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Pilot Study of Association of Bacteria on Breast Implants with Capsular Contracture▽

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Capsular contracture is the most common and frustrating complication in women who have undergone breast implantation. Its cause and, accordingly, treatment and prevention remain to be elucidated fully. The aim of this prospective observational pilot study was to test the hypothesis that the presence of bacteria on breast implants is associated with capsular contracture. We prospectively studied consecutive patients who underwent breast implant removal for reasons other than overt infection at the Mayo Clinic from February through September 2008. Removed breast implants were processed using a vortexing/sonication procedure and then subjected to semiquantitative culture. Twenty-seven of the 45 implants collected were removed due to significant capsular contracture, among which 9 (33%) had ≥20 CFU bacteria/10 ml sonicate fluid; 18 were removed for reasons other than significant capsular contracture, among which 1 (5%) had ≥20 CFU/10 ml sonicate fluid ($P = 0.034$). *Propionibacterium* species, coagulase-negative staphylococci, and *Corynebacterium* species were the microorganisms isolated. The results of this study demonstrate that there is a significant association between capsular contracture and the presence of bacteria on the implant. The role of these bacteria in the pathogenesis of capsular contracture deserves further study.

Breast implants are used for reconstruction after mastectomy and for breast augmentation. According to the American Society of Plastic Surgery, in 2007 breast augmentation became the leading cosmetic surgical procedure in the United States, with 347,500 procedures performed annually (http://www.plasticsurgery.org/media/statistics/index.cfm). Women who have undergone breast implantation may experience local complications during the ensuing years. Capsular contracture is the most common and frustrating complication (1, 3), with a reported incidence as high as 50 to 74% according to some studies (4, 9). Capsular contracture is classified according to the Baker classification system (14), as follows: grade I, breast absolutely natural; grade II, minimum contracture; grade III, moderate contracture; and grade IV, severe contracture. The cause of capsular contracture and, accordingly, its treatment and prevention remain to be elucidated fully. A number of factors, including foreign body reaction, hematoma, and periimplant infection, have been suggested (17). Several lines of evidence suggest, in a preliminary way, a role of subclinical infection in capsular contracture pathogenesis (7, 16, 21). Local skin flora (e.g., coagulase-negative staphylococci, *Propionibacterium acnes*, and *Corynebacterium* species) may gain access to breast implants during or following placement. Some have suggested that biofilms form on the implant, stimulating fibrosis around the implant and, ultimately, capsular contracture (6, 15, 17, 21). However, studies examining this issue have not used techniques to specifically sample implant-associated biofilms in a quantitative fashion; accordingly, findings of some prior studies may represent contamination.

We have developed a new technique which uses a combination of vortexing and sonication to sample biofilm bacteria on the surfaces of implants (20). We have shown that this technique is more sensitive than periprosthetic tissue culture for the diagnosis of prosthetic joint infection (20). Moreover, the combination of vortexing and sonication to disrupt bacterial biofilms, followed by culture, has been demonstrated to be a sensitive method for detecting bacteria adherent to bone cement and other surfaces (11). We hypothesized that bacterial biofilms are present in some patients with breast implant capsular contracture. We performed a prospective observational pilot study to test our hypothesis.

MATERIALS AND METHODS

Study subjects. We prospectively studied consecutive patients who underwent breast implant removal for reasons other than breast implant-associated infection at the Mayo Clinic from February through September 2008. Patient characteristics and breast implant-related events, including capsular contracture according to the Baker scale, were noted as judged by the treating plastic surgeon. Only participants who had granted permission to have their medical records reviewed for research purposes (Minnesota statute 144.335) were studied. Privacy was maintained by institutional procedures, and the same precautions used to protect patient clinical data were employed. This study was approved by the Institutional Review Board of the Mayo Clinic.

Sample collection and processing. For the purposes of the study, the surgeon aseptically placed the removed breast implant into a sterile 1-liter polypropylene straight-side wide-mouth jar (Nalgene, Lima, OH), using sterile technique. Up to three tissue specimens (the thickest portion of capsule, when present) were harvested using a cauterizer during surgery. The container and tissues were sent

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to the clinical microbiology laboratory for immediate processing for culture. A piece of tissue was sent for histopathological study.

For implant processing, we used the implant sonication procedure developed in our laboratory (18, 20). Briefly, 400 ml of Ringer's solution was added, and the container was vortexed for 30 s (Vortex Genie; Scientific Industries Inc., Bohemia, NY) and then subjected to sonication (frequency, 40 ± 2 kHz; power density, 0.22 ± 0.04 W/cm^2) in an Aquasonic model 750T ultrasound bath (VWR Scientific, Westchester, PA) for 5 min, followed by additional vortexing for 30 s. The resulting sonicate fluid was placed into each of eight conical 50-ml tubes; the tubes were centrifuged at 3,150 × *g* for 5 min (18). The supernatant was aspirated, leaving 0.5 ml remaining in each tube (100-fold concentration), and 0.1 ml of the sediment was plated onto aerobic and anaerobic sheep blood agar plates, which were incubated aerobically for 2 to 4 days and anaerobically for 14 days, respectively. The CFU per plate (corresponding to CFU/10 ml) were counted, and results were expressed as CFU/10 ml (CFU/plate). Results were reported as no growth, <20 CFU/10 ml (not considered significant), 20 to 50 CFU/10 ml, 51 to 100 CFU/10 ml, or >100 CFU/10 ml. For those implants yielding mixed flora, counts were applied to each organism type.

Tissue specimens were processed for culture as follows. Tissue was homogenized in 3 ml brain heart infusion broth for 1 min. Tissue homogenate was inoculated in aliquots of 0.5 ml onto aerobic blood, chocolate, and anaerobic blood agar and into thioglycolate broth (BD Diagnostic Systems, Sparks, MD). Aerobic and anaerobic sheep blood agar plates (BD Diagnostic Systems) were incubated at 35 to 37°C in 5 to 7% CO_2 aerobically and anaerobically for 2 to 4 and 7 days, respectively. Thioglycolate broth was subcultured if turbid. Microorganisms were enumerated and classified using routine microbiologic techniques.

Definitions. Breast implant-associated infection was defined as the presence of rapidly evolving pain and breast erythema with fever and/or fluid collection around the breast implant. Breast implant capsular contracture was defined as pain and/or asymmetry affecting the implant, with a Baker grade III or IV level of contracture and concordant histology. A significant positive implant culture was defined as ≥20 CFU/10 ml of sonicate fluid.

Patient characteristics. The patients were classified according to the following reasons for implantation: cosmetic, reconstructive after mastectomy for breast cancer, or prophylactic reconstruction after subcutaneous mastectomy for cancer prophylaxis among women at high risk for breast cancer. For all enrolled patients, the medical records were utilized to determine age, race, underlying disease(s), history of prior surgeries on the same breast (date and type), results of histopathologic and microbiologic studies, presence or absence of gross purulence (at the time of surgery), breast on which surgery was performed (i.e., left versus right), and reason for surgery (e.g., associated infection, capsular contracture, rupture of the implant, hematoma or bleeding, chronic pain, extrusion, or leakage of the implant).

Data analysis. Data were summarized using medians and ranges for continuous variables and frequencies and percentages for categorical variables. Comparisons between the demographic and implant characteristics were made using the Wilcoxon rank sum test or Fisher's exact test, as appropriate. We compared the rates of positive tests for microorganisms between the groups with and without capsular contracture by using Fisher's exact test. Calculations were performed using the statistical software package SAS (SAS Inc., NC). All tests were two sided, and *P* values of <0.05 were considered statistically significant.

RESULTS

This observational pilot study prospectively evaluated 45 implants and capsules removed and collected from 29 women over an 8-month period. The mean age at the time of explantation was 61 years (range, 37 to 74 years). Sixteen patients had an implant placed as part of reconstruction after ablative surgery for breast cancer. Nine patients had an implant placed during augmentation-mastopexy procedures. The remaining four patients had had a prophylactic mastectomy. Twenty-seven of the 45 implants collected (from 17 patients) were removed due to significant capsular contracture (Baker grade III or IV), while 18 implants were removed (from 12 patients) for reasons other than significant capsular contracture. The median implant life span from placement to removal was 16.4 years (range, 0.46 to 33.87 years). The patient demographics and implant characteristics, stratified by capsular contracture status, are shown in Table 1. There were no statistically significant differences among these characteristics between the two groups (all *P* values were >0.05).

TABLE 1. Subject demographics and implant characteristics

Characteristic	Value for group	
	Capsular contracture	Non-capsular-contracture
No. of implants (no. of patients)	27 (17)	18 (12)
Subject age (yr) (mean [range])	52 (37–72)	64 (37–74)
No. (%) of patients with reason for implantation		
Reconstructive	8 (47)	8 (67)
Cosmetic	7 (41)	2 (16)
Prophylactic	2 (12)	2 (17)
No. (%) of patients with implant type		
Silicone gel	25 (93)	15 (88)
Saline	2 (7)	3 (12)
Implant life span (yr) (mean [range])	16.4 (0.65–33.87)	14.8 (0.46–24.49)
No. (%) of patients with previous breast radiotherapy	2 (7)	4 (22)
No. (%) of patients with previous episode of capsular contracture	3 (11)	3 (17)

In 96% of the cases (43 of 45 implants), at least one piece of capsular tissue was harvested for culture and histopathology (one tissue was submitted for culture in 23 cases, two tissues were submitted in 9 cases, and three tissues were submitted in 11 cases). Table 2 shows the culture results for the implants and tissue capsules according to whether they were removed for capsular contracture or other reasons (excluding infection). There were nine significant positive implant cultures in the capsular contracture group. *Propionibacterium* species were the predominant isolates (seven implants), followed by coagulase-negative staphylococci (four implants). Five of these subjects also had a tissue culture positive with the same microorganism isolated from the implant. In the non-capsular-contracture group, there was a single significant positive implant culture; this subject had the same microorganism (coagulase-negative *Staphylococcus* species) isolated from tissue culture. Implant cultures were more frequently positive in the capsular contracture group than in the non-capsular-contracture group ($P = 0.034$) (Table 3). In Table 4, the histopathological features are shown. Dense fibrosis and inflammation were the most common findings for both groups of patients (i.e., capsular contracture and non-capsular-contracture groups). Calcification was present in 29% of the capsular contracture group versus none of the non-capsular-contracture patients. The presence of foreign material consistent with silicone was assessed in ~30% of patients in both groups.

DISCUSSION

The results of this study demonstrate that there is an association between capsular contracture and the presence of a significant amount of bacteria on explanted breast implants. *Propionibacterium* species and coagulase-negative staphylococci were the microorganisms most frequently involved. The role of these bacteria in the pathogenesis of capsular contracture deserves future study.

TABLE 2. Culture results for all implants removed

Patient no.	Implant no.	Capsular contracture (yes/no)[a]	Organism and quantitative implant culture result (CFU/10 ml)[b]	Organism and tissue culture result[b,c]
2	2	Yes (Baker IV)	0	ND
4	5	Yes (Baker IV)	0	ND
4	6	Yes (Baker IV)	CNS (51–100)	CNS (2/2)
5	7	Yes (Baker IV)	<20	Negative (1/1)
7	9	Yes (Baker IV)	*P. acnes* (20–50)	Negative (3/3)
7	10	Yes (Baker IV)	*P. acnes* (51–100)	Negative (3/3)
8	12	Yes (Baker IV)	0	Negative (1/1)
11	16	Yes (Baker IV)	<20	Negative (1/1)
11	17	Yes (Baker IV)	0	Negative (1/1)
12	18	Yes (Baker IV)	0	CNS (1/1)
12	19	Yes (Baker IV)	0	CNS (1/1)
14	22	Yes (Baker IV)	CNS (20–50), *P. acnes* (20–50)	CNS (2/3)
15	23	Yes (Baker IV)	0	Negative (1/1)
15	24	Yes (Baker III)	<20	Negative (1/1)
17	25	Yes (Baker IV)	*P. acnes* (>100)	*P. acnes* (2/2)
17	26	Yes (Baker IV)	<20	*P. acnes* (2/2), CNS (2/2)
18	27	Yes (Baker IV)	*Propionibacterium granulosum* (51–100), CNS (51–100)	CNS (2/2)
18	28	Yes (Baker IV)	*P. granulosum* (20–50)	CNS (2/2)
19	29	Yes (Baker IV)	0	Negative (2/2)
20	30	Yes (Baker III)	0	CNS (2/3)
20	31	Yes (Baker III)	CNS (>100), *Corynebacterium* species (20–50)	CNS (2/2)
24	38	Yes (Baker IV)	<20	Negative (1/1)
25	40	Yes (Baker III)	*P. acnes* (20–50)	CNS (1/3)
26	41	Yes (Baker III)	<20	Negative (3/3)
26	42	Yes (Baker III)	0	Negative (3/3)
29	47	Yes (Baker IV)	<20	CNS (1/3)
29	48	Yes (Baker III)	0	Negative (3/3)
1	1	No	0	Negative (2/2)
2	3	No	0	Negative (1/1)
3	4	No	<20	CNS (1/2)
6	8	No	0	Negative (1/1)
8	11	No	<20	Negative (1/1)
9	13	No	<20	Negative (1/1)
10	14	No	<20	Negative (1/1)
10	15	No	0	Negative (1/1)
13	20	No	0	Negative (1/1)
13	21	No	CNS (20–50)	*P. acnes* + CNS (1/1)
21	32	No	<20	Negative (1/1)
21	33	No	<20	Negative (1/1)
22	34	No	0	Negative (1/1)
22	35	No	<20	Negative (1/1)
27	43	No	<20	Negative (1/1)
27	44	No	<20	Negative (1/1)
28	45	No	0	Negative (3/3)
28	46	No	<20	Negative (3/3)

[a] For capsular contracture cases, the Baker grade is indicated.
[b] ND, not done; CNS, coagulase-negative *Staphylococcus* species.
[c] Number of positive or negative tissue cultures/number of tissues harvested.

There is evidence from experimental animal studies that staphylococci accelerate the development of capsular contracture (12, 19). Four studies (2, 7, 17, 21) have reported culturing of explanted breast implants (Table 5). A statistically significant correlation between a positive culture and symptomatic capsular contracture was found in three of these studies (7, 17, 21), although Ahn et al. (2) did not find a significant association. However, none of these studies used appropriate techniques to sample and/or quantitate implant-associated biofilms; instead, conventional culture methods (2), methods inadequate to recover *P. acnes* (7, 21), and/or prolonged implant sonication (which, although targeting biofilms, may compromise subsequent cultures as a result of microbial killing from prolonged exposure to ultrasound) (17) were used. In addition, these studies analyzed tissue expanders along with the implants or cultured only a portion of the implant (2, 7, 17, 21). Finally, none of these studies yielded quantitative bacterial counts associated with implants; accordingly, some of these findings might represent contamination.

There is a need to define and standardize criteria for significant positive cultures in the setting of a removed breast implant. The bacteriological diagnosis of infection generally depends upon the isolation of a recognized pathogen from a clinical specimen, whose nature and quality affect the validity and utility of the culture results. In the case of implantable medical devices, it can be challenging to determine, simply from the identity of the organism, whether the isolated microorganism is clinically significant or a contaminant derived from

TABLE 3. Comparison of culture results between implants removed for capsular contracture and implants removed for other reasons[c]

Patient group (*n*)	No. (% [95% exact binomial confidence interval]) of patients			
	Any positive implant culture[a]	Any positive tissue culture	Significant positive implant culture[b]	Significant positive implant culture[b] plus any positive tissue culture detecting the same microorganism
Capsular contracture (27)	16 (59 [39–78])	12 (44 [25–65])	9 (33 [17–54])	5 (19 [6–38])
No capsular contracture (18)	11 (61 [36–83])	2 (11 [1–35])	1 (6 [0.1–27])	1 (6 [0.1–27])

[a] <20 CFU/10 ml sonicate fluid.
[b] ≥20 CFU/10 ml sonicate fluid.
[c] For comparisons between the patient groups, *P* values were 1, 0.023, 0.034, and 0.38 for patients with any positive implant culture, those with any positive tissue culture, those with significant positive implant culture, and those with significant positive implant culture plus positive tissue culture detecting the same microorganism, respectively.

the skin of the patient, the medical staff obtaining the sample, or the laboratory staff processing it. This is of particular importance in the case of breast implant capsular contracture, because the pathogenesis of this condition is not completely defined; our findings may represent associated infection, implant colonization, and/or implant contamination. It has been suggested that "it may be idealistic to think that there might be an absolutely sterile breast implant, considering the proximity of skin and its appendages, the richness of endogenous flora in the surgical field, and the ability of many of those bacteria to form biofilms" (21). Nevertheless, we have found statistically significant differences between implants being removed for capsular contracture and those being removed for other conditions, suggesting that the presence of a significant number of bacteria on a breast implant is not normal. We also found statistically significant differences between tissue cultures; however, in the capsular contracture group, there were only five (of nine cases) concordant cases (i.e., the same microorganism isolated in the implant and tissue cultures). The tissue culture results from this study are difficult to interpret because in most cases only a single piece of tissue was submitted for culture. *Propionibacterium* species were the microorganisms most frequently isolated from breast implants. In a recent study (13), *P. acnes* was found significantly more often among patients with prosthetic shoulder infection than among patients with prosthetic hip or knee infection, suggesting an anatomical link between *P. acnes* and implants.

We found that a substantial proportion of implants (64%) obtained from patients who were diagnosed with capsular contracture on the basis of clinical and histological criteria failed to yield organisms in a significant amount, despite an extended culture regimen that included an enrichment broth suitable for the recovery of many fastidious organisms. These data reinforce the potential multifactorial nature of this disease (8).

TABLE 4. Histopathological features for all implants removed

Histopathological feature	No. (%) of implants with feature	
	Capsular contracture group (*n* = 24)[a]	Non-capsular-contracture group (*n* = 12)[b]
Inflammation	13 (54)	4 (33)
Dense fibrosis	12 (50)	6 (50)
Foreign body giant cell reaction	11 (46)	2 (17)
Calcification	7 (29)	0
Foreign material consistent with silicone	6 (25)	4 (33)
Benign tissue	5 (21)	3 (25)
Suture granuloma	2 (8)	0

[a] Tissue histopathology was not done for three implants.
[b] Tissue histopathology was not done for six implants.

This study has a number of strengths that should serve to make its findings relevant and important to anyone interested in capsular contracture diagnosis, prevention, and treatment. Our study included semiquantitative assessment of biofilm bacteria on the surfaces of removed breast implants and, in most cases, capsule tissue cultures. Implant culture using vortexing-sonication has been shown to be an accurate diagnostic test for prosthetic hip and knee infection (20). This technique was applied in a standardized way to all study subjects herein, and we observed larger numbers of bacteria when sonicated fluid cultures were compared to tissue cultures. This is the first prospective observational study to assess a possible link of bacterial biofilms to the etiopathogenesis of capsular contracture by using a technology to sample and quantitate bacterial biofilms on the implant surface.

Nonetheless, this study has some limitations. Given the small sample sizes, we could not calculate odds ratios. However, we have included 95% exact binomial confidence intervals in Table 3. A challenge associated with implant culture is prevention of contamination. Sources of contamination include the skin of the patient, the surgeon performing the removal, and the laboratory personnel transporting or processing the sample. We tried to overcome this issue by defining an implant culture positivity cutoff of ≥20 CFU/10 ml sonicate fluid. Of the subjects who did not have ≥20 CFU/10 ml sonicate fluid isolated, 16/27 (59%) capsular contracture subjects and 11/18 (61%) non-capsular-contracture subjects had a sonicate fluid culture with <20 CFU/10 ml ($P = 1.0$), suggesting that low positive results represent contamination. Another limitation is that microorganisms such as mycobacteria, fastidious bacteria, and certain fungi would not have been detected using our culture media and conditions. The generalization of the findings may be limited to the population of women coming to Mayo Clinic because of a breast implant-related condition. This study represents an analysis of breast implants in women subjected to removal surgery and so may inherently be biased and not truly representative of the population of women with breast implants, because these were patients who presented for surgery, and non-capsular-contracture implants were not collected from a pool of asymptomatic patients. The ideal design to study the role of biofilms in women with breast implant capsular contracture should include a control group of women

TABLE 5. Review of microbiological studies on breast implant capsular contracture

No. of implants/ no. of patients	Microbiological methods	No. of implants with positive culture/total no. of implants (%)		Predominant isolated microorganism	Reference
		Capsular contracture group	Non-capsular-contracture group		
55/40	Prolonged incubation with continuous agitation of a portion of the implant	15/27 (55)	5/28 (18)	*Staphylococcus epidermidis*	21
150/87	Prolonged incubation with continuous agitation of a portion of the implant	62/82 (75)	19/68 (28)	*S. epidermidis*	7
139/72	Standard culture of a portion of the implant	7/66 (10)	7/73 (9)	*Propionibacterium acnes*	2
48/27	Sonication for 20 min of a portion of the implant or the tissue capsule	24/48 (50) (implants), 17/19 (89) (tissue culture)	1/8 (12)	*S. epidermidis*	17

without any problem related with the breast implants. Clearly, such a study would not be feasible since it would involve surgery for healthy patients who do not need it. Thus, our study design was the scientifically and ethically strongest approach available to test our hypothesis.

If bacteria contribute to and/or cause capsular contracture, new strategies to prevent and treat this devastating complication, targeted at the associated bacteria, will need to be studied. Local delivery of antimicrobial agents is an attractive potential approach. Darouiche et al. (5) reported a significant decrease in the rates of contracture in a rabbit model when silicone implants impregnated with minocycline-rifampin were tested. Two studies (1, 4) have assessed the efficacy of local antibacterial agents (e.g., povidone-iodine, cephalothin, bacitracin, cephalexin, and gentamicin) in patients undergoing breast implant procedures. Such intervention was associated with a lower incidence of capsular contracture than those in other published reports not using antimicrobial irrigation (9, 10), indirectly suggesting that bacteria may be involved in the pathogenesis of capsular contracture.

In summary, bacteria are more frequently found on breast implants removed from women with capsular contracture than on breast implants removed from women with other causes of breast implant failure. The role of these bacteria in the pathogenesis of capsular contracture of breast implants deserves further study.

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R. Patel has an unlicensed U.S. patent pending for a method and an apparatus for sonication but has foregone her right to receive royalties in the event that the patent is licensed.

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