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The Detection of Bacteria and Matrix Proteins on Clinically Benign and Pathologic Implants

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Background: Bacterial contamination of breast implants causes infection, can lead to capsular contracture, and is implicated in breast implant-associated anaplastic large cell lymphoma. Bacteria, however, also colonize clinically benign breast implants and little is known about the biologic signals that trigger the switch from a benign to pathologic state.

Methods: Explanted smooth as well as Biocell and Siltex textured breast implants associated with clinically normal and pathologic conditions were analyzed in this observational study. Immunofluorescence and bacterial culture techniques were performed. To avoid sampling bias, implant surfaces >25 sq cm were analyzed.

Results: Bacteria were detected on 9 of 22 clinically normal explanted devices or periprosthetic capsules, including 40% of Biocell tissue expanders and 75% of Biocell textured implants. Staphylococcus epidermidis was identified in 67% of the bacteria-positive capsular contractures. Fibrinogen was present on 17 of 18, and collagen on 13 of 18 analyzed breast implants. S. epidermidis co-localized with collagen, while group B streptococci and Klebsiella pneumoniae co-localized with fibrinogen.

Conclusions: Bacteria are often detectable on clinically benign breast implants when a multimodal approach is applied to a substantial proportion of the device surface to avoid sampling bias. The impact of bacteria on breast implant pathology should be studied in the presence of an adequate negative control group to account for clinically benign bacteria. Disruption of the interaction of bacteria with matrix proteins coating the surface of breast implants may represent a nonantibiotic strategy for the prevention of breast implant bacterial contamination. (Plast Reconstr Surg Glob Open 2019;7:e2037; doi: 10.1097/GOX.0000000000002037; Published online 8 February 2019.)

INTRODUCTION

Bacterial contamination of breast implants can cause infection,1 capsular contracture (CC),2–4 and has been linked to breast implant-associated anaplastic large cell lymphoma (BIA-ALCL).5 Bacteria can also be identified on clinically benign breast implants, however, as they indefinitely abut pathologic tissue laden with a diverse array of microbes.6–10 Staphylococcus epidermidis is the most common bacterium found on both pathologic and nonpathologic implants, yet why complications manifest in some women and not others remains unknown.11 To establish the impact of bacterial contamination, including differing bacterial species, strains, abundance, or virulence factors, on breast implant pathology, detailed characterization of bacteria on clinically benign breast implants is needed to establish a negative control against which pathology can be compared.

The majority of bacterial infections of medical devices are associated with biofilms. Hallmarks of these infections...
tions include increased resistance to antibiotics and the host immune system, resulting in chronic infection, treatment failure, and often surgical intervention. For breast implants bacterial biofilm formation is a major concern. There is a large unmet need to understand the mechanisms by which bacteria colonize breast implants to form biofilms to develop effective drugs that can eradicate biofilm-associated infections. The extent to which bacteria become associated with breast implants is influenced by the surface characteristics of the device. Textured devices, whose contoured surfaces have increased surface area available for bacterial colonization, harbor significantly more bacteria than do smooth breast implant surfaces. However, recent studies show that medical devices become coated with host proteins that can be exploited by bacterial pathogens for colonization and biofilm formation. For example, Staphylococcus aureus, which causes the majority of implant-associated infections (IAI), utilizes a fibrinogen binding adhesion to colonize devices that become coated with fibrinogen, a common occurrence after placement of most kinds of medical devices. For breast prostheses, additional host ligands are deposited on the devices in the form of a collagen-rich capsule and this likely facilitates bacterial colonization. A granular understanding of the host-pathogen interactions that lead to breast implant colonization and the biological signals that trigger the switch from a benign to a pathologic state will inform future strategies to optimize breast implant design and establish antibiotic-sparing therapies that prevent problematic bacterial contamination.

The purpose of this observational study is 2-fold: (1) we examined the extent of which bacteria were associated with a series of explanted breast prostheses, with different surface characteristics, obtained from normal and pathologic clinical scenarios and used immunofluorescence techniques to characterize the bacteria associated with the devices; and (2) we evaluate, for the first time, matrix protein deposition on the breast device surface to determine the potential repertoire of available bacterial binding ligands. Characterization of bacterial binding mechanisms to breast implants may lead to the development of nonantibiotic antibacterial therapeutics for downstream clinical translation.

METHODS

Study Population

Cosmetic or reconstructive breast prostheses, either implants or tissue expanders (TE), explanted between March 2017 and March 2018 were analyzed under protocol #201703065 at the Washington University School of Medicine. We identified patients with CC, double capsules, seroma, and infection. Benign breast prostheses consisted of TE removed at the time of planned device exchange as well as the contralateral breast implant in patients with unilateral pathology where both devices were explanted. Breast prostheses and capsules were sharply removed using sterile technique, the surface between capsule and implant was marked with a suture, and samples were sectioned and immediately placed in a sterile container. In all cases, the entire breast prosthesis was removed, while the entire capsule was removed in cases of CC and double capsules, while the submuscular capsule was entirely removed but the acellular dermal matrix sling maintained in cases of submuscular TEs. In this manner, >25 sq cm of capsule and implant surface was made available for analysis. Device type was confirmed to be consistent with the medical record. Duration of implantation, device type, and clinical presentation were recorded.

Bacterial Culture and Identification

Explanted devices were divided into 3 sections, the largest section (>25 sq cm) was fixed for immunofluorescence staining and the 2 smaller pieces (~4–25 sq mm) were cultured for bacterial growth. One piece was sonicated for 10 minutes in phosphate buffered saline, plated on the rich media Brain Heart Infusion agar, and grown aerobically and anaerobically at 37°C for 48 hours. Individual bacterial species were evaluated for colony size, morphology, color, and bacterial load. The second piece was submerged in BHI and grown for 48 hours at 37°C. Cultures with visible microbial growth were restreaked onto BHI agar for single colonies. A representative isolate of each bacterial species was selected for identification via 16S sequencing, as described previously.

Immunofluorescence Staining

Immunofluorescence staining was performed as previously described. Briefly, the largest piece of the patient device was fixed, blocked, washed, and incubated with primary antibodies. Devices were then washed and incubated with secondary antibodies, which were then washed, dried, and imaged. All antibodies were tested against each isolated bacterial species to determine the optimal concentration for immunofluorescence detection. Infrared signal was examined using the Odyssey Imaging System (LI-COR Biosciences, Lincoln, Nebraska). Controls for auto-fluorescence included small pieces of each respective device in the absence of primary antibody were performed.

RESULTS

Study Population

Twenty-two clinically benign and 18 clinically pathologic breast prostheses were explanted from 35 women. Half of the breast prostheses placed were for reconstructive cases and the other half were for cosmetic purposes. Duration of implantation ranged from 3 months for some TEs to 540 months for severely contracted smooth, shaped, saline filled Dow Corning breast implants. Pocket irrigation with 50% Betadine was utilized in the TE cases, but this information was not reliably available for the other implants collected.

Bacteria Cultured from Benign Breast Implants

Patient samples were cultured to determine the bacterial abundance (Table 1), and 16S sequencing was utilized to identify the species (Fig. 1 and Supplemental Digital
Clinically from 8 women (Fig. 1 and Supplemental Digital Content). CCs were noted in 12 breast prostheses collected tent 1 Supplemental Digital Content 16S sequencing (Fig. 1 and ). Additionally, while capsular were not detected on the 3 clinically normal saline and Supplemental Digital Content 1. Bacteria were cultured from pathologic breast implants (Table 1) and species of bacteria identified on breast implants explanted from women in the absence of clinical pathology. Pathology categorized as CC, double capsule without seroma (seroma (-), double capsule with seroma (seroma (+)), or infection requiring explantation, http://links.lww.com/PRSGO/A990). Other Gram-positive bacteria, including other CNS, were found colonizing the rest (Supplemental Digital Content 2). Smooth-surface and Siltex and Biocell textured devices (both saline and silicone) were represented in the CCs analyzed (Fig. 1). Bacteria were inconsistently identified in smooth and textured devices complicated by CC (Fig. 1). Double capsules—defined as 2 distinct capsules between the device and the soft-tissue space with 1 capsule tenaciously adherent to the device surface—were exclusively identified in patients with Biocell textured prostheses (Fig. 1). CNS were identified in 2 of the 5 double capsules with or without seroma (Fig. 1 and Supplemental Digital Content 2). One TE was explanted for infection, and CNS was isolated (Fig. 1 and Supplemental Digital Content 2). Interestingly, the microbes isolated from the complicated prostheses were exclusively Gram-positive bacteria.

**Matrix Protein Deposition on Breast Implants**

Complicated (n = 5) and normal (n = 13) devices without any detectable bacteria were immunofluorescently stained for the presence of host proteins, including fibrinogen, a protein known to be deposited on other medical devices, and collagen type I and type III, proteins that make up the implant capsule. Fibrinogen was present on 5/5 and 12/13 clinically complicated and normal devices (Table 2 and Fig. 2). Collagen was detected on 4/5 and 9/13 clinically complicated and normal devices. All analyzed textured devices, including 14 Biocell and 2 Siltex, were coated with fibrinogen. Smooth surfaced breast implants included 1 that lacked matrix protein deposition, 1 coated with fibrinogen, and 2 coated with fibrinogen and collagen.

**Bacteria Co-localize with Deposited Matrix Proteins**

Breast prostheses with detectable bacteria were immunofluorescently stained with commercially available antibodies for the respective microbe (antibodies were not available for *Micrococcus, Bacillus*, or *Exiguobacterium*). *S. epidermidis* was detected on all devices from which the bacteria were isolated (Table 3). Additionally, since staphylococcal-collagen interactions have been implicated in breast IAI, we simultaneously stained the samples for col-

| Table 1. Bacterial Load Recovered from Clinically Normal and Complicated Patient Devices |
|-----------------------------------|-----------------|-------------------|-----------------|
| **Variable**                     | **Patient No.** | **Material**      | **CFU**        |
| **Clinically normal**            | 2               | Expander          | 2.0 x 10^3     |
|                                  | 3               | Expander          | 3.0 x 10^3     |
|                                  | 4               | Expander          | 1.3 x 10^3     |
| **Clinically complicated**       | 7               | Implant           | 1.0 x 10^3     |
|                                  | 23              | Implant           | 4.0 x 10^3     |
|                                  | 22              | Implant           | 1.0 x 10^3     |
|                                  | 25              | Capsule           | 2.0 x 10^3     |
|                                  | 27              | Capsule           | 5.0 x 10^3     |
|                                  | 32              | Left              | 1.3 x 10^3     |
| **Colony Identity**              | **CFU**         | **Material**      | **CFU**        |
| **M. luteus**                    | 2.0 x 10^3      | *K. pneumoniae*   | 1.69 x 10^3    |
| **S. epidermidis**               | 3.0 x 10^3      | *P. aeruginosa*   | 2.0 x 10^1     |
| **S. hominis**                   | 1.0 x 10^3      | *E. faecalis*     | 3.1 x 10^2     |
| **S. haemolyticus**              | 1.0 x 10^3      | *P. aeruginosa*   | 2.0 x 10^1     |
| **S. epidermidis**               | 1.0 x 10^3      | *S. hominis*      | 2.0 x 10^1     |
| **S. epidermidis**               | 2.0 x 10^1      | *K. pneumoniae*   | 1.69 x 10^3    |
| **Staphylococcus epidermidis**   | 1.3 x 10^3      | *S. hominis*      | 2.0 x 10^1     |
| **Staphylococcus epidermidis**   | 1.3 x 10^3      | *K. pneumoniae*   | 1.69 x 10^3    |

CFU, colony forming units.

Bacteria Cultured from Pathologic Breast Implants

Clinically complicated samples were cultured and bacterial abundance was assessed (Table 1) and species colonizing pathologic prostheses were determined via 16S sequencing (Fig. 1 and Supplemental Digital Content 1). CCs were noted in 12 breast prostheses collected from 8 women (Fig. 1 and Supplemental Digital Content 2) and *S. epidermidis* was identified in the majority (67%) of these cases (see figure, Supplemental Digital Content 2, which displays presence, absence, and species of bacteria identified on breast implants explanted from women in the absence of clinical pathology. Pathology categorized as CC, double capsule without seroma (seroma (-), double capsule with seroma (seroma (+)), or infection requiring explantation, http://links.lww.com/PRSGO/A990). Other Gram-positive bacteria, including other CNS, were found colonizing the rest (Supplemental Digital Content 2). Smooth-surface and Siltex and Biocell textured devices (both saline and silicone) were represented in the CCs analyzed (Fig. 1). Bacteria were inconsistently identified in smooth and textured devices complicated by CC (Fig. 1). Double capsules—defined as 2 distinct capsules between the device and the soft-tissue space with 1 capsule tenaciously adherent to the device surface—were exclusively identified in patients with Biocell textured prostheses (Fig. 1). CNS were identified in 2 of the 5 double capsules with or without seroma (Fig. 1 and Supplemental Digital Content 2). One TE was explanted for infection, and CNS was isolated (Fig. 1 and Supplemental Digital Content 2). Interestingly, the microbes isolated from the complicated prostheses were exclusively Gram-positive bacteria.

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Additionally, while capsular tissue was not routinely harvested for all specimens, there were 2 instances, one with a TE and one with an implant, where bacteria were retrieved from the prosthesis but not the capsule (Supplemental Digital Content 1).
plants, all were found to co-localize with matrix proteins (Tables 4, 5, Fig. 3).

**DISCUSSION**

Bacteria cause IAI, 1 have a role in CC, 2,3,30–32 and may play a role in the etiology of BIA-ALCL; however, the microbial species responsible and the host-pathogen interactions that result in these diverse complications are still being investigated. We have previously shown that the predominant Gram-positive and Gram-negative bacterial causes of breast IAI and explantation are *S. aureus* and *P. aeruginosa*, respectively. 1 *Ralstonia pickettii* has also been identified in a disproportionately high percentage of breast implants from patients with BIA-ALCL. 5 Additionally, while there is a growing body of evidence implicating bacterial coloniza-
tion of devices in the development of CC, including a strong correlation between Baker grade and positive breast implant and TE cultures and a reduction in CC rates after breast implant placement with the use of antibiotic pocket irrigation or impregnated mesh strategies, these findings are not universally consistent among reports, and the latter are limited to a mean of 2 years of follow-up or less. An elegant swine model, however, has demonstrated causation between *S. epidermidis* infection and CC. Together, these studies highlight the need to better understand the host-pathogen interactions that facilitate the development of pathologic implants in patients, including CC and BIA-ALCL, to implement truly effective interventions.

To understand how bacteria influence the development of breast implant-associated complications, it is critical to know which bacteria are present in a clinically benign scenario. However, data from this “negative control” group are scant, as evaluating normal breast implant colonization is challenging since it requires assessing either temporary TE or permanent implants explanted at the time of less common revision surgery due to malposition or when managing pathology on the contralateral side. Of the few small studies that address this question, Pajkos et al. identified bacteria in 1 of 8 (12.5%) clinically benign breast implants, Rieger et al. identified bacteria in 4 of 21 (19%) patients with Baker grade I and II capsules, and Hu et al. identified 7.6 × 10^5 bacteria/mg tissue in 3 clinically normal breast implants. Significantly, more bacteria were detected on pathologic implants than uncomplicated ones in these studies, suggesting bacterial abundance impacts the development of complications. The presence of bacteria on benign implants, though, requires further study to determine whether bacterial spe-
cies or strains, virulence factor production, or interactions with other bacteria or the host contribute to the development of complications.19,24,39–41

While this study is not adequately designed or powered to compare the bacterial abundance between clinically normal and complicated breast implants, it does provide important insights into the potential bacterial reservoir on uncomplicated devices. In this study, we detected bacteria in 41% of the clinically normal breast prostheses than previous reports.3–5 This is likely due to our combi-

Table 5. Bacteria Can Be Detected on Breast Implants with Pathology and Bacteria Co-localizes with Host Collagen Deposited

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Implant/Surface</th>
<th>Pathology</th>
<th>Host Protein Detected</th>
<th>Microbe Detected</th>
<th>Co-localize (+/-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>Implant/Biocell</td>
<td>Double capsule,</td>
<td>Collagen</td>
<td>S. epi</td>
<td>+</td>
</tr>
<tr>
<td>25</td>
<td>TE/Biocell</td>
<td>Seroma (-)ve</td>
<td>Collagen</td>
<td>CNS</td>
<td>+</td>
</tr>
<tr>
<td>26</td>
<td>Implant/Biocell</td>
<td>Infection</td>
<td>Collagen</td>
<td>CNS</td>
<td>+</td>
</tr>
<tr>
<td>29</td>
<td>Implant/smooth</td>
<td>Double capsule,</td>
<td>Collagen</td>
<td>CNS</td>
<td>+</td>
</tr>
<tr>
<td>30</td>
<td>Implant/Biocell</td>
<td>Seroma (+)ve</td>
<td>Collagen</td>
<td>S. epi</td>
<td>+</td>
</tr>
</tbody>
</table>

Fig. 3. Representative images of devices immunofluorescently stained for cultured bacteria and host proteins. A, Group B streptococcus (GBS) was cultured from the Biocell-textured silicone breast implant of patient 9, which was clinically normal. GBS was detected via immunofluorescence staining and it co-localized with the deposited host protein fibrinogen (Fg). Staphylococcus epidermidis (SE) was cultured from patient 22’s Biocell-textured silicone implant, which was clinically complicated by a double capsule without seroma. SE was visible on the device and co-localized with collagen (Cn). K. pneumoniae (KP) and SE were cultured from patients 23’s Biocell-textured TE, which was clinically normal. KP (C) and SE (D) were both visible on the device and co-localized with deposited Fg and Cn, respectively. Controls were pieces of each device treated the same, but without primary antibody. Commercially available primary antibodies: goat antifibrinogen (Cat # F8512); rabbit anticollagen (Cat. #234169, Calbiochem); mouse anti-S. epidermidis (Cat. # MA1-35788, ThermoFisher Scientific); rabbit anti-Pseudomonas (Cat. # ab68538, Abcam); rabbit anti-Protein A (for S. aureus) (P3775, Sigma-Aldrich); rabbit antienterococcus (Cat. # PA1-73120); rabbit anti-Klebsiella,29 and rabbit antigroup B streptococcus.29 Secondary antibodies: IRDye 800CW donkey antigoat, IRDye 800LT donkey antimouse, and IRDye 680LT donkey antirabbit (LI-COR Biosciences).
...ing standard sonication and plating, similar to previous studies, with liquid culturing techniques, to detect low colonization levels and/or bacteria firmly adherent to the implant. Of the clinically normal implants that were bacteria-positive, all had a Biocell-textured surface and were colonized exclusively by Gram-positive bacteria, with CNS the chief microorganism identified. For clinically normal TE, all colonized devices also had Biocell-textured surfaces; however, Gram-negative bacteria, including K. pneumoniae and P. aeruginosa, were found in addition to Gram-positive microorganisms. The wider array of bacteria present on TE may be due to the fact that the implant reconstruction paradigm differs significantly from aesthetic breast augmentation, with the traumatic dispersion of parenchymal and ductal bacteria from the breast and skin microbiome during mastectomy and reconstruction, longer operative times, and the potential for disease- or chemotherapy-induced immunosuppression. Recognizing the greater likelihood for bacterial contamination following TE breast reconstruction, a higher incidence of bacterial contamination with a more diverse group of microbes is not unexpected. Notably, the routine use of betadine irrigation of the skin and postmastectomy pocket before insertion of a TE with acellular dermal matrix may have been sufficient to reduce the contaminating bacteria below a clinically problematic threshold in these patients. Interestingly, similarly to what we found for normal implants, only Gram-positive bacteria, and primarily CNS, were found colonizing CC (50%) and double capsule (40%) specimens. While we cannot discount that some of the patients with colonized, clinically normal devices would eventually go on to develop complications like infection or CC, future studies elucidating the host-pathogen interactions that lead to pathologic implants will be critical for understanding why some women with colonized implants develop complications while others do not.

The presence of CNS, which are known skin colonizers, on both pathologic and benign implants supports the dogma that breast prosthesis contamination primarily occurs through contact with the skin microbiota during placement. However, even if skin contact can be minimized, breast implants are still susceptible to bacteria that reside in the breast parenchyma, which also contain CNS, among other Gram-positive bacteria. Thus, the breast represents a clean-contaminated surgical site. Importantly, the breast microbiome has greater microbial diversity than the skin, and is formed and evolved over time through the translocation of bacteria through mucosal membranes of the gut, oropharynx, and urogenital tract. Recent evidence suggests that the composition of the breast microbiome varies based on depth of biopsy, suggesting the important contributions of the nipple and skin microbiome to bacteria within the breast. Given the proximity of a breast implant to the colonized breast parenchyma, there is ample opportunity, even years past the time of initial device insertion, for bacteria to contact the implant surface and/or capsule. Thus, it is reasonable to expect that the bacteria contaminating implants is similar to the breast parenchyma microbiome because parenchymal microbes have the most direct pathway to the implant surface after placement.

Our previous work detailing the molecular mechanisms that result in catheter-associated urinary tract infections (CAUTI) has provided important insights into the host-pathogen interactions that facilitate disease and may lead to the development of therapeutics to treat these recalcitrant infections. This work showed that fibrinogen, which is recruited to the bladder following catheter-induced damage to the urothelium, is deposited on the urinary catheter surface and provides a critical binding substrate for Enterococcus faecalis and S. aureus adherence and biofilm formation. By developing a vaccine that specifically blocked the ability of the adhesive tip of the E. faecalis Ehp pilus to interact with fibrinogen, we could effectively prevent and treat enterococcal CAUTI in a mouse model. Thus, by identifying bacterial virulence mechanisms and dissecting the host-pathogen interactions that lead to CAUTI, it may be possible to design and develop effective therapeutics. Herein, we begin to translate this work to the breast implant paradigm. We found that fibrinogen was deposited on 17/18 and collagen on 13/18 of the breast implants analyzed in both clinically normal and abnormal cohorts (Tables 2, 3). Fibrinogen deposition likely stems from surgical manipulation of the breast parenchyma, which stimulates inflammation. Importantly, the deposition of host proteins, including fibrinogen and collagen, on breast prostheses has important implications for bacterial adherence and infection, as it is becoming increasingly clear that many bacteria encode proteins that bind to these factors to facilitate disease. Notably, we found that CNS co-localized with collagen on explanted breast implants. S. epidermidis elaborates SdrF and GehD, which interact with collagen, thus providing a potential mechanism for S. epidermidis breast implant adherence or biofilm formation. Interestingly, K. pneumoniae and Group B Streptococcus, which specifically block the ability of the adhesive tip of the E. faecalis Ehp pilus to interact with fibrinogen, could also be used to prevent and treat bacteremia associated with breast implantation.

CONCLUSIONS

We readily detected bacteria and matrix protein deposition on the smooth and textured surfaces of clinically normal and pathologic implants, explanted months to decades after insertion. Bacteria co-localized with matrix proteins, thus suggesting bacteria may preferentially adhere to host proteins instead of abiotic surfaces. Future studies examining bacteria-related breast implant pathology should analyze a sufficient percentage of the implant surface to avoid sampling bias, and include an adequately powered control group. Finally, several knowledge gaps in...
the field of breast implant bacteria require further study including identifying the signals, bacterial, or host, that trigger the transition from a normal, uncomplicated implant to a pathologic state and the role of matrix proteins, like collagen and fibrinogen, in implant contamination. The answers to these questions may lead to the development of novel nonantibiotic therapeutic strategies.

**ACKNOWLEDGMENTS**

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