The Impact of Postoperative Expansion Initiation Timing on Breast Expander Capsular Characteristics: A Prospective Combined Clinical and Scanning Electron Microscopy Study

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Background: In the first stage of expander-to-implant breast reconstruction, postoperative expansion is classically initiated at 10 to 14 days (conventional approach). The authors hypothesized that it may be beneficial to wait 6 weeks postoperatively before initiating serial expansion (delayed approach). Clinical and ultrastructural periprosthetic capsule analysis is first required before determining whether a delayed approach ultimately improves capsular tissue adherence and expansion process predictability.
Methods: Patients undergoing two-stage implant-based breast reconstruction were enrolled prospectively in this study. During expander-to-implant exchange, the clinical presence of "Velcro" effect, biofilm, and double capsule was noted. Periprosthetic capsule samples were also sent for scanning electron microscopic observation of three parameters: surface relief, cellularity, and biofilm. Samples were divided into four groups for data analysis (group 1, conventional/Biocell; group 2, delayed/Biocell; group 3, conventional/Siltex; and group 4, delayed/Siltex).
Results: Fifty-six breast reconstructions were included. Each group comprised between 13 and 15 breasts. In group 1, no cases exhibited the Velcro effect and there was a 53.8 percent incidence of both biofilm and double capsule. In group 2, all cases demonstrated the Velcro effect and there were no incidences of biofilm or double capsule. Group 3 and group 4 cases did not exhibit a Velcro effect or double-capsule formation; however, biofilm was present in up to 20.0 percent. All group 2 samples revealed more pronounced three-dimensional relief on scanning electron microscopy.
Conclusions: Variations in expansion protocols can lead to observable modifications in periprosthetic capsular architecture. There may be real benefits to delaying expander inflation until 6 weeks postoperatively with Biocell expanders. (*Plast. Reconstr. Surg.* 135: 967, 2015.)

Two-stage expander-to-implant breast reconstruction is the most commonly used technique for postmastectomy breast reconstruction. According to the American Society of Plastic Surgeons 2012 Statistics Report, 70.5 percent of breast reconstructions were achieved using expanders.

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To the best of our knowledge, nearly all two-stage implant-based breast reconstruction outcome studies available in the literature are based on patients undergoing tissue expansion according to the current conventional approach, with initiation at 10 to 14 days postoperatively.[1,2] We hypothesize that there are benefits to waiting at least 6 weeks before initiation

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of postoperative serial expansion because this may permit improved capsular tissue adherence into the textured expander implant shell and, ultimately, lead to a safer and more predictable expansion process. The rationale for such a delayed approach rests partly on the principles evoked by Levenson et al. in their classic experiments on wound healing—the most rapid gain in wound strength takes place over the first 42 days,[3] at which point the wound has roughly 70 percent of the tensile strength of normal skin and net collagen synthesis has ceased.[4] These principles may reasonably be applied to periprosthetic capsule evolution. According to Kronowitz, a mature scar capsule requires at least 4 weeks to form.[5] Other authors note that a longer overall expansion process leads to enhanced capsule maturation and tissue adherence, resulting in a softened, relaxed state of the expanded tissue envelope.[6] A combined clinical and ultrastructural evaluation of periprosthetic capsular characteristics following both conventional and delayed expansion approaches is necessary to warrant future clinical outcome comparison studies.

Scanning electron microscopy is a powerful tool that has been applied extensively in the study of periprosthetic capsules. Previous studies have used conventional high-vacuum scanning electron microscopy, which necessitates a drying and metallization process, to define implant surface characteristics and their effects on corresponding periprosthetic capsular tissue; findings included the demonstration of a capsular "Velcro" effect with Biocell textured implants.[7–9] Our group has recently demonstrated that conventional high-vacuum scanning electron microscopy is superior to newer environmental scanning electron microscopy technology for the assessment of breast periprosthetic capsules, despite the ability to directly examine wet, nonconductive biological tissue samples with the latter. High-vacuum scanning electron microscopy allows excellent assessment of capsule three-dimensional relief, cellularity, and biofilm presence.[10]

In this study, we aim to prospectively investigate, using conventional high-vacuum scanning electron microscopy, whether differing expansion protocols lead to observable modifications in capsular formation around both Biocell (Allergan, Inc., Irvine, Calif.) and Siltex (Mentor Worldwide, Santa Barbara, Calif.) expander prostheses. Intraoperative observations regarding expander periprosthetic capsular adhesiveness and biofilm presence will equally be considered.

PATIENTS AND METHODS

Patients with breast cancer undergoing two-stage implant-based breast reconstruction were prospectively included in this study before second-stage expander-to–permanent implant exchange surgery. All included patients were treated at the same university hospital center by one of five plastic surgeons specializing in breast reconstruction. Each surgeon adhered to his or her usual standard surgical technique. Prophylactic antibiotics were administered at induction (first-generation cephalosporin or, if allergic, clindamycin). Skin preparation was performed using the standard solution of chlorhexidine with alcohol. Dissection of the subpectoral pocket was performed with electrocautery. Subpectoral fascia and serratus muscle were elevated to provide lower pole coverage and, thereby, total submuscular coverage. Before insertion into the submuscular breast pocket, all expander implants and submuscular breast pockets were bathed and irrigated with bacitracin solution, respectively. Muscle closure was performed with absorbable suture. Skin closure was performed in two layers with absorbable sutures. All surgeons, with one exception, installed Jackson-Pratt drains (submuscular and subcutaneous planes) as part of their approach. All surgeons also followed their own respective postoperative management approach—specifically, timing of first postoperative expander inflation—over the course of this study.

Baseline demographic data were collected for all included patients and medical charts were reviewed for pertinent risk factors, including radiotherapy status. Regarding the first-stage expander insertion procedure, the following variables were documented: type of mastectomy, expander prosthesis model and size, type of implant coverage, incidence of drain insertion, and volume of intraoperative expander filling. The timing of first postoperative saline inflation, total duration of the expansion process (from first to last saline inflation), final expansion volume, and first-stage complications were equally noted.

During the second-stage expander-to–permanent implant exchange operation, the surgeon documented the presence or absence of a clinically observable Velcro effect, double capsule, and biofilm in the periprosthetic capsular tissue. The Velcro effect was considered positive when the capsule was adherent to the implant surface in such a way that it required forceps to peel it off, thus simulating the feel of separating two actual Velcro (Velcro Industries, Amsterdam, The Netherlands)

surfaces apart. Double capsules were characterized as two distinct capsular layers: a partial or complete inner layer in contact with the implant surface and an outer layer in contact with the surrounding breast tissue. Biofilm was considered clinically positive when a slimy, reflective layer of film was visualized within the implant/capsule interface.

A 1-cc sample of periprosthetic capsule adjacent to the prosthesis dome was then submitted to biopsy; the implant side of the sample was subsequently tagged with a suture. Notably, for cases demonstrating double-capsule formation, the outer capsule was the one submitted to biopsy; the inner surface was subsequently tagged with a suture as described above. Capsule samples were then fixed in a solution of glutaraldehyde 2% and sodium cacodylate 0.1 M to stabilize the cellular structures at a pH of 7.3. All samples were then stored in a refrigerator at 4°C for a minimum of 24 hours. Capsule samples were analyzed gradually as the study progressed.

In preparation for analysis under scanning electron microscopy in high-vacuum mode, a 3 × 3-mm portion of each sample was cut, cleaned, and subjected to a 20-minute air-drying process. Gold coating was then performed using an Agar manual sputter coater (Agar Scientific, Essex, United Kingdom). Sample analysis under scanning electron microscopy was performed in blinded fashion on the tagged implant side of the capsule specimens and the following three parameters were assessed: texture (surface relief characterization), cellularity (cell count and characterization), and biofilm (presence or absence), as described previously by our team.[10] Energy dispersive x-ray microanalysis was conducted in all samples for chemical element composition measurements. All observations were performed using a Quanta 200 FEG microscope (FEI, Hillsboro, Ore.) with energy dispersive x-ray analysis detector and XT Docu (FEI) image-acquisition software. All samples were studied under high-vacuum modality with magnifications of 100×, 400×, 1600×, and 3000×, and collected images were subsequently assembled in Adobe Photoshop CS6 Extended (Adobe Systems, Inc., San Jose, Calif.).

For sample texture three-dimensional relief analysis, capsular peak-and-trough dimensions were measured using the Imagan2 computerized image analysis system (Kompira, Strathclyde, United Kingdom). Microscopic fields were examined with a 100× phase-contrast objective for capsule structural peaks and troughs and 50× objective for density. The fields were then observed with a video camera and corresponding images were displayed on the connected monitor. The maximum diameters, heights/depths, and densities were computed for 100 randomly selected peak or trough points in each capsule sample. A binary pattern description was subsequently assigned to all analyzed samples, as either a minimal-texture (flat/linear fibrotic) or a high-texture (rough/nonlinear) pattern. All cell counts were performed manually at 100× magnification and densities were determined using the scales provided by the image acquisition software. Biofilm was deemed positive when a characteristic acellular layer covering areas of capsule cellular and fibrotic components was identified. For analysis purposes, studied patient samples were subsequently categorized into four distinct groups, constituted on the basis of expander prosthesis type and time delay until the first postoperative saline inflation:

Group 1: Allergan Biocell, first postoperative expansion at 2 weeks or less (conventional approach).
Group 2: Allergan Biocell, first postoperative expansion at 6 weeks or more (delayed approach).
Group 3: Mentor Siltex, first postoperative expansion at 2 weeks or less (conventional approach).
Group 4: Mentor Siltex, first postoperative expansion at 6 weeks or more (delayed approach).

RESULTS

A total of 48 patients were included in this study, for a total of 56 breast reconstructions; there were 40 unilateral and eight bilateral reconstructions. Each of the four study groups comprised between 13 and 15 breast reconstructions. Patient demographic and clinical data were comparable between groups (Table 1). All radiotherapy treatment, when applicable, occurred before expander insertion. All patients had undergone skin-sparing mastectomies; there were no cases of nipple-sparing mastectomies. Implant coverage was total submuscular in all cases. No acellular dermis products were used in this study. Other pertinent clinical data regarding first-stage operative and postoperative management details are summarized in Table 2.

Thorough scanning electron microscopic analysis of each group of samples revealed observable differences with regard to the aforementioned study parameters. Scanning electron microscopic observations were correlated with clinical findings

Table 1. Demographic and Clinical Data, by Group

Group	No. of Breast Reconstructions	Mean Age (yr)	Diabetes (%)	Active Smokers (%)	Past Chemotherapy (%)	Past Radiotherapy (%)
1	13	55.6	7.7	23.1	23.1	23.1
2	15	55.1	6.7	26.7	20	26.7
3	15	57.3	0	13.3	20	20
4	13	55.5	0	15.4	23.1	23.1

Group 1, Biocell, first postoperative expansion at 2 wk or less; group 2, Biocell, first postoperative expansion at 6 wk or more; group 3, Siltex, first postoperative expansion at 2 wk or less; group 4, Siltex, first postoperative expansion 6 wk or more.

Table 2. First Stage and Expansion Process Clinical Data, by Group

Group	Mean Expander Prosthesis Volume (ml)	Incidence of Drain Insertion (% of breasts)	Mean Intraoperative Expansion (% of capacity)	Final Expansion Volume (% of capacity)	Mean Delay until First Postoperative Expansion (days)	Mean Interval between First and Last Postoperative Expansion (days)	Overall Complication Rate (%)
1	444	84.6	28.3	112.4	13	81.8	7.7
2	400	86.7	28.5	103.4	52	80	13.3
3	390	86.7	30	102.7	13.5	72.3	13.3
4	450	84.6	31.8	101.1	51.2	69.9	7.7

Group 1, Biocell, first postoperative expansion at 2 wk or less; group 2, Biocell, first postoperative expansion at 6 wk or more; group 3, Siltex, first postoperative expansion at 2 wk or less; group 4, Siltex, first postoperative expansion 6 wk or more.

made during second-stage expander-to-implant exchange surgery.

In group 1, there were no clinically observable cases of the Velcro effect. This clinical finding was well corroborated by scanning electron microscopic observations; there was minimal three-dimensional texture in group 1 samples (Fig. 1, *left*) compared with group 2 samples (Fig. 1, *right*). Furthermore, cellularity was minimal and energy dispersive x-ray analysis revealed silicone in the capsular tissue. In 53.8 percent of breast capsules, a biofilm was clinically identifiable and confirmed under scanning electron microscopy (Fig. 2). Importantly, seven cases of double capsule (53.8 percent) were noted intraoperatively, associated with the same cases possessing a biofilm (Fig. 3).

In group 2, all cases exhibited a clinical Velcro effect, and a high level of three-dimensional texture was noted in the corresponding capsular tissue samples under scanning electron microscopy (Fig. 1, *right*). A high cellularity was observed in group 2 samples, with over 40 cells per 40 μm^2; up to 45 percent of cells had features consistent with those of echinocytes, which are erythrocytes with modified cellular membranes. Importantly, no biofilms were identifiable by the surgeon at the time of expander-to-implant exchange in these

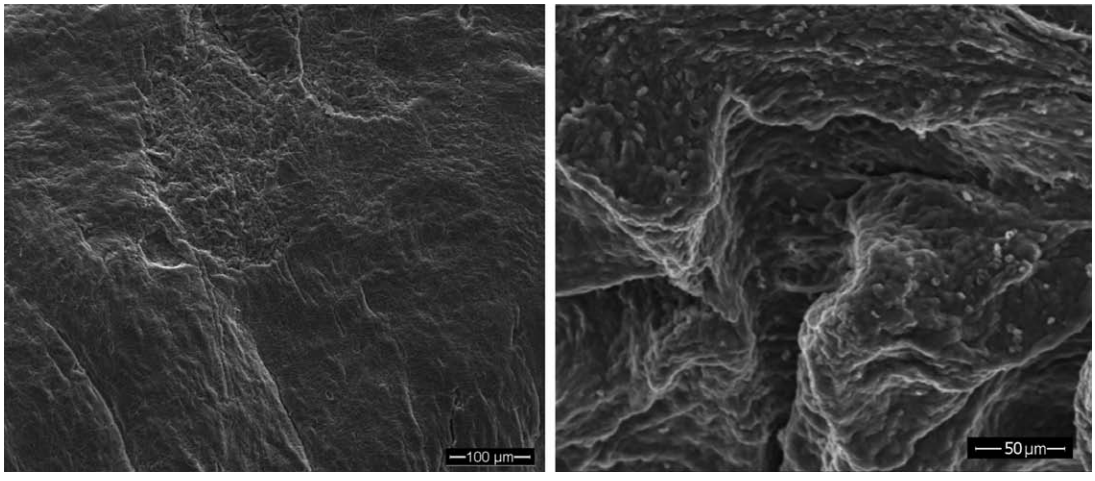

Fig. 1. (*Left*) Example of minimal three-dimensional capsule relief in a conventional Biocell sample (group 1). (*Right*) Example of pronounced three-dimensional capsule relief in a delayed Biocell sample (group 2).

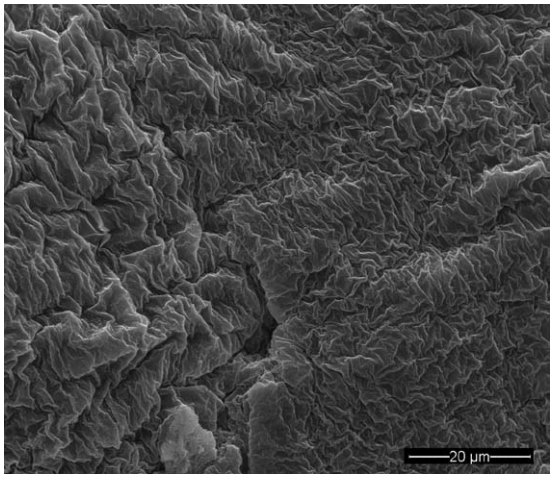

Fig. 2. Biofilm presence on the inner surface of capsule tissue in a conventional Biocell sample (group 1).

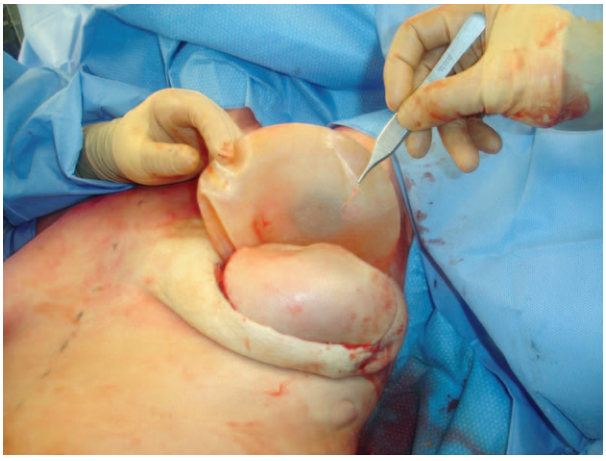

Fig. 3. Demonstration of a double capsule around a Biocell implant from the conventional group (group 1). The inner adherent capsule is being grasped with the forceps.

cases; the subsequent scanning electron microscopic analyses confirmed this lack of biofilm. No cases of clinical double capsule were observed intraoperatively.

In group 3, no cases of clinical Velcro effect were noted. On scanning electron microscopy, texture was minimal and revealed linear fibrotic patterns (Fig. 4, *left*). Scanning electron microscopic observations also revealed low cellularity with fewer than five cells per 40 μm^2. There were three cases of clinical biofilm in group 3 samples (20.0 percent), and this was consistent with parallel scanning electron microscopic observations. No cases of clinical double capsule were observed intraoperatively.

In group 4, there continued to be an absence of clinically observable Velcro effect in the samples. On scanning electron microscopy, texture level and cellularity remained low (fewer than five cells per 40 μm^2) and were comparable to group 3 samples in these respects (Fig. 4, *right*). Biofilm was observed in two breast capsules intraoperatively (15.4 percent); again, these findings were corroborated by subsequent scanning electron microscopic analyses (Fig. 5). No cases of clinical double capsule were observed intraoperatively. Clinical intraoperative observations for all groups are summarized in Table 3.

Overall first-stage complication rates were comparable between the groups and ranged from 7.7 to 13.3 percent. Complications included delayed wound healing, infection, and seroma. There were no cases of implant exposition or premature expander removal in this series.

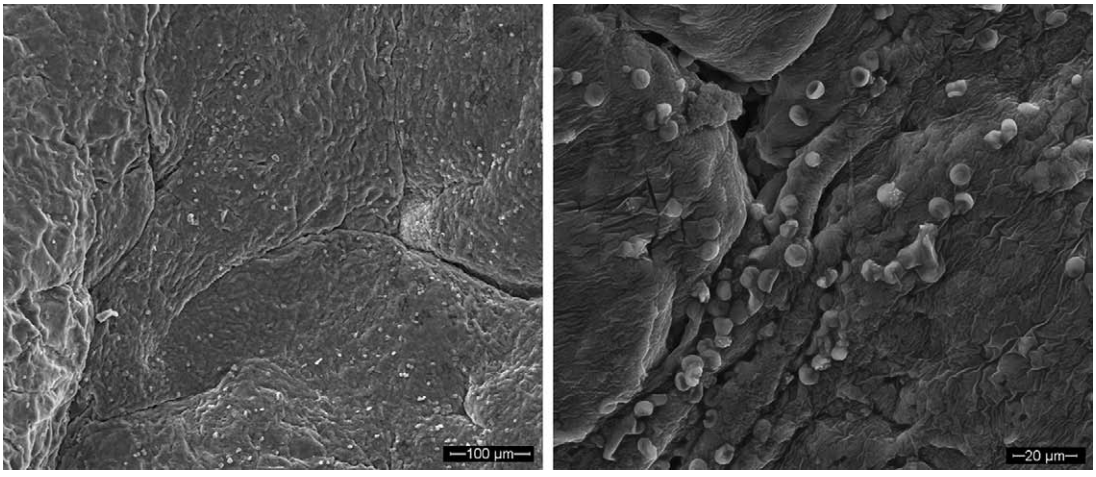

Fig. 4. (*Left*) Example of linear fibrotic pattern in a conventional Siltex sample (group 3). (*Right*) Example of a periprosthetic capsule in a delayed Siltex sample (group 4). Three-dimensional capsule relief was comparable to that observed in group 3 samples.

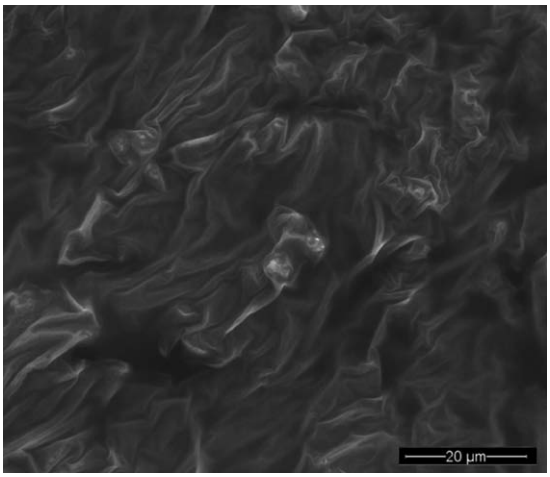

Fig. 5. Example of biofilm in a delayed Siltex sample (group 4).

Table 3. Summary of Intraoperative Clinical Observations, by Group

Group	Velcro Effect (%)	Biofilm (%)	Double Capsule (%)
1	0	53.8	53.8
2	100	0	0
3	0	20	0
4	0	15.4	0

Group 1, Biocell, first postoperative expansion at 2 wk or less; group 2, Biocell, first postoperative expansion at 6 wk or more; group 3, Siltex, first postoperative expansion at 2 wk or less; group 4, Siltex, first postoperative expansion 6 wk or more.

DISCUSSION

The Mentor Siltex texturing is a patterned surface created as a negative contact imprint off of a texturing foam. In contrast, the Allergan Biocell surface is a more aggressive open-pore textured surface created using a lost-salt technique, with the elastomer shell being placed on a bed of finely graded salt and subsequently exposed to light pressure. The latter manufacturing process increases the depth of the depressions and also creates a stilted edge (Table 4). The scanning electron microscopic analyses reveal noteworthy ultrastructural differences between the conventional and delayed Biocell groups (groups 1 and 2). In the delayed group (group 2), the capsule texture three-dimensional surface relief was notably more pronounced (rough/nonlinear); therefore, on an ultrastructural level, the capsules exhibited features compatible with superior tissue adherence, which was corroborated clinically by the respective surgeons' intraoperative observations (only group 2 capsules exhibited the Velcro effect). This may imply that a longer healing and evolution period for the periprosthetic capsule before postoperative expansion initiation is necessary for a more stable scar response, and may potentially lead to better predictability of the expansion process. In the conventional and delayed Siltex groups (groups 3 and 4), no discernible differences in the capsular architecture, characterized by a linear fibrotic pattern and lack of ingrowth into the expander implant surface, were observed. The consistent finding of linear fibrosis associated with Siltex texturing is nothing new or surprising. Previous authors have established that a critical pore size is necessary to accommodate tissue ingrowth into textured silicone implants and other surfaces.[11–14] Studies in plastic surgery have not demonstrated features of overt capsular ingrowth or clinical adherence in Siltex implants, which lack the porous features of the more aggressively textured Biocell textured surface.[7–9,15] Because this study focuses solely on the first stage of expander-to-implant breast reconstruction, it is not possible to ascertain the ultimate clinical impact of the more pronounced three-dimensional texture relief observed in group 2 (delayed Biocell) samples with respect to complication rates and aesthetic outcomes. However, our overall first-stage complication rates were similar among the four groups studied and comparable to rates of 8.5 to 11 percent cited in the literature.[16,17]

The finding of double capsules in the conventional Biocell group (group 1) deserves special mention. Maxwell et al. define a "double capsule" to be capsular adherence in two layers (inner adherent to device, and outer adherent to surrounding tissue). The authors go on to list the possible causes of this presentation: foreign body reaction, oversized breast implant pockets, micromotions, mechanical shear, trauma, and infection/biofilm.[18] In a large complications review of breast augmentation and mastopexy-augmentation cases, Hall-Findlay reports findings of double capsule in 14 patients who underwent reoperation

Table 4. Overview of Textured Implant Surface Ultrastructural Topography

Implant	Ultrastructural Texture Feature	Diameter (μm)	Height or Depth (μm)	Density/ 1.5 mm^2
Biocell	Depressions	600–800	Depth, 150–200	8
Siltex	Nodules	70–150	Height, 40–100	15

with Biocell textured permanent implants, with three cases presenting in the context of late seromas. Hall-Findlay proposes a mechanical cause, suggesting that the double capsule results from incomplete adherence of the capsule, with subsequent serous fluid production secondary to shear forces at the implant-capsule interface; seeding of cells from the seroma then leads to development of a new distinct inner capsule.[19]

The senior author's (M.A.D.) belief is that to truly optimize the use of the aggressively textured Biocell expanders in breast reconstruction, a longer delay before initiating postoperative saline inflation is desirable because it allows sufficient maturation and adherence of the developing capsule, as demonstrated clinically and ultrastructurally in our study. We observed seven cases of double capsule in the conventional Biocell expansion group (group 1); we believe that this finding indicates that the immature capsule, which we were able to appreciate under scanning electron microscopy as being minimally textured (flat/linear fibrotic pattern), is at higher risk of separating from the implant expander shell, thereby creating a potential space with fluid production caused by mechanical shear forces. Consequently, a partial or complete new adherent inner capsule develops. In the specific context of expander implants described here, shear stresses and micromotions caused by expander inflation most probably provoked this separation.

A related question that needs to be addressed is what role, if any, does biofilm play in the development of double capsules? Interestingly, Hall-Findlay reports the finding of a *Staphylococcus epidermidis* biofilm within a double capsule sample sent for scanning electron microscopic analysis. In our series, biofilms were observed in all the double capsule cases. Importantly, none of our patients had any evidence of either infection or seroma intraoperatively during expander-to-implant exchange. We theorize that biofilms are simply associated with double capsule formation. Weaver et al. conducted a thought-provoking in vitro study of biofilms in a model meant to replicate the catheter microenvironment; they demonstrate that fluid shear stress induces biofilm formation in certain strains of *Staphylococcus epidermidis*.[20] The prospect of extrapolating these findings to the breast periprosthetic environment is appealing. The mechanical shear forces and seroma fluid production discussed may potentially promote periprosthetic biofilm formation; this could explain the uncharacteristically high incidence of biofilm in the conventional Biocell group (group 1).

Although a total of five cases of biofilm formation were found in the Siltex groups (groups 3 and 4), there were no incidences of clinical double capsule. Regardless of the presence or absence of biofilms, no double capsule formation would be expected in the Siltex groups because, in our experience, the periprosthetic capsule never truly adheres to this textured shell. Even if consequential quantities of periprosthetic fluid were to be produced because of shear forces, the nature of the Siltex surface would be unlikely to accommodate seeding of cells and eventual inner capsule development.

CONCLUSIONS

Our scanning electron microscopic analyses demonstrate that variations in expansion protocols can lead to modifications in periprosthetic capsular architecture. The more pronounced three-dimensional texture of capsules in the delayed Biocell (group 2) group suggest that there may be real benefits to delaying the first postoperative saline inflation until 6 weeks after Biocell expander implant insertion. Using our suggested delayed approach allows capsule maturation and may optimize the Biocell textured implant's ability to accommodate capsular adherence. With respect to capsular architecture, the benefits of delayed postoperative expansion initiation do not seem to extend to Siltex type expanders. Future prospective clinical trials are needed to determine the ultimate clinical impact

CODING PERSPECTIVE

This information provided by Dr. Raymund Janevicius is intended to provide coding guidance.

19357	Breast reconstruction, immediate or delayed, with tissue expander, including subsequent expansion
11970	Replacement of tissue expander with permanent prosthesis

- Code 19357 is global and includes creation of a pocket for the tissue expander, placement of the expander, straightforward wound closure, and all expansions during the 90-day postoperative global period.
- Creation of a submuscular pocket for the tissue expander is not considered a

"muscle flap" and is not reported separately with code 15734.
- Code 19357 is used for both immediate and delayed breast reconstructions.
- If expansions are performed after the 90-day global period, they may be reported separately, including supplies.
- Replacement of the tissue expander is reported with code 11970. This code is global and includes removal of the tissue expander and the port, some capsular adjustments, and placement of the permanent prosthesis.
- If extensive capsular work is performed, this must be well-documented in the operative report. Extensive capsulotomies (19370) or capsulectomies (19371) may be reported separately. Many payers, however, will not reimburse for these codes with code 11970 and prefer reporting code 19342 alone to describe tissue expander removal, capsular adjustments, and placement of the permanent prosthesis.
- It is imperative to preauthorize all breast reconstructive procedures in writing before performing surgery.

of our findings on patients undergoing two-stage expander-to-implant breast reconstruction.

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