and a specificity of 57.1%. When compared to Musculoskeletal Infection Society criteria, sensitivity remained at 100% while specificity was 80%. Biofilm accumulation was patchy and heterogeneous throughout different prostheses, though notably the non-articulating surfaces between the tibial tray and polyethylene insert showed consistent growth. On individual components, ridges and edges consistently harbored biofilm, while growth elsewhere was case dependent. ISC successfully identified microbial growth with high sensitivity while also revealing that biofilm growth was commonly localized to particular locations. Understanding where biofilm formation occurs most often on implanted hardware will help guide debridement, retention choices, and implant design.

Key words: Mapping biofilm; periprosthetic joint infection; total joint arthroplasty; implant surface culture; *Staphylococcus aureus*.

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INTRODUCTION

Periprosthetic joint infection (PJI) is a serious complication of primary total joint arthroplasty (TJA), with an incidence of 1.7–2.3% [1]. Bacterial biofilms [2,3] are known to play a major role in these infections due to their difficulty in diagnosis and treatment [4,5].

The most common PJI pathogens are Gram-positive cocci, namely *Staphylococcus aureus* (31%), though other main players include coagulase-negative *staphylococci*, *enterococcus*, and Gram-

negative bacteria [6]. Up to 22% of PJIs are culture negative by conventional clinical microbiology [6,7].

Orthopedic hardware implanted during TJA, such as metals, polymers, and even antibiotic impregnated bone cement, have been shown to provide surfaces for biofilm growth [8,9]. However, there is a significant gap in addressing whether certain areas of a prosthetic joint prosthesis may be more susceptible to biofilm formation than others. Specifically, the effect of biofilm attachment and formation on larger surface features, such as ridges, edges, surface elevations, rough patches, and holes, was studied, since these areas were hypothesized to be more prone to bacterial biofilm accumulation as they may provide protected niches. This study

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aimed to identify specific surface features of explanted orthopedic hardware which may harbor bacterial biofilms. We collected explanted knee and hip components from 14 consecutive PJI cases indicated for revision and cultured directly from the components using a modified “agar encasement” method [10], here referred to as “implant surface culture (ISC),” in which hardware was immersed in agar followed by incubation [11,12] and daily observation for outgrowth from the component.

METHODS

Specimen retrieval

In all, 53 orthopedic prosthetic components were collected from 14 consecutive adult total knee arthroplasty (TKA) and total hip arthroplasty (THA) revision cases at our institution (in accordance with an institutional review board approved protocol). Components consisted of metal (titanium or cobalt–chromium alloy), ultra-high molecular weight polyethylene (UHMWPE), and polymethylmethacrylate (PMMA). There was no inclusion or exclusion criteria other than PJI suspicion. All cases involved removal of major prosthetic implants, including components of a total knee system (femoral component, tibial tray, polyethylene tibial insert, and patellar component) and those of a total hip prosthetic (femoral stem, femoral head, acetabular component, and polyethylene acetabular insert). Components explanted during the second stage of two-stage total joint revisions (after prior prosthesis removal and debridement) were also included in the study to identify persisting PJI.

Study participants were consented prior to surgery and patient demographics have been listed (Table 1). Upon hardware removal, all components were immediately loosely wrapped in a sterile towel, lightly wetted with surgical saline, and transported from the operating room to the laboratory where processing was completed. All samples were transported within 1 h of the surgery and processed immediately thereafter.

Table 1. Patient demographic and clinical information from 14 total joint arthroplasty (TJA) revision surgery cases at The Ohio State Wexner Medical Center East Hospital in the Department of Orthopedics

Patient demographics			Number
Total number of cases			14
Sex	Male		7
	Female		7
Surgical Site	Knee (85.7%)	Right	9
		Left	2
		Bilateral	1
	Hip (14.3%)	Right	1
		Left	1
Patient height and weight			Value
Height (inches)	Median		65
	Mean		66
Weight (kg)	Median		92.9
	Mean		94.1

Implant surface culture

ISC is an adaptation of an established “agar overlay method,” which has been conventionally used to assess growth on the surface and subsurface of an agar layer as well as to create a uniform lawn of bacteria for antimicrobial assays [10]. Explanted components were first rinsed with phosphate-buffered saline (PBS; Dulbecco's, Gibco, USA) in sterile, 1-liter vacuum filtration units (Fisherbrand®, USA) to remove planktonic bacteria, blood, and tissue debris. Smaller components were rinsed twice with 25 mL PBS, while larger components were rinsed in a similar manner using 50 mL. After rinsing, components were coated with 60°C molten 1.5% brain heart infusion (BHI) agar (Becton, Dickinson, and Company, USA). BHI agar was chosen as a non-selective media to recover a broader range of potential pathogens. Smaller components were submerged in the molten agar for 5 s, removed, and placed in a sterile glass container (Pyrex, USA) to allow the agar to solidify. Larger components had molten agar pipetted gently over the entire surface until a thin agar coating of around 1–4 mm was achieved. Though this coating did not provide a consistent and uniform agar encasement across all components, effort was made to coat all aspects of larger components in the most minimal fashion, but without leaving any dry spots lacking adequate coverage. After allowing the agar to set, a small amount of additional molten agar was pipetted over each component to cover any neglected areas. All components were then incubated for 9 days at 37°C, 5% CO₂, a time frame shown to adequately allow for biofilm development [13].

Growth observation and intra-implant biofilm mapping

Explanted materials were photographed every 24 h for visible bacterial growth on the implant itself or in the surrounding agar using a 12-megapixel camera (Apple Inc., USA). The development of outgrowth in the form of single colonies from surfaces within the agar coating of the various implants was minimum requirement necessary as evidence for the existence of biofilm. Imaging helped pinpoint specific areas of biofilm formation and distinguish daily changes in biofilm growth from initial tissue, cement, or host debris attached to the surfaces.

If bacterial outgrowth was seen, a single colony was picked, streaked to isolation, and subsequently converted to glycerol stocks to assemble a clinical culture bank. Colony samples were also sent for Sanger sequencing of the V1–V9 regions of 16s rRNA after colony lysis and PCR amplification, with the specific microbes present identified by bioinformatics (Genewiz, Azenta Life Sciences, South Plainfield, NJ). If *S. aureus* was suspected from clinical culturing, a colony taken from the implant was plated onto mannitol salt agar, a medium selective for *S. aureus* [13]. If able to ferment mannitol (evidenced by the presence of yellow colonies on the medium after incubation), the specimen was considered positive for *S. aureus*.

Musculoskeletal infection society criteria for PJI diagnosis and culture comparison

Patient medical charts were reviewed retrospectively for relevant clinical information, namely microbiological

results from intraoperative tissue cultures, which were taken from areas close in proximity to the implanted hardware during the procedure and cultured in a hospital clinical laboratory. The data were compared with ISC outcomes. If identified, the species from clinical testing was also noted. PJI diagnosis was assessed as positive or negative based on the 2013 Musculoskeletal Infection Society (MSIS) definition. This criterion states that PJI exists if one major criterion or three minor criteria are present. Major criteria include having two positive periprosthetic cultures with phenotypically identical organisms or having a sinus tract communicating with the joint. Minor criteria involve (1) elevated C-reactive protein and erythrocyte sedimentation rate, (2) elevated synovial fluid white blood cell count or positive change on leukocyte esterase test strip, (3) elevated synovial fluid polymorphonuclear neutrophil percentage, (4) positive histological analysis of periprosthetic tissue, and (5) a single positive culture [14]. Though newer PJI definition criteria exist that may be more sensitive for diagnosis [15], the diagnostic information from patients involved in this study better aligned with the 2013 MSIS criteria. This criterion also provided a more concrete outcome of PJI positive or negative, which was desired in the current study for simplicity when comparing to ISC and clinical microbiology. Sensitivity and Specificity measures of ISC were calculated in comparison to clinical microbiology and MSIS diagnosis. Sensitivity represented the true-positive rate while specificity denoted the true-negative measure. For clinical culturing and ISC, the presence or absence of growth was used as positive and negative measures, respectively.

RESULTS

Efficacy of ISC

The presence or absence of bacterial outgrowth from ISC was compared against clinical microbiological culturing results and PJI diagnosis using MSIS criteria (Table 2). Of the 14 cases, 10 were ISC positive and 4 were negative for growth. Of the 10 positive ISC cases, identified organisms included *S. aureus* (3), other staphylococci species (2; *S. epidermidis* & *S. warneri*), *Enterococcus faecalis* (2), *Candida albicans* (1), and *Kocuria rhizophila* (1). The final positive case yielded three microbes by 16s rRNA sequencing, including *Pseudomonas aeruginosa*, *Morganella morganii*, and *Enterococcus faecalis*. Comparing ISC to clinical culturing results, 7 cases were concordantly positive, 4 cases were concordantly negatives, and 3 cases were ISC positive but clinical culture negative. This resulted in a calculated sensitivity of 100% and a specificity of 57.1% of ISC compared to clinical culture.

When MSIS status was considered as the gold standard, 9 cases presented as true positive, 4 were true negative, and one case (12) was a false positive. This gave ISC a sensitivity of 100% and a specificity of 80%, compared to 77.8% and 100% from clinical culture, respectively. Two cases (11 and 14) were clinical culture negative but met the MSIS criteria for

PJI diagnosis and were positive by ISC. Taking all diagnostic methods into account, 7 cases were positive by all three measures, 4 were negative for all three, 2 (cases 11 & 14) were ISC and MSIS positive but clinical culture negative, and 1 case (12) was ISC positive but clinical culture and MSIS negative (Table 2).

Biofilm mapping over entire orthopedic prostheses

At least one hardware component displayed bacterial growth in 10 of the 14 cases using ISC, though many cases had growth on multiple components and 3 cases had growth on all components (Table 2). Hardware was examined to localize bacterial growth to specific areas over the entire prosthetic joint (Figs. 1 and 2). Sparse amounts of growth were observed on components retrieved from the two THA revision cases, with colonies located in the surrounding agar of the femoral stem in case 12 and the UHMWPE acetabular insert in case 11 (Fig. 1). There was no colony outgrowth on the implants themselves from the two hip revision cases.

In the TKA revision cases that were ISC positive, outgrowth was pinpointed to specific areas of the entire prosthesis. Biofilm was commonly observed on the non-articulating surfaces of the tibial tray that interfaced directly with the polyethylene tibial insert. All six of the tibial trays retrieved that were ISC positive displayed growth on this surface. Outgrowth was also observed on both the non-articulating and articulating surfaces of polyethylene tibial inserts. Case 3 displayed an example of growth on the articulating surface of the insert, while case 6 depicts growth on the surface that directly abuts the tibial tray (Fig. 2).

Other growth patterns among the total knee prostheses were heterogeneous—no one component grew biofilm in every case. Likewise, no single component was exempt from biofilm accumulation in every case. Some cases were evidenced by patchy growth throughout each individual component and flooding the surrounding agar (case 6, Fig. 2), while others displayed one or two small areas of growth on a single component. The four cases that were deemed ISC negative exhibited no bacterial growth on any component (Fig. 3).

Biofilm localization to features of individual orthopedic components

Outgrowth was also mapped to surface features of individual components. In case 6 (one of the most complex PJI) case, the hinged knee system grew abundant colonies of *S. aureus* that covered almost the entirety of the component. In other cases,

Table 2. Information from 14 total joint arthroplasty (TJA) revision cases, including surgical procedure and site, number and name of the removed components, growth status of implant surface culture, growth status of clinical microbiology culturing, and MSIS status. Positive MSIS status is based on the 2013 definition and represents the presence of one major criterion, or three minor criteria outlined previously in the methods. Component material is indicated in parentheses. All components not indicated were various types of metal alloy hardware.

Case number	Surgical procedure	Surgical site	Components retrieved	Implant surface culture (ISC) status		Clinical microbiology culturing status		MSIS status for PJI diagnosis
				Positive/negative	Organism	Positive/negative	Organism	
1	Total Knee Explant with Placement of Antibiotic Spacer	Knee— Right	4—Femoral Component, Tibial Insert (UHMWPE), Tibial Tray, Patellar Component (UHMWPE)	Negative	N/a	Negative	N/a	Negative
2	Total Knee Explant with Placement of Antibiotic Spacer	Knee— Bilateral	8—Femoral Components (2), Tibial Inserts (2; UHMWPE), Tibial Trays (2), Patellar Components (2; UHMWPE)	Positive (5/8)	<i>Staphylococcus aureus</i>	Positive	<i>Staphylococcus aureus</i>	Positive
3	Total Knee Explant with Placement of Antibiotic Spacer	Knee— Right	4—Femoral Component, Tibial Insert (UHMWPE), Tibial Tray, Other Component	Positive (3/4)	<i>Staphylococcus epidermidis</i>	Positive	<i>Cutibacterium acnes</i>	Positive
4	Total Knee Explant with I&D and Placement of Antibiotic Spacer	Knee— Right	4—Femoral Component, Tibial Insert (UHMWPE), Tibial Tray, Patellar Component (UHMWPE)	Positive (1/4)	<i>Staphylococcus aureus</i>	Positive	<i>Clostridium septicum</i> , <i>Staphylococcus lugdunensis</i>	Positive
5	Total Knee Explant with Placement of Antibiotic Spacer	Knee— Right	4—Hinged Femoral/ Tibial Component, Tibial Tray, Femoral Stem, Tibial Stem	Positive (4/4)	<i>Pseudomonas aeruginosa</i> , <i>Morganella morganii</i> , <i>Enterococcus faecalis</i>	Positive	<i>Pseudomonas aeruginosa</i>	Positive
6	Total Knee Explant with Placement of Antibiotic Spacer and gastroc flap	Knee— Left	3—Large Hinged Femoral/Tibial Knee System, Tibial Tray, Tibial Insert (UHMWPE)	Positive (3/3)	<i>Staphylococcus aureus</i>	Positive	<i>Methicillin Resistant S. aureus (MRSA)</i>	Positive
7	Aseptic Polyethylene Exchange	Knee— Right	1—Tibial Insert (UHMWPE)	Negative	N/a	Negative	N/a	Negative
8	Total Knee Explant with Placement of Antibiotic Spacer	Knee— Right	5—Femoral Component, Tibial Insert (UHMWPE), Tibial Tray, Patellar Components (2; UHMWPE)	Negative	N/a	Negative	N/a	Negative
9	Total Knee Explant with I&D and Placement of Antibiotic Spacer	Knee— Left	8—Femoral Component, Tibial Insert (UHMWPE), Tibial Tray, Tibial Stem, Femoral Stem, Screw, Other Small Components (2)	Positive (8/8)	<i>Enterococcus faecalis</i>	Positive	<i>Candida Albicans</i>	Positive
10	Removal of Antibiotic Spacer and Re- implantation of Hardware	Knee— Left	2—Tibial Insert (UHMWPE) with attached PMMA Spacer, Femoral Component	Negative	N/a	Negative	N/a	Negative
11	Total Hip Explant with Placement of Antibiotic Spacer	Hip—Left	4—Cut Femoral Stem, Femoral Head, Acetabular Socket Component, Acetabular Insert (UHMWPE)	Positive (1/4)	<i>Enterococcus faecalis</i>	Negative	N/a	Positive
12	Removal of Antibiotic Spacer and Re- implantation of Hardware	Hip— Right	4—Femoral Stem, Femoral Head, Acetabular Insert (UHMWPE) with attached PMMA Spacer, Acetabular Liner Component	Positive (1/4)	<i>Kocuria rhizophila</i>	Negative	N/a	Negative

Table 2 (continued)

Case number	Surgical procedure	Surgical site	Components retrieved	Implant surface culture (ISC) status		Clinical microbiology culturing status		MSIS status for PJI diagnosis
				Positive/negative	Organism	Positive/negative	Organism	
13	Antibiotic Spacer Removal and Replacement, Muscle Flap and Skin Graft Closure	Knee—Right	1—Femoral Component	Positive (1/1)	<i>Candida Albicans</i>	Positive	<i>Candida Albicans</i>	Positive
14	Debridement, Antibiotics, & Implant Retention (DAIR) with Polyethylene Exchange	Knee—Right	1—Tibial Insert (UHMWPE)	Positive (1/1)	<i>Staphylococcus warneri</i>	Negative	N/a	Positive

MSIS, Musculoskeletal Infection Society; UHMWPE, ultra-high molecular weight polyethylene

however, biofilm was localized to specific areas, including ridges, contours, and edges of individual components. Case 9 showed striking biofilm growth on edges of the femoral component articulating surface and growth along the raised ridge of the femoral stem, while case 13 showed substantial growth along the side edge of the femoral component (Fig. 2). In case 6, a zoomed image shows *S. aureus* growth on the side edge of a tibial insert, with prominent biofilm was present at the change in contour (Fig. 2).

DISCUSSION

ISC displays promise in identifying biofilm on both known culture-positive and culture-negative PJIs. Two cases (11 and 14, identified as *E. faecalis* and *S. warneri*, respectively) that were ISC positive were also MSIS positive for PJI but clinical culture negative, implying that ISC may be useful in culturing pathogens from patients with confirmed PJI but unable to be detected from intraoperative culture samples. This aligns with a recent study by Jiang *et al.* [16] where the “ISC” method was used to in fracture device-related infections (FDRI) and found to have an increased sensitivity and shorter mean culture time when compared with traditional culturing methods, suggesting this technique is a possible adjunct to traditional culturing. However, a larger population size is required to confirm the ability ISC to more effectively culture from PJI cases. The current study consisted of a limited size of 14 cases from both male and female patients (Table 1), all of which were Caucasian.

Only case 12 was considered a false positive when compared to MSIS criteria and clinical culturing, with the femoral stem harvesting colony growth in the surrounding agar (Fig. 1), subsequently identified as *Kocuria rhizophila*, an

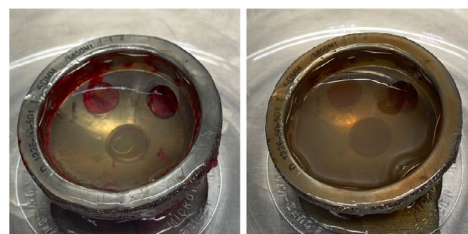
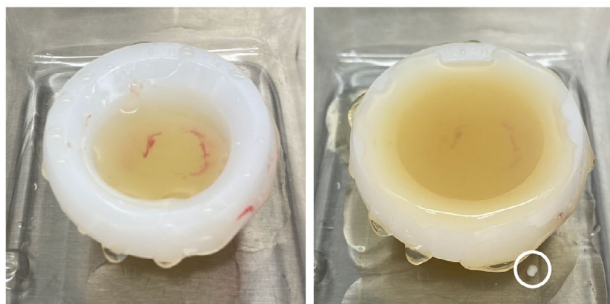
environmental gram-positive coccus from the same family as staphylococcus. *Kocuria* is not often cultured in PJI but is present in normal skin flora and mucous membranes of humans [17]. This case was a second stage of a two-stage total hip revision, which involved removal of an antibiotic spacer and placement of a fresh hip prosthesis. The patient was on prior antibiotics following the first stage and showed negative synovial fluid cultures prior to the procedure, suggesting a possibility of it being a contaminant.

Though case 11 was also ISC positive (*E. faecalis*) and negative on both clinical tissue culture and MSIS diagnosis, this growth was not considered to be a contaminant. Two large colonies were visible in the surrounding agar of the acetabular insert near the base of the component (Fig. 1). This raises less concern for contamination, as erroneous growth would be expected to be more widespread and less likely to be in close proximity to the component. In addition, the patient in case 11 had an extensive prior PJI history resulting in two prior revisions. Though no specific organism was identified clinically from the first revision, clinical suspicion of infection remained high enough to perform a second even after 6 weeks of IV antibiotics. Enterococcal PJI has also been shown to have a high rate of treatment failure [18], correlating clinically with this case.

There were three cases (3, 4, and 9) where the organisms identified through ISC did not align with clinical microbiology. This discordance could be explained by contamination during explant handling and processing, although if this were the circumstance, growth would be expected to be prevalent among the surrounding agar from the implants. Instead, growth was limited to the top of the components, sometimes extending into the surrounding agar but not widespread. In case 9, ISC outgrowth appeared to mirror the morphology of the clinically identified *C. albicans* (Fig. 2), growing

Case

11



12

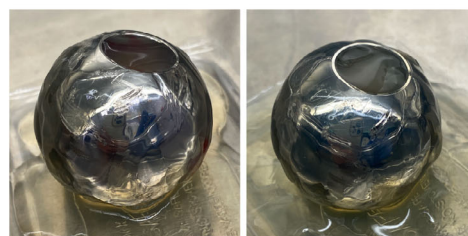


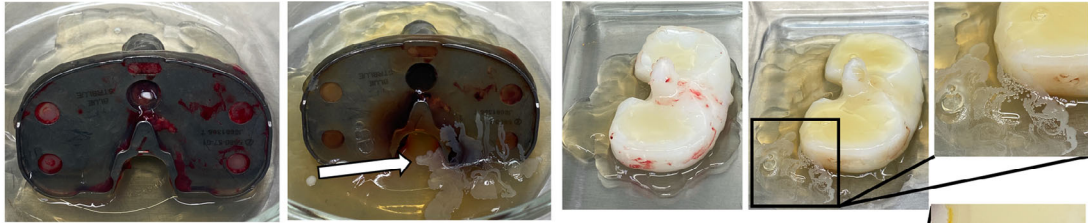
Fig. 1. Images showing example components from two total hip arthroplasty (THA) revision cases both before (left) and after incubation (right). Arrows and circles indicate colony growth. Images without arrows depict that no growth was observed.

in a yeast-like pattern; however, ISC 16s rRNA identified *E. faecalis*, suggesting a polymicrobial infection. In cases 3 and 4, *Cutibacterium acnes* and *Clostridium septicum*, respectively, were identified from clinical microbiology but not ISC. ISC does allow for the recovery of anaerobes, but this is conventionally done only at request of the surgeon for an anaerobic incubation. A limitation of our method is that we can only perform one type of media and one incubation type. Since facultative anaerobes are the most common pathogens, we chose to incubate under conditions of 5% CO₂ and 37°C. However, we point out that our method has previously allowed for the isolation of a fastidious anaerobe, *Bacteroides fragilis*, after incubation at 37°C, and this organism was also isolated from a

clinical microbiology anaerobic culture [12]. We hypothesize that since the colonies are growing embedded by an agar overlay, rather than directly exposed to an oxic atmosphere, there may be hypoxic conditions that allow the growth of anaerobes. We recognize that control incubations with spiked anaerobes need to be conducted to test this hypothesis. Also in case 3, *S. epidermidis* was cultured from ISC, but not from intraoperative clinical cultures. On further chart review, however, this patient had *S. epidermidis* cultured from intraoperative samples of a prior, separate revision in the same knee, suggesting that this organism may have persisted from that previous revision but was not picked up on clinical culturing in the most recent procedure.

Case

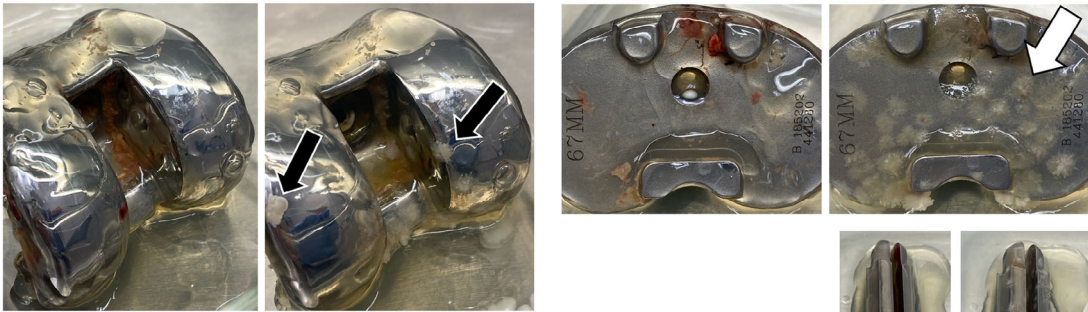
3



6



9



13

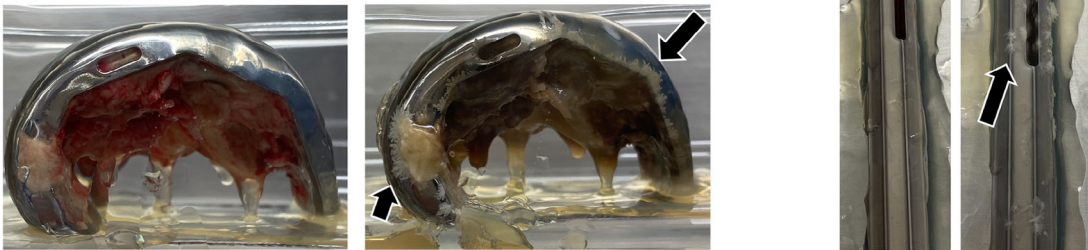


Fig. 2. Examples of components that exhibited biofilm growth from four of the 8 ISC-positive TKA revision cases. Images show components both before (left) and after incubation (right). White arrows indicate growth on a specific, non-articulating surface of many tibial tray components. Black arrows depict growth on edges and ridges of various components. Circles highlight colony outgrowth from individual infected explants. Boxes are used to zoom in on certain areas of components. (ISC, Implant surface culture; TKA, Total knee arthroplasty).

In case 5, three separate organisms were identified from ISC. One of these organisms (*P. aeruginosa*) matched clinical findings suggesting that ISC may be more effective in identifying polymicrobial infections.

Another notable finding relates to the different appearances of *S. aureus* growth present in case 6.

On the hinged knee component, there were gold *S. aureus* colonies, but also many small white colonies (Fig. 2) identified as *S. aureus* by 16s rRNA. This suggests the presence of small colony variants (SCVs) growing alongside wild-type colonies as seen in a 2014 case report [19]. An analysis of five hip-associated PJI containing *S. aureus* SCVs by Sendi

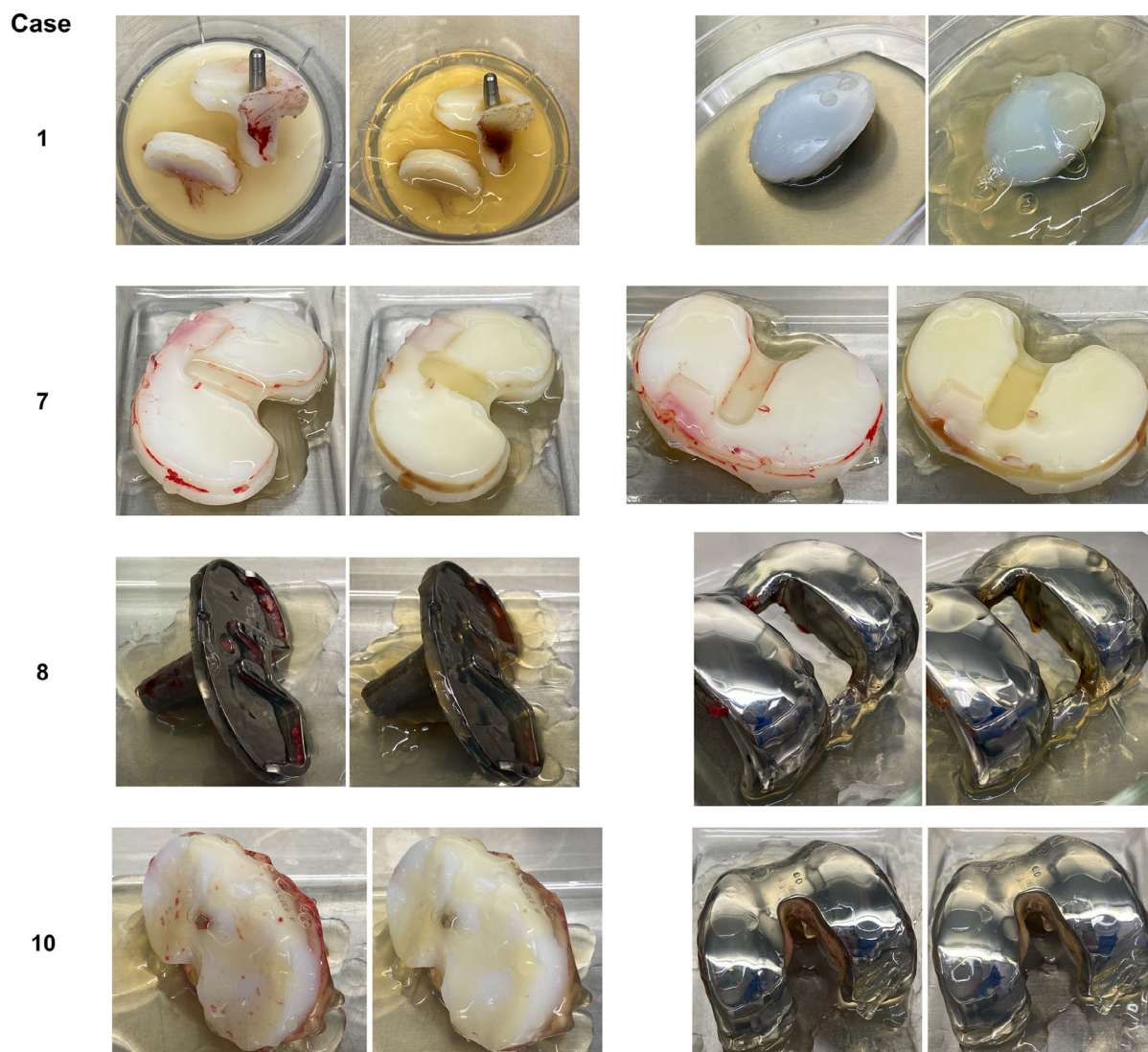


Fig. 3. Examples of components that were ISC negative for biofilm growth from four total TKA revision cases. Images show components both before (left) and after incubation (right). Case 1 shows a polyethylene tibial (left) and patellar component (right). Case 7 shows a single tibial insert, while case 8 shows a tibial tray and tibial insert. Case 10 shows a tibial insert and femoral knee component. (ISC, Implant surface culture; TKA, Total knee arthroplasty).

et al. revealed similar morphology and discussed the presence of prior revision or antibiotic treatment failure in all cases [20]. Interestingly, the patient in case 6 experienced two prior unsuccessful two-stage revision procedures before this most recent explant, further supporting the presence of SCVs and the ability to identify these variants morphologically using ISC. The occurrence of SCVs also helps explain the difficulty in treating this patient with antibiotics.

With respect to identifying specific locations on the hardware that may be more prone to biofilm, ISC demonstrated the ability to reveal patterns of growth in the context of the whole knee prosthesis. All TKA revisions that were both ISC positive and

included a tibial tray (six total) showed biofilm formation on the non-articulating surface interfacing with the tibial insert, suggesting that this area may be more susceptible to bacterial accumulation. During total knee explant procedures, the UHMWPE tibial insert is often placed after the attachment of the tibial tray to the surrounding bone surface. The gap between the tray and polyethylene insert varies slightly by manufacturer and the type of locking mechanism; however, the space between the two components is estimated to be 1 micron, with 2–5 μm allowances along the edges and locking mechanism to allow for insertion and extraction. This space possibly offers microbes protection from

antibiotics and host defense cells that may not be able to diffuse or migrate into this area. Prior work has described the ability of *S. aureus* to colonize the sub-micron areas of bone osteocyte canicular networks during chronic osteomyelitis [21,22]. In the current study, a similar mechanism may explain why we found biofilm in this tight gap. Most cases that displayed growth in the mating surfaces of the gap did so around the edges and near the locking mechanism (Fig. 2; cases 3, 6). In debridement, antibiotics, and implant retention procedures, the polyethylene insert is removed, the non-articulating surface of the tibial tray is exposed, the area is debrided, and a new insert is placed. Our results suggest more attention be paid to scrubbing techniques or cleaning solutions to reduce possible colonization on these surfaces.

ISC also revealed evidence that biofilms may be more likely to form on certain surface features; however, this association was difficult to quantify statistically. There were multiple instances of biofilm adhered to edges or ridges of total knee components, specifically femoral components, UHMWPE tibial inserts, and even a femoral stem (Fig. 2). Similar patterns have been demonstrated for laboratory biofilms grown *in vitro* [23].

ISC has several limitations. First, there is an increased possibility for contamination compared to traditional culturing methods due to the many steps required for processing, which include transporting components, preparing agar, and pouring agar over components in a sterile manner. Second, only one type of agar and incubation condition can be used per component. Since there are a limited number of components removed, this makes testing for selective pathogens with different media or environmental conditions difficult. Third, though previous limitations with a past “candle dip” method [24] regarding visualization of outgrowth on components had been greatly improved with the use of less agar replenishment over the 9 days of incubation, there remains the inability to produce a perfectly even coating around each component with zero surrounding agar accumulating. Also, certain areas on components pooled agar more while shiny metallic surfaces were difficult to keep saturated. Lastly, though previous work has shown the ability to produce *in vitro* biofilm growth over sterile components of similar nature [23], the lack of true positive controls is a main limitation of this method.

CONCLUSIONS

While limitations existed, ISC functioned similarly in culturing pathogens compared to clinical culturing and MSIS criteria for diagnosing PJI. Though

growth was often heterogeneous and patchy on different components of the prosthesis, the method revealed areas in the prosthetic joint articulation that could be more vulnerable to biofilm formation, namely the non-articulating surfaces of the tibial tray and insert. There was reasonably precise localization of biofilm outgrowth, which was often pinpointed to edges, ridges, and other sharp changes in contours on various knee implants. While unlikely to be adopted as a routine method due to the extensive handling, processing, and physical size of the culture containers, this diagnostic method could be useful in several types of revision cases to better obtain the location and extent of biofilm existence and inform for more efficient washout procedures as well as implant design.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest for this study.

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