High Prevalence of Biofilms on Retrieved Implants from Aseptic Pseudarthrosis Cases

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Abstract:

Introduction: Recent literature has associated pseudarthrosis and pedicle screw loosening with subchronic infection at the pedicle of the vertebra. The positive culture results of a previous retrieval analysis show that such patients have a high frequency of bacterial contamination. The objective of this study is to visually capture the architecture of these undiagnosed infections, which have been described in other studies as biofilms on supposedly "aseptic" screw loosening.

Methods: Explants from 10 consecutive patients undergoing revision spine surgery for pseudarthrosis were collected and fixed in glutaraldehyde solution. Each of these implants was imaged thoroughly by using scanning electron microscopy and x-ray spectroscopy to evaluate the architecture of the biofilm. Additionally, eight patient swabs from tissues around the implants were sent for cultures to assess bacterial infiltration in tissues beyond the biofilm. The implants were also analyzed using energy dispersive x-ray spectroscopy. The exclusion criteria included clinically diagnosed infection (current or previous) and/or mechanical failure of the implant due to falls/accidents.

Results: The study was successful in capturing the visual architecture of the biofilm on retrieved implants. A total of 77% of pseudarthrosis cases presented with loose pedicle screws, which were diagnosed by a preoperative computed tomography scan showing radiolucency along the screw track and were confirmed intraoperatively, and 72% of the cases showed biofilm on explants.

Conclusions: In the absence of the clinical presentation of infection, impregnated bacteria could form a biofilm around an implant, and this biofilm can remain undetected via contemporary diagnostic methods, including swabbing. Implant biofilm is frequently present in "aseptic" pseudarthrosis cases.

Keywords:

Pseudarthrosis, screw loosening, biofilm, occult infection, undiagnosed infection, subchronic infection, surgical site infection, aseptic revision, readmission

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Introduction

Revision surgery is a common occurrence among patients undergoing spinal fusion procedure and can be challenging to resolve satisfactorily[1)]. Patient factors such as smoking, obesity, diabetes, and autoimmune diseases can inhibit successful spine fusion, thus causing the reoccurrence of back pain[2,3)]. Some of the known causes for revision surgery include surgical site infection (SSI), implant-related failure, pseudarthrosis, adjacent segment diseases, degenerative disease progression, nerve impingement recurrence, surgical error, and incorrect initial diagnoses[4)]. Aseptic screw loosening is a common implant-related failure, which occurs in conjunction with pseudarthrosis[5,6)]. Although it was previously

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deemed a mechanical failure at the screw-bone interface in the absence of solid fusion, recent studies have highlighted a possible biological pathway. The proposed biological path includes the presence of occult infection in the form of bacterial growth around the implant with no known clinical symptoms of SSI[7-11]. This is consistent with other studies that demonstrated a positive bacterial biodose impregnation at the screw-bone interface during spine sugery[12-15]. In addition to screw loosening, studies have shown a high rate of delayed or late-onset infection only in cases with instrumentation[16-19]. These studies support the biological pathway hypothesis, which states that many bacterial species that are known to cause infection can remain dormant in implant-associated biofilms and would later cause screw loosening or delayed/late-onset infection. Nevertheless, the exact architecture of such implant-associated biofilms is yet to be determined because all previous studies have relied on bacterial cultures, wet-lab procedures, or animal studies. The objective of the current study is to characterize the supposedly "aseptic" pedicle screw loosening in patients undergoing revision surgery and to determine both the frequency and visual architecture of biofilms on implant surfaces.

Material and Methods

This study prospectively collected pedicle screw explants from 10 consecutive patients who underwent revision spine surgery for pseudarthrosis in 2019. The exclusion criteria were as follows: age<18 years; surgery for causes related to trauma, tumors, or primary infection; use of cemented screws; an anterior-only approach; and lack of radiographic data (to determine the presence or absence of radiolucent rim and confirm screw loosening). The preoperative data collected were age, gender, comorbidities, radiographic assessment, and period between index and revision surgery. Each of the collected explants was fixed in 3% glutaraldehyde solution, followed by gold sputter coating, which involves the vapor deposition of a nanolayer of gold to increase the electrical conductivity required for high-resolution imaging via electron microscopy. The samples were subsequently analyzed using a scanning electron microscope (FEI Quanta 3D FEG Environmental Scanning Electron Microscope and Focused Ion Beam). In samples wherein biofilms were identified with certainty, energy dispersive x-ray spectroscopy was performed to identify the regional distribution of mineralization, such as calcium and phosphate elemental groups. Additionally, in eight patients, intraoperative cultures were sent from debrided tissues surrounding the implants, as well as culture swabs from within the bony defect after implant removal, to assess for viable bacterial infiltration in the tissues beyond the biofilm. Pearson's correlation coefficient statistics was used to measure the linear correlation between the two paired variables, namely, screw loosening and biofilm.

Results

This study was successful in capturing the visual architecture of the biofilm on retrieved implants. Electron microscopy revealed that there are various presentations of the biofilm architecture (Fig. 1, 2). A total of 77% of pseudarthrosis cases presented with loose pedicle screws, and 72% of cases showed biofilms on the implants. Statistical tests demonstrated that the confirmed cases of screw loosening (as identified by radiographic halo) and the detection of biofilms were positively correlated. However, by normal standards, the association between the two variables would not be considered statistically significant: $r(8)=.52$ and $p=.18$. Areas with biofilms always tested negative for calcium phosphate (bone mineralization), whereas areas without biofilms tested positive for calcium phosphate (Fig. 3). Intraoperative tissue and swab cultures of the surrounding screws did not demonstrate bacteria growth on cultures. Table 1 summarizes the data collected in these 10 patients.

Discussion

This study is the first to visually capture the exact architecture of implant-associated biofilms in patients undergoing revision surgery for "aseptic" pedicle screw loosening. Recent studies have demonstrated an association between screw loosening and the presence of bacterial biodose[10,11]. However, in the current study, the correlation between confirmed screw loosening and presence of biofilm was not statistically significant. This could be the result of the small sample size. Nonetheless, this study sheds light on the surreptitious dynamics of implant and impregnated microbes and possibly explains the sudden onset of delayed and late infection responses. The proclivity of bacteria to grow in conjunction with metal implants has been well characterized in the past by using a mouse osteomyelitis model[16]. The current study showed that *Propionibacterium acnes* was absent from the control group (no implants) six months after bacterial inoculation. By contrast, the implant group had biofilm formation, thus allowing the inoculated bacteria to thrive. The negative results from swabs even in the presence of visual evidence of biofilm was expected because there was no systemic response to the underlying infection. In addition, previous studies showed that methods for isolating microbial organisms often result in suboptimal yield. The results of the current study highlight the importance of keeping the implantable devices free from any bacterial biodose to the best of our capability. Recent studies have shown that repeated reprocessing and intraoperative exposure is a main source of such a biodose and other foreign bodies[12,14,15]. Literature shows that delayed-onset infection can occur from 90 days to a year from the date of surgery and constitutes between 15% to 35% of all reported infections[19]. It also shows that late-onset infection, which occurs after a period of one year from surgery, is the least studied infection type owing to the lack of long-term follow-up[19]. The few longer-term studies

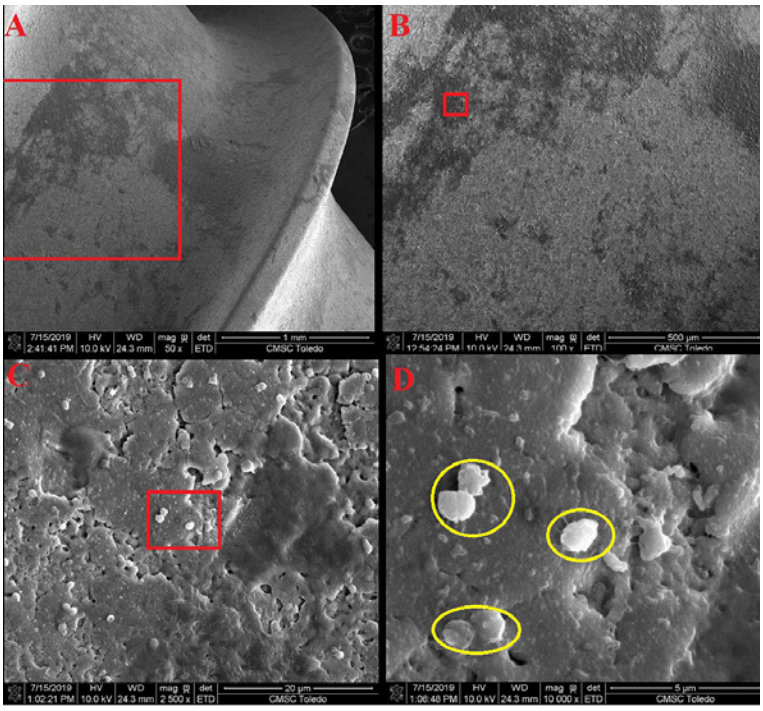

Figure 1. Retrieval sample 1 showing biofilms on spinal implants under an electron microscope. A, B, C, and D show the magnified images of the biofilm on the pedicle screw shaft. The yellow circle shows the biofilm-encapsulated bacterial cells.

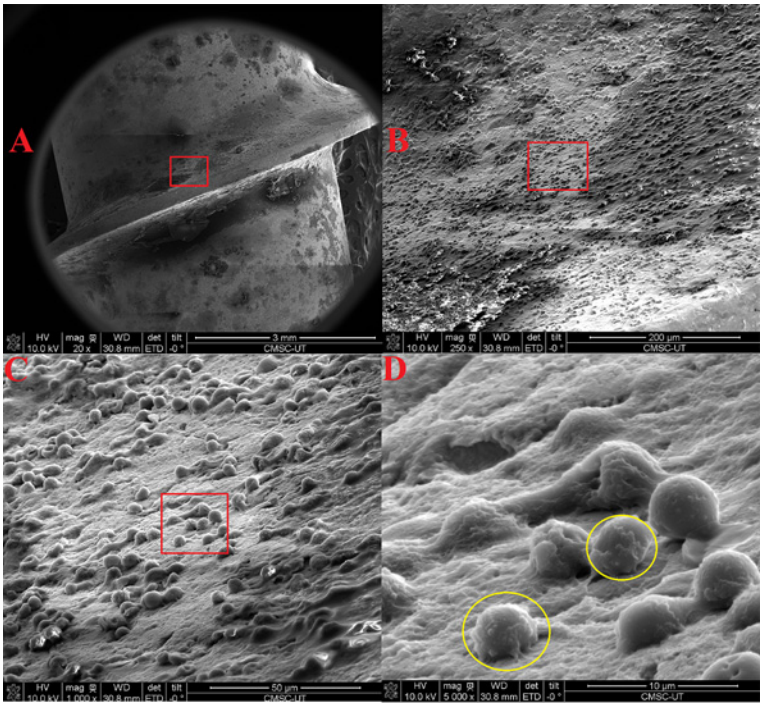

Figure 2. Retrieval sample 2 showing biofilms on spinal implants under an electron microscope. A, B, C, and D show the subsequently magnified image of the biofilm on the pedicle screw shaft. The yellow circle shows the biofilm-encapsulated bacterial cells.

(>6 years) that considered late-onset infection have shown an average time to infection detection of 56 to 80 months postsurgery with a total incidence of 9.7%[19]. A previous hypothesis suggests that in several susceptible patients, the

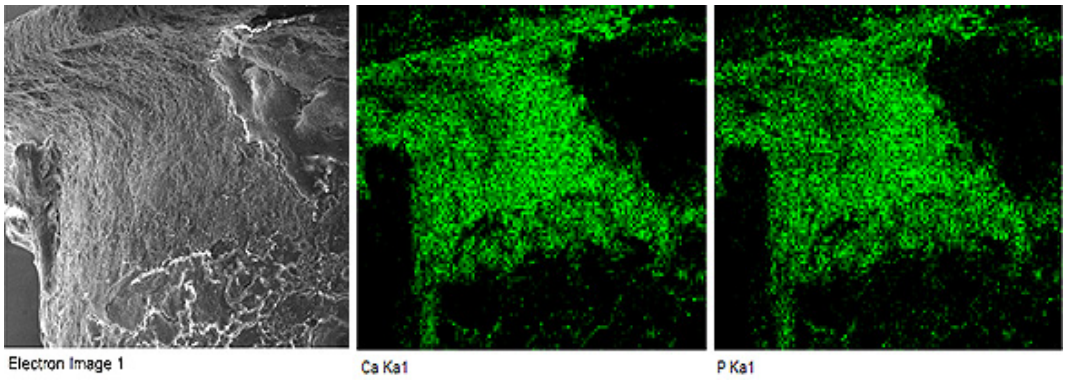

Figure 3. Energy dispersive x-ray spectroscopy areas. The biofilms always tested negative for calcium phosphate (bone mineralization), whereas areas without biofilms tested positive for calcium phosphate (bone mineralization). Ca: Calcium; P: Phosphorus.

Table 1. Patient Demographics, Surgery Duration (between Index and Revision), Comorbidities, Biofilm Detection, and Confirmation of Screw Loosening.

				Revision Surgery			Bio-film		Duration	Confirmed Loosened Screw	
Study Number	Date of Surgery	Age	Gender	Comorbidities	New Patient? (y/n)	Images Halo (y/n)	Y	N	Years	Y	N
1	6/25/2019	43	M	Asthma, Fibromyalgia	Y	Y	1	0	4	1	0
2	8/8/2019	60	M	HTN	Y	Y	1	0	Unknown >1 year	1	0
3	8/13/2019	57	M	HTN, HLD, CAD	Y	N	1	0	9	0	1
4	9/5/2019	87	M	GERD, HLD, BPH	N	N	1	0	4	1	0
5	10/18/2019	37	F	Anxiety, Depression, PTSD, Migraines, HTN	N	Y	1	0	2	1	0
6	10/22/2019	67	F	Asthma, HLD, HTN	Y	N	0	1	3	0	1
7	10/29/2019	53	F	COPD, Bipolar, Asthma, GERD, HTN	N	Y	0	1	5	1	0
8	11/1/2019	56	F	GERD	N	Y	1	0	5	1	0
9	11/22/2019	64	F	CAD, HTN, HLD, Hep C, CHF, CKD, OSA	N	N	0	1	8	0	1
10	11/19/2019	69	M	HTN, Hypothyroidism	N	Y	1	0	46 days	1	0

HTN=hypertension, HLD=hyperlipidemia, CAD=coronary artery disease, GERD=gastroesophageal reflux disorder, BPH=benign prostatic hyperplasia, PTSD =post-traumatic stress disorder, COPD=chronic obstructive pulmonary disorder, Hep C=hepatitis C, CHF=congestive heart failure, CKD=chronic kidney disease, OSA=obstructive sleep apnea

bacteria from initial surgery could lie dormant and thrive via biofilm formation on the implant[20]. The current study provides preliminary data that support this hypothesis as a possible onset mechanism for delayed and late infection. We postulate that the two alternate pathways of failure could include screw loosening or delayed/late infection. For the infection pathway, the existing occult biofilm could release bacterial colonies, thus causing a sudden onset of delayed or late infection response in a patient. Alternatively, instead of a systemic infection response, there could be a local response where the implant and bone begin to disassociate, thus leading to screw loosening and failed fusion. This process is referred to as occult infection phenomenon.

The major limitation of this study is its relatively small sample size and the statistical nonsignificant correlation between screw loosening and biofilms. However, the study provides the first visual evidence of implant biofilm architecture with a prevalence rate of 72% in "aseptic" pseudarthrosis cases. Another limitation includes unknown bacterial species owing to negative swab cultures. This study also highlights the importance of keeping screw/screw-bone interfaces devoid of bioload because of the propensity of bacterial inoculation to form biofilms around the implant. Such biofilms can remain undetected by contemporary diagnostic methods, including swabbing.

Conflicts of Interest: AA is a consultant for Spinal Balance Inc., an editorial board member of *Clinical Spine Surgery*, and an editorial board member of *Spine*. VG received royalties from Paradigm Spine, Joimax, Globus Medical,

Endosphere LLC, and Butterfly LLC and is a stockholder of Spinal Balance and Osteonovus. AA reports consultancy from Spinal Balance Inc., Editorial Board Membership from Clinical Spine Surgery, Editorial Board Membership from Spine, outside the submitted work; VG reports royalties from paradigm spine, Joimax, globus medical, endosphere LLC, butterfly LLC, Stock ownership from Spinal Balance, Osteonovus; JW reports royalties from Biomet, Seaspine, Amedica, Synthes, Investments/Options from Bone Biologics, Pearldiver, Electrocore, Surgitech, Board of Directors for North American Spine Society, AO Foundation, Cervical Spine Research Society, Society for Brain Mapping and Therapeutics, American Orthopaedic Association, Editorial Boards on Spine, The Spine Journal, Clinical Spine Surgery, Global Spine Journal, and Fellowship Funding (paid to institution) from AO Foundation; NA reports consultancy from Medtronic, Globus, GYS Tech, Spinal Balance, royalties from Medtronic, Elsevier, Globus, stocks from AF cell, Theracell, Atlas Spine, Paradigm spine, Bonovo, Medtronic, Globus, GYS Tech, Spinal Balance, SAB from Theracell, Globus, GYS Tech, Spinal Balance, speaker for DePuy Synthes, Stryker, and Editor for Gray's Anatomy; SG reports consultant for Benvenue Medical, Intrinsic Therapeutic, Magnifi Group, Medtronic, SI Bone, Spinal Kinetics, Institutional Support from DePuy (Johnson & Johnson), Globus, Synthes, royalties from DePuy (Johnson & Johnson), and stock from SI Bone & Spinal Kinetics. Rest of the coauthors have nothing to disclose.

Ethical Approval: The IRB reviewed the protocol and exempted the study from requiring IRB approval because no patient data were disclosed. All laboratory analyses were performed on the retrieved implants, which are usually disposed of.

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Author Contributions: Each author satisfied the four criteria defined by ICMJE.

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