Do Bacteria and Biofilm Play a Role in Double-Capsule Formation around Macrotextured Implants?

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Background: The double capsule is a complication mostly described in aggressive macrotextured implants. Mechanical shear stress applied onto an immature periprosthetic capsule has been linked to their formation. The authors aim to demonstrate the role of bacterial phenotype and biofilm in the development of the double capsule.

Methods: Seven double capsules formed at the interface of macrotextured breast expander implants were studied using scanning electron microscopy. Two samples for each surface of the inner capsule layer (the prosthesis interface and the intercapsular space) were analyzed for bacteria cell size, bacterial density, and biofilm deposition.

Results: Although all routine bacterial cultures were negative, the prosthesis interface had both higher bacteria load and biofilm deposition compared with the intercapsular space (Mann-Whitney *U* test, $p = 0.004$ and $p = 0.008$, respectively). Moreover, bacteria cell sizes were significantly smaller at the prosthesis interface in six of seven samples. Comparison of bacteria density and biofilm dispersion showed an increase of biofilm extracellular matrix deposition over 2000 cells/mm^2 (linear regression, $p = 0.0025$). These results indicate a common trend among bacteria species.

Conclusions: Bacterial expression between the different surfaces of the double capsule displays significant differences; bacteria at the prosthesis interface are mostly in a biofilm state, whereas they demonstrate a planktonic phenotype at the intercapsular space. When a sufficient amount of bacteria are present at a specific location, quorum sensing may trigger a biofilm phenotypic switch in planktonic bacteria cells. Biofilm formation may alter capsule formation through immune response, thereby weakening capsule strength and facilitating extracellular matrix delamination and double-capsule formation. (*Plast. Reconstr. Surg.* 140: 878, 2017.)

CLINICAL QUESTION/LEVEL OF EVIDENCE: Therapeutic, V.

Double-capsule formation around textured breast implants refers to the development of two distinct fibrous layers around an implant separated by an intercapsular space. This recently described phenomenon was the first in a string of manifestations largely associated with macrotextured implants; subsequently, cases of late seroma and breast implant–associated anaplastic large-cell lymphoma were also described in the literature.[1–5] Our team recently demonstrated

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evidence of mechanical delamination within the capsule itself and showed that early inflation of macrotextured expander implants results in a higher incidence of double capsule.[6,7] It was also found that the double-capsule phenomenon largely occurred in the absence of any particular clinical expression.

Although the pathophysiology has yet to be fully elucidated, multiple hypotheses have been proposed. Although mechanical influences may be the predominant drivers of double-capsule formation, we cannot exclude involvement of biofilm. We hypothesize that bacterial contamination contributes to double-capsule development and that it is more likely found at the prosthesis interface. The aim of this study was to evaluate, for the first time, the presence of different bacterial phenotypes and associated biofilm within the different surfaces and compartments of the double capsule using scanning electron microscopy.

PATIENTS AND METHODS

Patients with breast cancer undergoing two-stage implant-based breast reconstruction using Biocell 133-MV implants (Allergan, Inc., Dublin, Ireland) were prospectively included. The institutional review board approved the protocol and written consent was obtained from all patients. Our research team is guided by the ethical principles regarding research involving human subjects and has respected the principles set forth in the report of the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (the "Belmont Report") throughout the course of this research. Our team also adhered to the rules and regulations of its hospital center ethics board (Centre Hospitalier de l'Université de Montréal Ethics Committee), which approved this study. During the first stage, a partially inflated expander was placed underneath the thoracic muscles and serially inflated with saline postoperatively. At the time of expander removal, the surgeon macroscopically assessed the presence of double capsule; two representative samples for each surface of the inner capsule layer (the prosthesis interface and intercapsular space) were then harvested using a validated methodology.[6] Scanning electron microscopic observations were made from 400× to 3000× magnification on each sample to assess capsule texture, bacterial density (cells per square millimeter), bacterial cell size, and biofilm quantification (Van Heerden score).[8] The "Velcro effect" was evaluated in all cases, and routine microbiological analyses were performed.[9,10]

Statistical analyses were performed using the Mann-Whitney *U* test for non-Gaussian distributions and the *t* test for Gaussian distribution. One-way analysis of variance was used to compare multiple columns, followed by a Tukey posttest. For all statistical tests, the α risk was defined at 5 percent.

RESULTS

Of the 40 patients prospectively enrolled in the study, seven patients with macroscopic double capsule were identified and included for further analysis as described. These latter patients had a mean age of 50 years (range, 41 to 58 years), and the delay before the first postoperative inflation was 4 ± 2.7 weeks. Two specimens per breast were collected from the prosthesis interface and from the intercapsular space aspect of both the inner and outer capsule layers for subsequent scanning electron microscopic analysis. The clinical Velcro effect was noted in all cases; all routine microbiological analyses were negative. [**See Figure, Supplemental Digital Content 1**, which shows scanning electron microscopic expression of double capsule. (*Above, left*) Prosthesis interface with peeled inner capsule from an en bloc intraoperative sample. (*Above, right*) Inner capsule demonstrating negative imprint of prosthesis texture which manifests clinically as the Velcro effect of macrotextured prosthesis. (*Below*) Intercapsular space from two distinct capsular samples showing red and white human cells and bacteria, ***http://links.lww.com/PRS/C386***.]

During scanning electron microscopic analysis, 2383 spherical objects measuring 0.56 to 7.5 μm in diameter were identified, presenting a bimodal distribution. The lower limit of the larger cell group was evaluated at 4 μm (mean ± SD, 6.2 ± 1.7 μm). To differentiate between bacterial cells and human cells, the upper threshold for bacterial cell size was set at 4.5 μm (2 SD below the mean of the larger cell group). Bacteria cell size followed a normal distribution when transformed by the natural logarithm. [**See Figure, Supplemental Digital Content 2**, which shows an analysis of spherical objects found on capsular samples. (*Above, left*) Quantile-quantile plot (*above, right*) and frequency graph of all objects. (*Center, left*) Quantile-quantile plot (*center, right*) and frequency graph of objects filtered to exclude 97 percent of human cells. (*Below, left*) Quantile-quantile plot (*below, right*) and frequency graph of natural logarithm of objects filtered to exclude 97 percent of human cells, ***http://links.lww.com/PRS/C387***.]

Analysis of bacteria size revealed a discrepancy between patients at the prosthesis interface location. A smaller cell size for samples from patients 4, 5, and 6 and larger size for patients 1, 2, 3, and 7 were found, thereby representing distinct bacteria strains (analysis of variance, $p < 0.0001$) (Fig. 1).

A larger amount of bacteria was identified on the prosthesis interface surface of the inner capsule layer when compared to the intercapsular space aspect, as evaluated by the bacterial density (Mann-Whitney *U* test, $p = 0.0039$) and the biofilm spreading (Mann-Whitney *U* test, $p = 0.0079$) (Fig. 2).

For each sample, comparison of bacteria size (based on capsular location), revealed a smaller mean cell size at the prosthesis interface for subjects 2, 5, and 6; the same trend was noted in samples from patients 3, 4, and 7. On the contrary, the first patient displayed a smaller bacteria size at the intercapsular space (not significant). [**See Figure, Supplemental Digital Content 3**, which shows an individual comparison of spherical objects size. Either the *p* value of the *t* test is reported or denoted as not significant. *PI*, prosthesis interface; *ICS*, intercapsular space, ***http://links.lww.com/PRS/C388***. **See Figure, Supplemental Digital Content 4**, which shows a side-by-side comparison of two subjects that are representative of the study. (*Above*) Subject 5. (*Below*) Subject 7. (*Left*) Intercapsular space. (Right) Prosthesis interface. Bacteria cells are significantly more represented at the prosthesis interface than at the intercapsular space interface, ***http://links.lww.com/PRS/C389***.] A comparative analysis of bacterial density and biofilm scores demonstrated a significant correlation between both variables, with a rapid increase in biofilm production above a bacterial density of 2000 cells/mm² ($p = 0.0025$) (Fig. 3).

DISCUSSION

Despite negative routine microbiological cultures, the presence of bacteria on breast capsules has consistently been demonstrated by scanning electron microscopic analysis. Our results indicate that in the setting of double capsules, bacterial density and biofilm scores are significantly greater on the prosthesis interface aspect of the inner capsule compared with its intercapsular space–facing surface. Furthermore, there is an observable trend of smaller bacteria size at the prosthesis interface compared with the intercapsular space, which is further reflected by the reduced biofilm at the intercapsular space. The phenotypic state of bacteria cells varies by surface of the inner capsule layer in our scanning electron microscopic observations, reflecting a vegetative phenotype at the prosthesis interface and planktonic cells at the intercapsular space. This finding suggests that bacterial contamination occurs during initial implant placement. Allan et al. inoculated six breast implants inserted into a porcine model with 10^6 *Staphylococcus epidermidis*. Two of the implants were noted to have double capsule with associated biofilms, which led the authors to suggest that chronic infection with a bacterial biofilm may play a significant role in double-capsule development.[11]

The inability of swab cultures to detect bacteria in collected samples may result from several

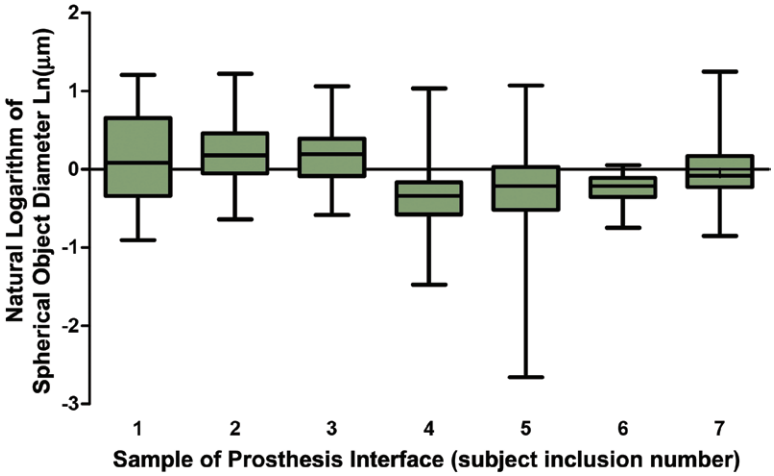

Fig. 1. Comparison of bacteria size of different samples at the prosthesis interface (natural logarithm transformation of bacteria size; the analysis of variance test is very significant, $p < 0.0001$).

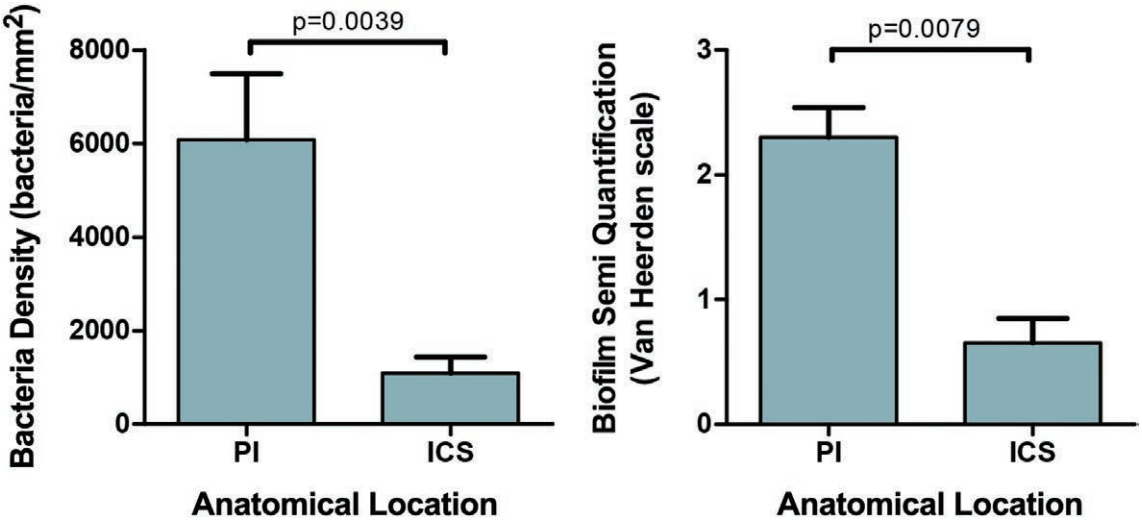

Fig. 2. Comparison of bacteria density (*left*) and biofilm coverage (*right*) according to the Van Heerden scale at the prosthesis interface (*PI*) and intercapsular space (*ICS*).

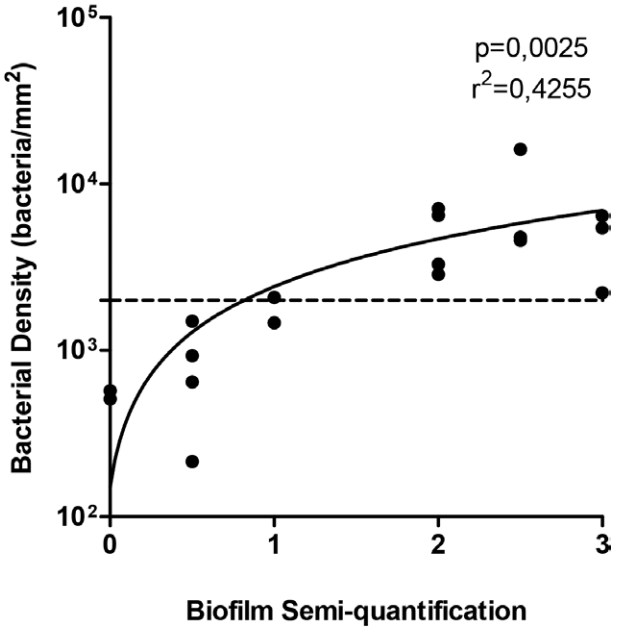

Fig. 3. Relationship between bacterial density and biofilm coverage. The 2000 cells/mm^2 is a visual limit between lowly and highly spread biofilms.

factors.[12,13] This limitation has been previously discussed in the literature in a variety of clinical settings.[14–16] Biofilm formation around implanted medical devices is nearly ubiquitous and has been associated with significant morbidity and mortality.[17,18] Following irreversible binding of bacterial cells to the implant surface, cellular proliferation and extracellular matrix formation result in the creation of biofilm. This matrix acts as a shelter for bacterial colonies, providing protection against host immune responses.[19–21] Implant texture appears to have a primary role in biofilm formation. Macrotextured implants have been shown to carry a higher load of bacteria when compared to smooth implants.[22–24]

Our scanning electron microscopic analyses demonstrate higher bacterial deposition and biofilm presence at the prosthesis interface surface of the inner layer of the double capsule in clinically noninfected macrotextured Biocell devices, thus representing a quiescent infection.[25] Our study further shows a quantifiable increase in biofilm production when bacterial density exceeds 2000 cells/mm^2. This threshold could be an indicator of quorum sensing, a natural cell signaling system used by bacteria to quantify their own metabolism and that of other bacterial cells in the local environment.[26] When the pathway is activated, bacteria cells express genes associated with cell proliferation and toxin production, among others. We hypothesize that bacteria cells are released into the intercapsular space as planktonic cells and may develop into biofilm when a population threshold is attained.

Bacteria within biofilms exhibit unique phenotypic characteristics compared with their counterparts in the planktonic state, including increased resistance to antibiotics and host immune responses. *S. aureus* and *S. epidermidis*, two of the most commonly identified bacteria in breast capsule tissue, have evolved mechanisms to alter the innate immune responses of keratinocytes and also fine-tune their secretome in relation to their phenotypic state.[27–29] For example, pyrazinones such as tyrvalin, phevalin (also

known as aureusimine A and B, respectively), and leuvalin have been found in staphylococci and in numerous Gram-positive bacteria, suggesting a constitutive gene of bacteria found within the skin microbiota. The secretion of these peptides is augmented in biofilm phenotypes of *S. aureus*. Notably, in the skin, these peptides are implicated in epithelial and phagocyte cell escape from phagosomes, leading to bacterial cell stabilization within the cutaneous tissue.[30]

Another potential pathway for host tissue modulation by skin microbiota is through the alteration of both human transcription factor expression and cytokine expression, among others. This phenomenon was demonstrated by *S. aureus*–produced biofilms which, when compared to planktonic *S. aureus*, significantly reduced human keratinocyte viability and increased human keratinocyte apoptosis.[31] Also, *S. aureus* mutants, lacking the toxin associated with the accessory gene regulator virulence gene, are able to tweak the internal clearance mechanism of keratinocytes by activating autophagy, which in turn reduces inflammasome activation, reduces caspase-1 activation and, finally, results in their phagocyte-mediated destruction.[32]

Thus, using a wide variety of pathways, commensal bacteria of the skin are able to evade innate and adaptive immunity. These mechanisms are also activated within the breast capsule, where these bacteria originating from the skin microbiota use the same mechanisms to alter their environment for survival. Periprosthetic capsule strength may therefore be weakened, thus facilitating extracellular matrix delamination and the development of double capsule. Planktonic bacteria cells released by the mature biofilm are most probably found at the intercapsular space secondarily, where they can potentially trigger an infection and/or manifest as late seromas.

CONCLUSIONS

The authors investigated the association of different bacterial phenotypes and biofilm in double-capsule formation around macrotextured Biocell breast expander implants. Of 40 prospectively enrolled patients undergoing two-stage implant-based breast reconstruction, seven were found to have macroscopic presence of double capsule at the time of expander-to-implant exchange surgery. Two representative capsule specimens were collected per breast from the prosthesis interface and intercapsular space aspects of both the inner and outer capsule layers intraoperatively. Analyses for capsule texture, bacterial density (cells per square millimeter), bacterial cell size, and biofilm quantification (Van Heerden score) were conducted using scanning electron microscopy. Although all routine microbiological cultures were negative, the prosthesis interface had a higher bacterial load and biofilm deposition than the intercapsular space. A trend toward smaller bacterial size was noted at the prosthesis interface. There was a correlation between bacterial density and biofilm, with a rapid increase in biofilm production above a density of 2000 cells/mm^2 (p = 0.0025). The results suggest that bacteria cells are released from the prosthesis interface to the outer surface of the original single-layer breast capsule as planktonic cells that subsequently, triggered through quorum sensing, undergo a phenotypic switch, enabling biofilm production. Biofilm may weaken periprosthetic capsule strength, thereby facilitating extracellular matrix delamination and double-capsule formation.

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