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.

INTRODUCTION

Bacterial contamination of breast implants can cause infection, capsular contracture (CC), and has been linked to breast implant-associated anaplastic large cell lymphoma (BIA-ALCL). Bacteria can also be identified on clinically benign breast implants, however, as they indefinitely abut pathogenic tissue. The majority of bacterial infections of medical devices are associated with biofilms. Hallmarks of these infections, especially in breast implants, include biofilms, presence of different bacterial species, strains, and 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.

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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 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 #201703063 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 Variable from 8 women (Fig. 1 and 2). CCs were noted in 12 breast prostheses collected via 16S sequencing (Fig. 1 and Supplemental Digital Content 1). 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). Bacteria were cultured on 5/5 and 12/13 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.

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 12/13 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 colocalization. Interestingly, 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 was isolated from 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.

<table>
<thead>
<tr>
<th>Table 1. Bacterial Load Recovered from Clinically Normal and Complicated Patient Devices</th>
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<tbody>
<tr>
<td>Variable</td>
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<tr>
<td>Clinically normal</td>
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<td></td>
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<td></td>
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<td>Clinically complicated</td>
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CFU, colony forming units.
plants, all were found to co-localize with matrix proteins (Tables 4, 5, Fig. 3).

**DISCUSSION**

Bacteria cause IAI, have a role in CC, 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. *Ralstonia pickettii* has also been identified in a disproportionately high percentage of breast implants from patients with BIA-ALCL. Additionally, while there is a growing body of evidence implicating bacterial coloniza-

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**Table 2. Clinically Normal Breast Implants without Detectable Bacteria Stained for Deposited Fibrinogen and Collagen**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Implant</th>
<th>Texturing (+/-)</th>
<th>Fibrinogen</th>
<th>Collagen</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I</td>
<td>Smooth</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>TE</td>
<td>Biocell texture</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>TE</td>
<td>Biocell texture</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>TE</td>
<td>Biocell texture</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>TE</td>
<td>Biocell texture</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>13</td>
<td>TE</td>
<td>Biocell texture</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>17</td>
<td>I</td>
<td>Siltex texture</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>18</td>
<td>I</td>
<td>Smooth</td>
<td>-</td>
<td>-</td>
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<tr>
<td>19</td>
<td>TE</td>
<td>Biocell texture</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>20</td>
<td>TE</td>
<td>Biocell texture</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>21</td>
<td>TE</td>
<td>Biocell texture</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>23</td>
<td>TE</td>
<td>Biocell texture</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>24</td>
<td>I</td>
<td>Smooth</td>
<td>+</td>
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</table>
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—higher than previous reports.3–5 This is likely due to our combin-
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.1,42 Recognizing the greater likelihood for bacterial contamination following TE breast reconstruction,42 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.13,26 Interestingly, similarly to what we found for normal implants, only Gram-positive bacteria, and primarily CNS, were found colonizing CC (50%) and doub-}

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**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.

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