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Université de Montréal

Applying Scanning Electron Microscopy for the Ultrastructural and Clinical Analysis of Periprosthetic Capsules in Implant-Based Breast Reconstruction

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Résumé

La reconstruction en deux étapes par expanseur et implant est la technique la plus répandue pour la reconstruction mammmaire post mastectomie. La formation d'une capsule périprothétique est une réponse physiologique universelle à tout corps étranger présent dans le corps humain; par contre, la formation d'une capsule pathologique mène souvent à des complications et par conséquent à des résultats esthétiques sous-optimaux. Le microscope électronique à balayage (MEB) est un outil puissant qui permet d'effectuer une évaluation sans pareille de la topographie ultrastructurelle de spécimens.

Le premier objectif de cette thèse est de comparer le MEB conventionnel (Hi-Vac) à une technologie plus récente, soit le MEB environnemental (ESEM), afin de déterminer si cette dernière mène à une évaluation supérieure des tissus capsulaires du sein. Le deuxième objectif est d'appliquer la modalité de MEB supérieure et d'étudier les modifications ultrastructurelles des capsules périprothétiques chez les femmes subissant différents protocoles d'expansion de tissus dans le contexte de reconstruction mammaire prothétique. Deux études prospectives ont été réalisées afin de répondre à nos objectifs de recherche. Dix patientes ont été incluses dans la première, et 48 dans la seconde. La modalité Hi-Vac s'est avérée supérieure pour l'analyse compréhensive de tissus capsulaires mammaires. En employant le mode Hi-Vac dans notre protocole de recherche établi, un relief 3-D plus prononcé à été observé autour des expanseurs BIOCELL® dans le groupe d'approche d'intervention retardée (6 semaines). Des

changements significatifs n'ont pas été observés au niveau des capsules SILTEX® dans les groupes d'approche d'intervention précoce (2 semaines) ni retardée.

Mots clés:

- Microscopie électronique à balayage (MEB)
- Microscopie électronique à balayage environnemental (ESEM)
- Cancer du sein
- Reconstruction mammaire prothétique
- Expanseurs mammaires
- Implants mammaires
- Expansion tissulaire
- Capsule periprothétique
- Contracture capsulaire

Abstract

Two-stage implant-based (expander to implant) breast reconstruction is the most frequently applied technique following total mastectomy. While the periprosthetic capsule is a normal physiologic response to any foreign body, pathological capsule formation often leads complications and suboptimal aesthetic results. The scanning electron microscope (SEM) is a powerful tool that offers unparalleled assessment of capsule ultrastructural topography.

The first research aim was to compare conventional high-vacuum (Hi-Vac) SEM with newer environmental scanning electron microscopy (ESEM) technology to determine whether the latter offers superior assessment of breast capsular tissue. The second aim was to apply the most optimal SEM mode to study periprosthetic capsule ultrastructural modifications in women undergoing differing expansion protocols during the first stage of implant-based reconstruction. Ten patients were prospectively included in the first study and 48 prospectively included into the second. Conventional Hi-Vac mode was deemed superior for the comprehensive analysis of breast capsular tissue. Using Hi-Vac mode within the established study protocol, a more pronounced capsular 3-D relief was observed around BIOCELL® expanders when the first postoperative saline inflation took place at 6 weeks following expander insertion (delayed approach). No significant changes were observed with SILTEX® expander capsules in both early (2 weeks) and delayed approach groups.

Key words:

- Scanning Electron Microscopy (SEM)
- Environmental Scanning Electron Microscopy (ESEM)
- Breast Cancer
- Implant-based Breast Reconstruction
- Breast Expander Implants
- Breast Implants
- Tissue Expansion
- Periprosthetic Capsule
- Capsular Contracture

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List of Abbreviations and Acronyms

AFM: Atomic force microscopy

ASPS: American Society of Plastic Surgeons

DMS: Dimethylsiloxane

CTGF: Connective tissue growth factor

ECM: Extracellular matrix

EDX: Energy-dispersive X-ray spectroscopy

ESEM: Environmental scanning electron microscope

ESR: Erythrocyte sedimentation rate

ETD: Everhart-Thornley secondary electron detector

FBR: Foreign-body reaction

FDA: U.S. Food and Drug Administration

FEG: Field-emission gun

GSED: Gaseous scanning electron detector

Hi-Vac: High-vacuum

IBR: Implant-based breast reconstruction

IFN-γ: Interferon gamma

IL: Interleukin

PDMS: Polydimethylsiloxane

PIA: Polysaccharide intercellular adhesion

RH: Relative humidity

SEM: Scanning electron microscope

TEM: Transmission electron microscope

TGF-β: Transforming growth factor beta

Th1: T-helper 1

TNFα: Tumour necrosis factor alpha

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Introduction

The Scanning Electron Microscope: History and Basic Concepts

The modern day scanning electron microscope (SEM) stems largely from the original work of Hans Busch on charged particle trajectories in axially symmetric electric and magnetic fields. In 1926, he theorized that magnetic fields could be used to direct electrons in a manner analogous to light passing through a lens in an optical microscope (figure 1)[1]. German scientists Max Knoll and Ernst Ruska built upon these principles of geometrical electron optics, eventually leading to the development of the first transmission electron microscope (TEM), circa 1931 (figure 2). Von Ardenne published the principles underlying the SEM in the late 1930s and constructed an instrument that mainly intended to overcome chromatic distortion, which occurred with when relatively thick specimens were examined with TEM. Important research subsequently carried out by the Cambridge University Engineering Department, starting in 1948 and led by Charles Oatley, culminated in the marketing of the first commercial SEM, “Stereoscan 1”, in 1965[2, 3]. Ernst Ruska would eventually be awarded the Nobel Prize in Physics in 1986 for the revolutionary foundations he established.

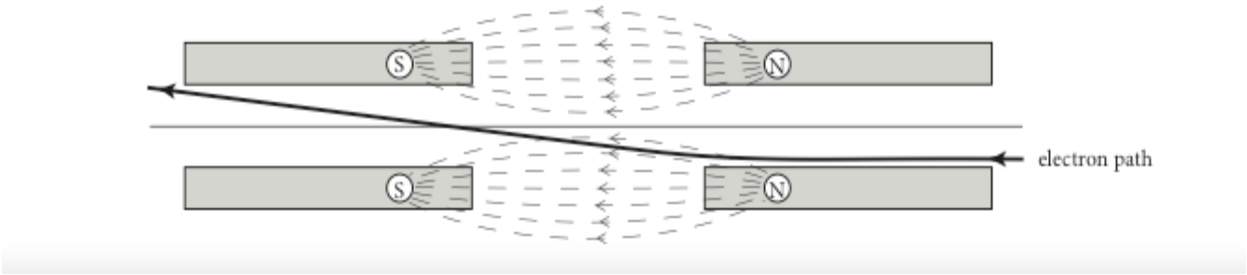

Figure 1: The electromagnetic lens: the magnetic field exerts a focus action on a moving electron.
(From: https://bsp.med.harvard.edu/node/221)

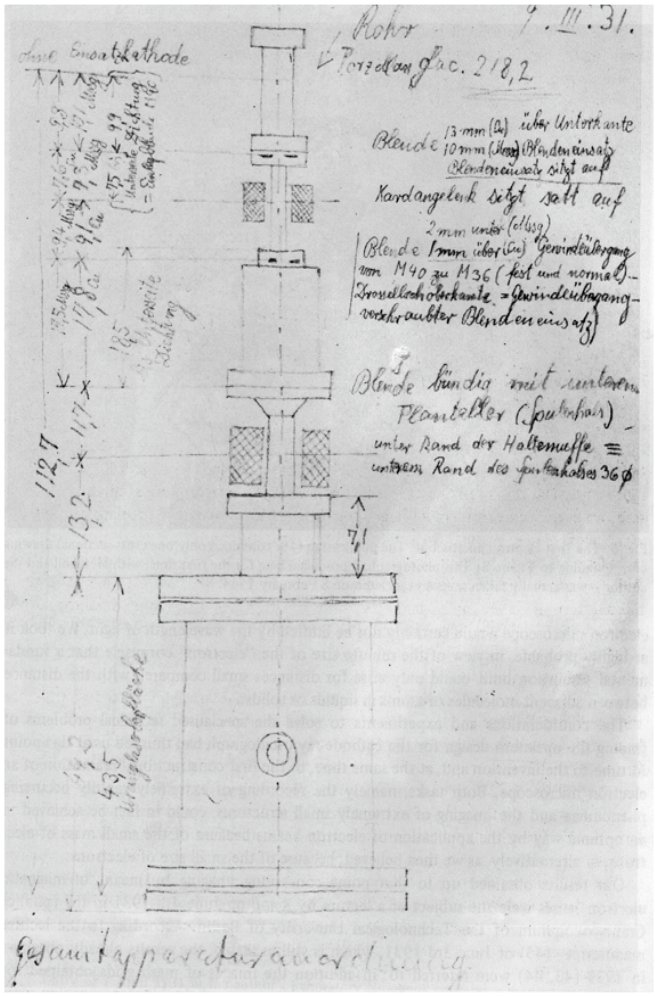

Figure 2: Sketch of the first TEM prototype, originally from Ruska's laboratory notebook.
(From: "The Early Development of Electron Lenses and Electron Microscopy" by Ernst Ruska)

TEM is a microscopy technique in which a beam of electrons is transmitted through a thin specimen and yields a projection of the complete sample, including internal information. The image is formed from the interaction of the electrons transmitted through this specimen. On the other hand, SEM produces images of a sample by scanning its surface with a focused beam of electrons. The beam of electrons interact with the sample surface, which emits secondary electrons that can then be recorded and provide details about the specimen's 3-D surface topography and composition (figures 3 and 4). Although SEM has a lower resolution than TEM, it allows for a larger sample area to be analysed at one time and is not limited to thinner cuts.

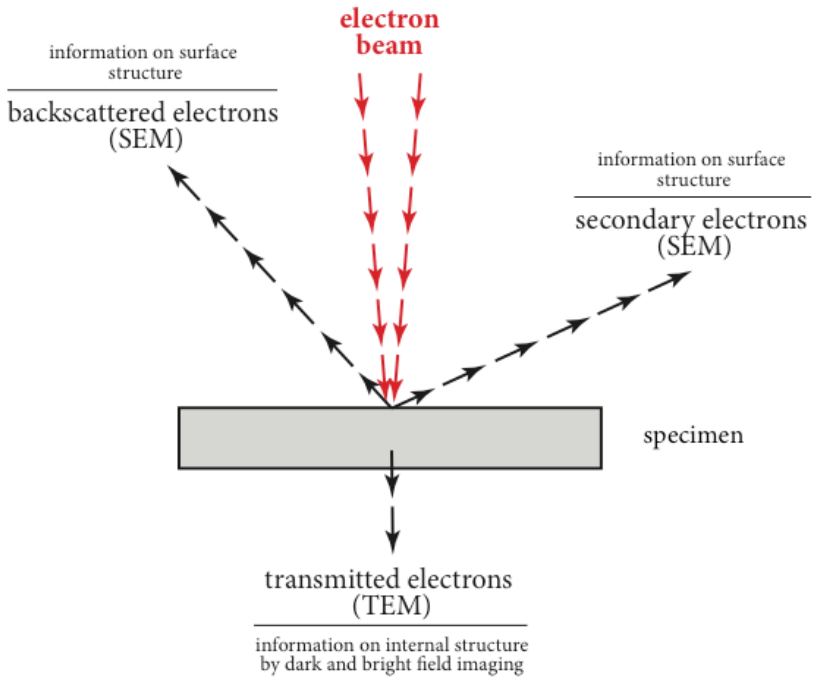

Figure 3: Representation of information produced by the interaction between specimen matter and the electron beam.

(From: https://bsp.med.harvard.edu/node/221)

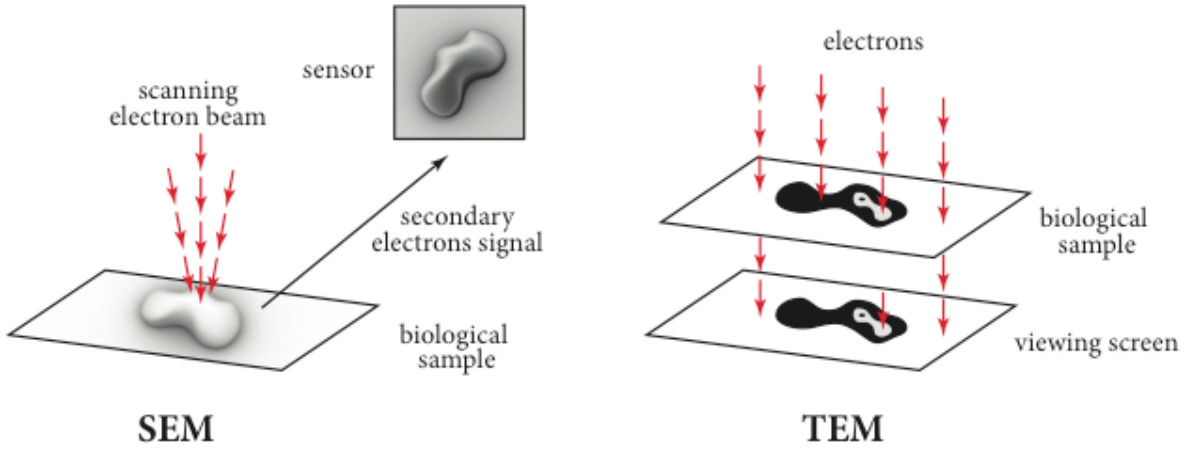

Figure 4: Illustration of differing principles behind scanning and transmission electron microscopes.
(From: https://bsp.med.harvard.edu/node/221)

Furthermore, the SEM may serve to obtain surface images of practically any type of solid material with up to approximately 500,000X magnification. Energy-dispersive X-ray Spectroscopy (EDX), a technique used for specimen chemical element analysis and characterization, is also possible when the SEM is equipped with the appropriate detectors. Conventional, or high-vacuum (Hi-Vac), SEM functions in a chamber with pressure settings typically at 10^{-5} mbar (0.001 Pa). Conventional SEM requires a vacuum for the generation and propagation of the electron beam, which will spread and attenuate in a gaseous environment [4]. For specimen imaging and microanalysis under such Hi-Vac conditions, specimens must be dry and electrically conductive. Conventional preparation therefore implies that biological specimens must undergo fixation, cleansing, drying and surface metallization processes prior to analysis.

The first environmental scanning electron microscope (ESEM) was commercialized in

the late 1980s; importantly, this particular form of SEM allows a gaseous environment in its specimen chamber, thereby allowing the examination of practically any type of specimen surface whether it be wet/dry or insulating/conducting [5, 6]. ESEM mode introduces high water vapour pressure in the specimen chamber (typically <26 mbar [2600 Pa]) rendering it possible to achieve high levels of humidity. Under such settings, wet or hydrated specimens (e.g. cells, plant samples, human tissue samples) will not dry or introduce artifacts; they can be observed in real-time under controlled environmental conditions. In theory, when compared to Hi-Vac, ESEM offers several novel features and interesting advantages, which are summarized in table I. Figure 5 shows a modern SEM machine with environmental mode capabilities.

ESEM Features and Advantages
Gas ionization in the sample chamber eliminates the charging artifacts (typically seen with nonconductive samples). Specimens do not need to be coated with a conductive film.
Can image wet, dirty and oily samples
Can acquire electron images from samples as hot as 1000°C
Delicate structures can be imaged with minimal risk of alteration/damage as the need for conductive coating is obviated
Can acquire x-ray data from insulating samples at high accelerating voltage
Eliminating the need for sample preparation, makes it possible to investigate specimen in dynamic processes (e.g. tension, compression, deformation)

Table I: Summary of ESEM features and theoretical advantages.

Figure 5: Modern SEM with environmental mode capabilities and EDAX detector (Quanta 200 FEG Environmental Scanning Electron Microscope [FEI Company, Hillsboro, OR, USA]).

Breast Cancer: Epidemiology

Breast cancer is the most common cancer among North American women with over 256 000 new cases expected in 2013. In Canada alone, an estimated 23 855 new breast cancer diagnoses are expected, accounting for more than a quarter of all cancers in women. Breast cancer represents the second leading cause of cancer death in women (figure 6)[7, 8]. Treatment often includes a combination of chemotherapy, radiotherapy

and surgery. Total mastectomy leaves significant physical and psychological sequelae that can be partially alleviated by breast reconstructive surgery.

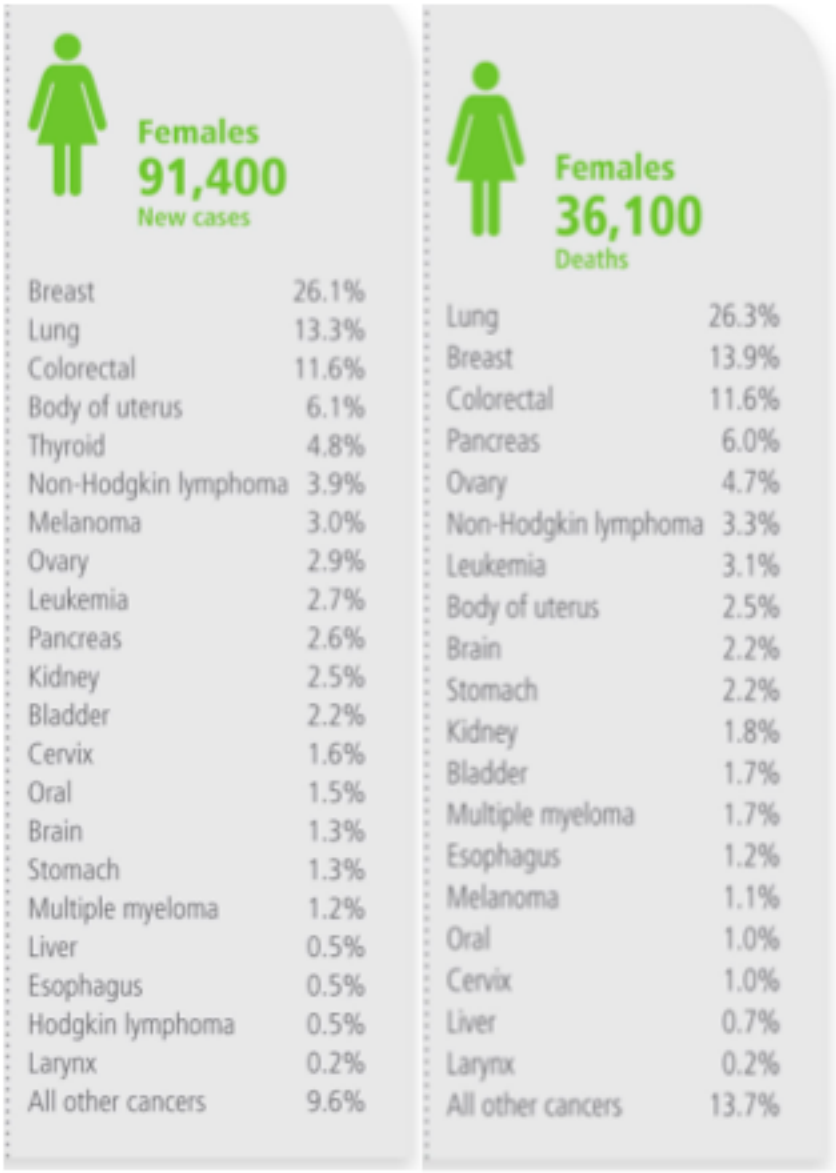

Figure 6: Percent distribution of estimated new cancer cases and cancer deaths in females, Canada, 2013.

(Analyses by: Chronic Disease Surveillance and Monitoring Division, CCDP, Public Health Agency of Canada Data source: Canadian Cancer Registry database and Canadian Vital Statistics Death database at Statistics Canada)

Principles of Expander-Implant Breast Reconstruction

According to the American Society of Plastic Surgeons (ASPS) 2012 statistics report, 70.5% of breast reconstructions were achieved with expanders and implants while an additional 8.1% were completed with implants alone[9]. Furthermore, between 1998 and 2008, the rate of implant use in the U.S. for breast reconstruction rose by 203%[10]. Therefore, only about one-fifth of patients underwent autologous breast reconstruction with either local or distant muscle and/or soft tissue. An interesting survey published in 2009 demonstrated that about two-thirds of North American female plastic surgeons would choose an implant-based approach to breast reconstruction for themselves[11].

The goal of breast reconstructive surgery is to recreate, as closely as possible, two symmetrical, proportionally sized breast mounds. Ancillary procedures, nipple and areolar reconstruction in particular, complete the surgical process. Implant-based breast reconstruction (IBR) can be performed in either an immediate or delayed fashion. Many of the earliest procedures consisted of immediate reconstructions with definitive implant alongside mastectomy surgery[12, 13]. More recently, planned post-operative radiotherapy has often been cited as a reason for performing the oncological resection (mastectomy) and the breast reconstruction as separate procedures, in other words, as part of a delayed breast reconstruction plan.

In terms of reconstructive technique, most commonly, a two-step (expander to implant) approach is employed in IBR. In this procedure, an initial expander prosthesis is placed

under the pectoralis major muscle through the mastectomy incision; this expander will be partially inflated intraoperatively with saline and is followed by sequential inflations postoperatively over several weeks to a few months until the desired tissue expansion is achieved. A second-stage surgery is subsequently performed in order to exchange this expander for the definitive prosthesis. In certain select cases, the Becker combined expander-implant may be used. In doing so, tissue expansion is performed without requiring two surgeries under general anesthesia; only the injection port must eventually be removed, which is easily done under local anesthesia. Doubts regarding the efficacy of Becker implants in breast reconstruction have recently arisen; Simpali et al. cite a 64% explantation rate in their study[14].

As indicated by the 2012 ASPS survey, a certain number of patients may benefit from a single-staged reconstruction with regular permanent breast implants, thereby obviating the tissue expansion process. Such reconstructions require a sufficiently sized post-mastectomy skin envelope as well as a reasonable amount of available pectoralis major muscle for implant coverage. An inferior sling of acellular dermis may also be employed in order to enhance implant coverage[15].

IBR techniques are known for their relatively high complication rates. Recent series reveal first-stage complication rates ranging from 8.5 to 11%[16, 17]. Overall complication rates after both stages of expander to implant reconstruction of 17.6% and higher have been reported in the literature[18]. Complication rates are even more markedly elevated in patients undergoing radiotherapy; in their systematic review of all

types of IBR surgeries on irradiated breasts, Momoh et al. cite pooled major complication and failure rates of 49% and 19%, respectively[19]. The high rate of morbidity and consequent economic costs to society associated with IBR render it a true public health issue[20].

The Evolution of Breast Implants and Expanders:

The modern development of breast implants as well as breast augmentation and reconstruction techniques were inspired by Czerny; in 1895, he described a surgical procedure implicating transfer of a trunk lipoma to the breast area[21]. In 1930, Schwarzmann suggested the use of glass balls as breast implants; a practice that was advocated by Thorek in "certain cases", at least up until 1942[22]. However, it is the latter half of the 20th century that is most notable for the true development and use of synthetic medical products for breast surgery and related procedures. F.S. Kipping of Nottingham University conducted pioneering research on silicon polymers; from 1899-1944, he published 54 papers regarding silicon-carbon chemistry; others subsequently built upon his work for more practical purposes. In 1943, liquid silicone was developed during World War II for potential use in military aircraft. The invention of silicone rubber followed in 1945[23]. Following the end of the war effort, the medical field would become a prime beneficiary of silicone's many potential applications. Silicone is a highly pure polymer of dimethylsiloxane (DMS) and is based on the element silicon (figure 7); it

may be produced in the form of oils, gels or elastomers (rubber). The polymer chains vary in length, with longer chains correlating with greater substance viscosity.

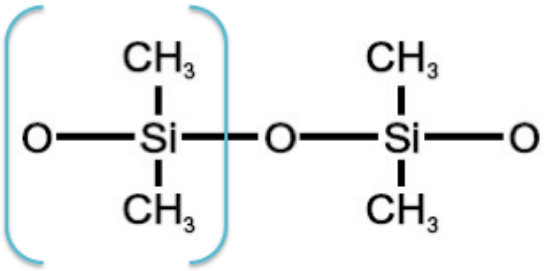

Figure 7: Chemical structure of silicone; DMS molecule demarcated in brackets.

Uchida, based on work conducted by Japanese scientists on silicone-containing fluid mixtures in the 1940s, reported a case of free silicone injection into the breast in 1961[24]; this complication-ridden practice has since been ceased in developed countries. Around the same time, from the 1950s until the early 60s, solid materials such as polyurethane, Teflon, and polyvinyl alcohol formaldehyde (the Ivalon sponge) were used as breast implant devices[25].

Silicone Gel Breast Implants

Silicone implants, as currently engineered, were first utilized in the early 1960s. Importantly, all modern implants share two basic features. Firstly, they have an outer silicone elastomer shell, which can be single or double, smooth or textured, and even coated with polyurethane foam. Secondly, they are also filled with either silicone gels of varying viscosity or normal saline[26].

According to Scales, the ideal implant should have the following characteristics[27]:

- Impermeable to tissue fluid
- Chemically inert
- Nonirritant (does not cause inflammatory or foreign body reaction)
- Noncarcinogenic
- Nonallergenic
- Resistant to mechanical stresses
- Capable of being manufactured to the desired form
- Sterilizable

In 1962, The Dow Corning Corporation started manufacturing the first generation of breast implants, which was pioneered by Cronin and Gerow. These teardrop-shaped implants were composed of a relatively thick outer shell in order to resist possible rupture and leakage. The contained gel was particularly viscous in order to preserve the intended form of the implant. Notably, the original model included a posterior Dacron patch in order to help stabilize the implant against the chest wall tissue. The Cronin-Gerow implant was plagued by capsular contracture complications. By the beginning of the 1970s, the evidence was clear; studies reported in multiple conferences and publications showed pathological capsule formation rates greater than 50% in certain series[24]. H.L. Silver noted that capsulotomy failed to counter thickened capsular formation; in his experience, complete recurrence was the norm[28]. Furthermore, it soon became clear that the Dacron patch served as an unhelpful nidus of inflammation;

the patch was omitted from the subsequent generation of implants in the early 1970s.

The second-generation implants were round in shape and featured both a thinner exterior shell and more liquefied inner gel consistency. However, the trade-offs for this more natural, "responsive" implant were an increased risk of shell rupture, a phenomenon of silicone gel "bleed" as well as diffusion of small amounts of the gel's silicone oil fraction. Several authors subsequently reported their clinical and laboratory findings, which revealed exacerbated pathological capsule formation and intense foreign body reactions (FBR) in cases of breast implant leakage[29-32].

In the early 1980s, a third generation of "low bleed" implants were developed[33, 34]. These round gel-filled implants possessed two layers of high-performance elastomer with a thin fluorosilicone barrier coat in between[25]. The Silastic® II model, by the Dow Corning Corporation, was one such "low bleed"' third-generation implant that demonstrated improved strength and lower contracture rates in early animal experiments and human clinical case series[33, 34].

Fourth-generation implants represented a particularly significant advance for two reasons: the addition of "anatomic" shaping and textured surfacing. McGhan Medical Corporation (now Allergan, Inc.) was the first to introduce texturization in 1987 with the BIOCELL® surface, created by the "lost salt technique" in which the implant shell is applied with pressure onto a layer of fine salt. Mentor Corporation responded the following year with its SILTEX® surface, which is made via negative contact imprinting

from textured foam. The SILTEX® surface is considered to be a less aggressive form of texturization than its BIOCELL® counterpart[35, 36].

Fifth-generation implants introduced the concept of firmer, thicker "cohesive" gel along with a greater selection of volume and shape options with respect to height, width and projection parameters (Figure 8). "Highly cohesive" silicone gel, essentially obtained by a higher concentration of cross-linking between silicone chains, is a relatively recent addition to the North American market. These latest silicone gel implants are said to have superior shape retention, less rippling, and lower risk of leakage, albeit being firmer and therefore slightly less natural in feel. Health Canada has approved the Allergan NATRELLE™ Style 410 model and the analogous Mentor MemoryShape™ since 2006[37, 38]. The U.S. Food and Drug Administration (FDA) approved both aforementioned products in February 2013 and June 2013, respectively[39, 40]. Recent studies confirm the safety and effectiveness of these enhanced cohesive implants, while corroborating benefits of form stability claimed by the manufacturers[41-44]. The five implant generations are summarized in table II.

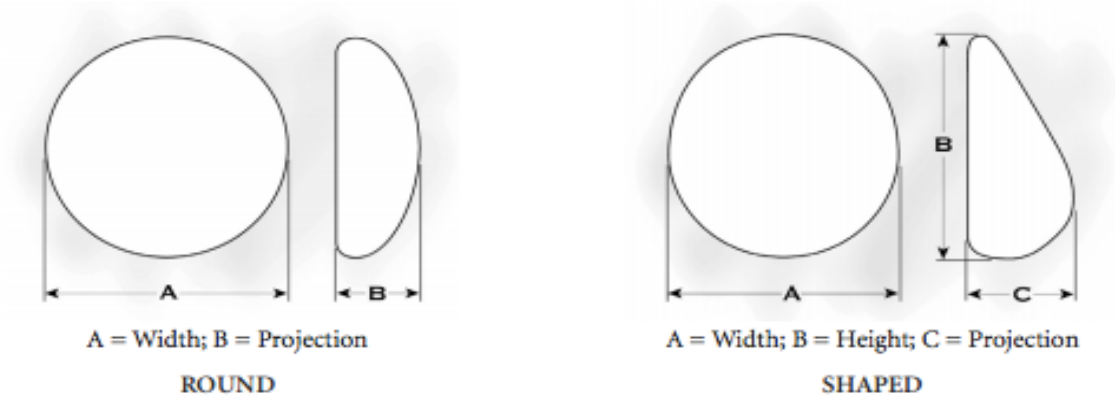

Figure 8: Illustration of parameters in round and anatomic-shaped breast implants.
(From: Allergan Medical Corporation, Directions for Use - NATRELLE™ Silicone-Filled Breast Implants)

Inflatable Saline-Filled Implants

In 1965, French plastic surgeon H.G. Arion reported the use of an inflatable saline-filled implant with silicone elastomer shell[22]. The development of such an inflatable device was motivated by the desire to minimize the size of incisions needed for implant insertion. Deflation proved to be a significant drawback of these initial implants; the original French implant produced by the Simaplast Company had a deflation rate of approximately 75% at 3 years, ultimately leading to its withdrawal from the market[45]. Several manufacturers, including the Dow Corning and Heyer Schulte corporations, developed their own inflatable implant models over the course of the next decade, with reported deflation rates as high as 16%[46-56]. Current saline implants produced by American manufacturers Allergan and Mentor now have diaphragm valves as well as thicker, room temperature vulcanized silicone shells, both of which eliminate much of the deflation risk that characterized earlier models.

Generation (with approximate dates)	Characteristics
1st Generation (1962-1970)	• Thick, two-piece shell • Smooth surface, Dacron patches • Teardrop shape • Viscous silicone gel
2nd Generation (1970-1982)	• Thin, slightly permeable shell • Smooth surface (no Dacron patches) • Round shape • Less viscous silicone gel
3rd Generation (1982-1992)	• Thick, strong, "low bleed" shell • Smooth surface • Round Shape • More viscous silicone gel
4th Generation (1986-present)	• Thick, strong "low bleed" shell • Smooth or textured surface • Round or anatomic-shape • More viscous silicone gel
5th Generation (1993-present)	• Thick, strong, "low bleed" shell • Smooth or textured surface • Round or anatomic-shape (various) • Cohesive or highly cohesive gel

Table II: Summary of silicone gel implant generation features.
(Adapted from Maxwell and Gabriel[45])

Breast Expander Implants

Austad and Radovan independently developed the original silicone soft tissue expanders, with both eventually publishing their respective results in 1982[57, 58]. Present-day inflatable breast expander implants are based on the same aforementioned manufacturing concepts and principles of saline implants for aesthetic breast surgery. Moreover, they are also produced with textured surfacing. In the North American market, virtually all expanders used are anatomic-shaped implants from either Allergan or Mentor, with BIOCELL® and SILTEX® textured surfaces, respectively. An overview of some of the available anatomic and round profiled breast implant products is presented in figures 9 to 11.

Lastly, Hartley's double-lumen implant merits brief mention; the device, conceived in the mid-1970s, consisted of a silicone gel-filled core lumen completely surrounded by an inflatable saline-filled shell[59]. The modern day parallel of Hartley's novel implant is the Mentor Becker, which is a combination breast expander and implant used in breast reconstruction. The Becker implant is a "reverse double-lumen" implant; the core lumen is inflatable with saline, and the outer shell is silicone gel-filled. The implant includes a removable connected external port valve for inflation.

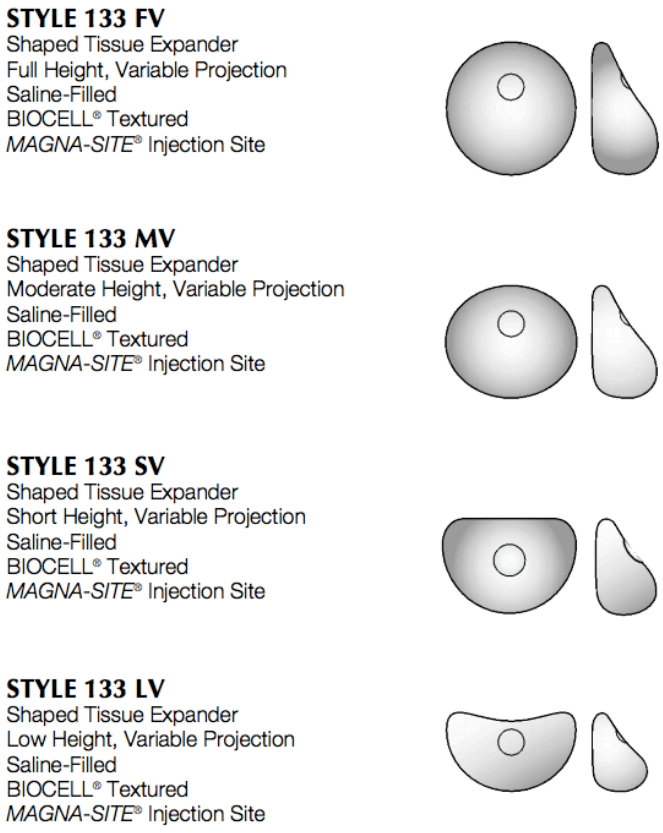

Figure 9: Examples of available profiles for Allergan's Style 133V Series Tissue Expanders with BIOCELL® textured surface and MAGNA-SITE® integrated injection site.

(From: Allergan Medical Corporation, Directions for Use - NATRELLE™ 133 Tissue Expanders)

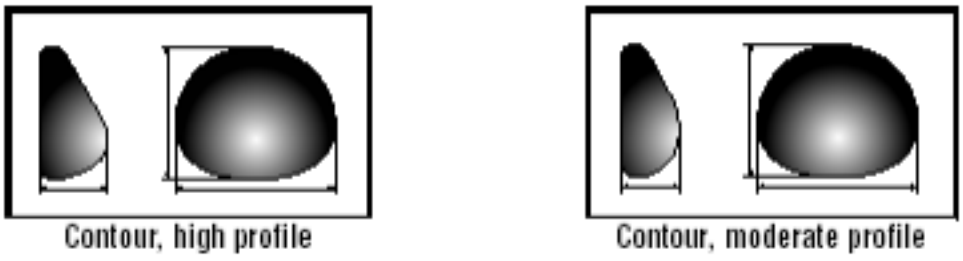

Figure 10: Available profiles for Mentor's contour (anatomic-shaped) saline-filled permanent breast implants, manufactured with either smooth or SILTEX® textured surface.

(From: Mentor Corporation, Directions for Use – Saline-filled & Spectrum™ Breast Implants)

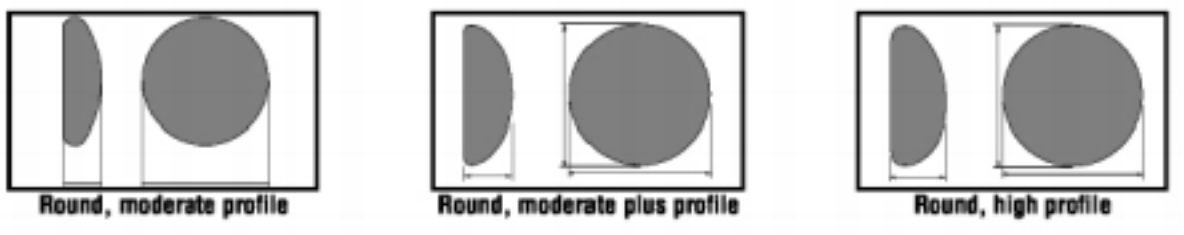

Figure 11: Available profiles for Mentor's round saline-filled permanent breast implants, manufactured with either smooth or SILTEX® textured surface.
(From: Mentor Corporation, Directions for Use – Saline-filled & Spectrum™ Breast Implants)

Silicone Gel Implants and the FDA: the Controversy

In 1976, the U.S. Congress passed the *Medical Device Regulation Act*, thereby giving the FDA regulatory authority over medical devices, including breast implants, as was already the case for medications. With regards to breast implants that were already marketed at the time of the new law, their continued use was permitted as the FDA undertook the task of formally reviewing their safety and efficacy. In 1992, the FDA called for a U.S. moratorium on the use of silicone gel breast implants, citing the absence of adequate data proving their safety and effectiveness. Concerns about a potential link between these implants and immune-related disorders played a role in the decision[60, 61]. As part of this ruling, controlled use of silicone gel-filled implants was permitted under certain circumstances, namely for breast reconstruction cases and limited clinical research trials. Saline-filled implants were not placed under restriction. Subsequent epidemiological investigations found no evidence of increased risk of connective tissue diseases in women with silicone gel breast implants[62]. In 2006, restrictions on silicone gel implants were lifted, conditional upon the conduction of post-approval studies and

FDA monitoring. In Canada, medical devices are regulated by Health Canada's Therapeutic Products Directorate and are subject to the *Medical Devices Regulations* under the *Food and Drugs Act*.

The Periprosthetic Capsule: An Overview

The Concept of Capsular Contracture

The periprosthetic capsule is a normal physiological response to any foreign object inserted into the human body. An implanted prosthesis is surrounded by young scar tissue composed largely of fibrin and phagocytes[63]. Progressive collagen synthesis and local inflammation resolution lead to a mature scar capsule, which takes at least 4 weeks to form[64, 65].

Pathological breast periprosthetic capsule formation is known as capsular contracture and was described by Baker as 4 clinical grades (table III)[66]. In grade I, the breast remains soft without obvious changes in size and shape, while grade IV describes a hard and painful breast that may include chest wall deformation. Very severe cases may require revision surgery with an alternate autologous tissue-based technique. Capsular contracture usually develops over the course of the weeks to months following implantation; up to 92% of cases occur within the first year[35, 67-69]. Long-term severe contracture (Baker grades III and IV) rates range from 10.4 to 29.5% in reconstructive series[70-72]. With respect to patients receiving radiotherapy post-

reconstruction, a recent meta-analysis revealed a pooled severe contracture rate of 32%[19]. Capsular contracture develops less frequently in aesthetic breast augmentation patients; recent series with latest generation highly cohesive implants show rates below 10% over medium-term follow-up (roughly 3-6 years)[41-43, 73].

Baker Grade	Description
I	• Breast is soft, natural-looking • Implant non-palpable
II	• Breast slightly firm • Implant palpable, not visible
III	• Breast moderately firm • Implant easily palpable and is visible
IV	• Breast hard and painful • Implant easily visible and distorts breast

Table III: Baker Classification.
(Adapted From: Baker, JL. Classification of Spherical Contractures. [66])

Risk factors for capsular contracture include: periprosthetic infection, postoperative tissue hypoxia, hematoma, seroma, radiation, implant shell breakdown, silicone gel leakage, pregnancy and genetic predisposition. Inflammation, whether of infectious or sterile etiology, is the common denominator of the aforementioned factors[67, 74].

Uncontrolled inflammation has deleterious tissue effects, including excessive tissue fibrosis, which is a key feature of capsular contracture. The inflammatory process that is specifically involved in breast capsule formation is poorly understood and no effective targeted medical treatments exist. Allergan's BIOCELL® and Mentor`s SILTEX® are generally considered effective at reducing the incidence of capsular contracture[75]; however, according to the current literature, this advantage has only been convincingly demonstrated with saline and gel-filled textured implants placed within subglandular pockets[76, 77].

Immunobiology of Tissue Fibrosis

Tissue fibrosis is the body's natural response to stresses such as infections, toxins, drugs, trauma and recurrent inflammation due to chronic disease[78]. All fibrotic diseases have been linked to increased activity of the Transforming Growth Factor β1 (TGF-β1) cytokine pathway[79]. Other cytokines and growth factors implicated in fibrotic disease include connective tissue growth factor (CTGF), interleukin-1 (IL-1), IL-4, IL-6, IL-13 and Tumor Necrosis Factor α (TNFα)[78]. Fibrosis may also result from a deficient anti-fibrotic response. For example, interferon γ (IFN-γ) is secreted by T-helper 1 (Th1) lymphocytes and has been implicated in early inflammation resolution[80].

Furthermore, fibroblasts secrete extracellular matrix (ECM) proteins and are responsive to cytokines. TGF-β induces a phenotypic switch from resting fibroblasts to myofibroblasts, thereby conferring the ability to secrete large amounts of protein and cause local microenvironment contraction. Myofibroblasts have been observed in

pathological periprosthetic capsules where they form dense bands[81]. Therefore, myofibroblasts are not only present in the periprosthetic capsule but are induced and maintained by a specific inflammatory microenvironment, thereby contributing to capsular contracture and, ultimately, reconstructive failure.

Radiotherapy Effects on Capsular Tissue

Breast radiation therapy treats the cancer but also induces genetic damage of parenchymal cells. Following the acute inflammatory reaction, which lasts a few months, the long-term sequelae of chronic inflammation ensue, manifested by tissue atrophy, fibrosis, redness and telangiectasia[82, 83]. In parallel, biological analysis reveals vascular alterations, leukocytic infiltration and a cascade of cytokine interactions. However, the pathophysiological mechanisms of fibrosis in radiated tissues remain unknown.

Clinically, IBR in the context of radiotherapy is plagued by markedly higher complication rates, including a 400 to 1000% increased risk of capsular contracture, leading some authors to advocate limited use of implants in irradiated patients[19, 84].

Impact of Subclinical infections on Capsule Formation

Periprosthetic infection, by skin flora in particular, has been demonstrated in contracted capsular tissue, with a *Staphylococcus epidermidis*-positive culture results correlating significantly with capsular contracture grades III and IV[85]. Regardless of whether the infection is overt or subclinical, the innate immune system is stimulated in some fashion

by the involved pathogens. In certain cases, the bacteria have gone undetected by traditional culture and have only been identified by electron microscopy[86]. Pathogens also have the ability to produce biofilm at the interface with a foreign body; consequently, these bacterial organisms successfully isolate themselves from the reaches of both the immune system and antibiotics[87]. Biofilm can confer up to a thousandfold increase in bacterial resistance to antibiotics and disinfectants; furthermore, its ECM can obstruct macrophage phagocytosis[88].

In a recent study of breast capsule bacteria, *Propionibacterium acnes and S. epidermidis* were the most commonly isolated bacteria. The former often goes undetected by routine cultures, largely because of its excessively slow rate of growth[89, 90]. Notably, positive *S. epidermidis and P. acnes* culture results are often interpreted to be contamination. A recognized causal link between biofilm formation and capsular contracture exists; however, bacterial presence does not inevitably trigger pathological capsule development[86, 90]. Nonetheless, weakly pathogenic bacteria may induce substantial capsular contracture by stimulating the fibroblast innate immune response[85, 91-95].

Implant-Capsule Interface: Mechanical Factors

The SEM is a powerful tool that has been extensively applied in the study of breast periprosthetic capsules. SEM offers unparalleled assessment of both specimen surface topography and cellularity; it can also reveal biofilm and bacterial organism presence that is often otherwise undetectable[85, 86, 96]. Additionally, EDX allows for specimen chemical element analysis. Periprosthetic capsules behave differently in response to

varying surface textures[35, 36]. Other mechanical factors such as implant micromotions and externally applied shear stress, expander inflation for example, also impact capsule formation. Also, despite improved reliability of implant shell integrity, foreign body inflammatory reactions to silicone deposits in periprosthetic tissue have been observed with even the latest generation of breast expanders and implants. Detailed structural, cellular and chemical descriptions of implant surfaces and corresponding periprosthetic capsules are essential to understanding normal and pathological capsule evolution.

SEM as a Study Tool for Breast Implants and Capsules: A Review of the 21st Century

Since the 1990s, interest in the ultrastructural characteristics of implant surfaces and periprosthetic capsules, normal and pathological, has grown; SEM has progressively become an invaluable tool in this area of research. Rubino et al. tangentially split capsule samples derived from textured subpectoral implants removed because of Baker grade III contracture. Using conventional SEM, they identified a 5-layer structure, which included an inner and outer "vascular layer". Interestingly, this multilayer architecture was not identifiable in their control samples derived from non-contracted implants[97].

The ultrastructural features of the BIOCELL® and SILTEX® implant surfaces have been described and depicted in detail. Danino et al. confirmed the BIOCELL® surface`s ability to accommodate some degree of tissue ingrowth by illustrating the mirror-image capsular tissue response, or the "Velcro effect". On the other hand, a more linear, non-

adherent fibrotic pattern was observed in the SILTEX® capsule samples[35]. Also using SEM, Barr et al. conducted a survey of Mentor SILTEX®, Allergan BIOCELL®, Allergan smooth, Cereplas CEREFORM® and Polytech Microthane® (micropolyurethane) implant surfaces. The images presented added further insights into the different nanoscale topographic subtleties of these products[98].

Prasad et al. studied the relationships between surface topography and wound healing in a cell culture medium of mouse fibroblasts with the hopes of gaining insights on capsular formation. They prepared silicone elastomer samples with varying surface roughness; the degrees and differences of sample roughness were qualitatively analysed using SEM and quantitatively using adjunct tools such as atomic force microscopy (AFM). Interestingly, using a PicoGreen® assay, fibroblast growth was found to be decreased with increases in surface roughness. Additionally, smooth silicone surfaces demonstrated significantly higher concentrations of fibroblasts [99]. Studies by Dalby et al. have demonstrated that cellular filopodia, sensory protrustions, are capable of sensing nanoislands as small as 10 nm[100]. Such observations highlight the relevance of specific implant surface characteristics with respect to the natural history of the adherent capsule and may have implications for future product manufacturing techniques.

Other studies, while using SEM as a study tool, have yielded important findings in capsule analysis with more palpable clinical implications. SEM has played an important role in the analysis of biofilms in breast capsule samples. Pajkos et al. demonstrated

extensive amorphous biological deposits on breast implants removed for capsular contracture; interestingly, SEM images were able to reveal biofilm on one capsule sample despite a negative bacterial culture. The authors posited that bacterial biofilm induces and accelerates capsule formation[85]. Tamboto et al. furthered this hypothesis in their study inoculating *S. epidermidis* around BIOCELL® gel-filled implants in porcine models. Coccoidal cells encased in a glycocalyx matrix were observed with SEM on all biofilm-positive capsule samples; bacterial culture was negative in 19% of biofilms. The authors noted a fourfold increased risk of developing contracture when comparing the inoculated and the control groups[86]. In-vitro experiments by Van Heerden et al. on the effects of various antibacterial-coating agents on biofilm formation yielded interesting conclusions that may be directly applicable to clinical practice. Using SEM to grade the formation of biofilm on both textured and smooth silicone discs, they noted that chloramphenicol, fusidic acid and oxytetracyclin/polymyxin B sulphate ointments were superior to mupirocin, silver sulfadiazine and neomycin/chlorhexidine in resisting biofilm formation over a 7 day period[101].

Especially over the last decade, a myriad of important studies have led to a better understanding of the periprosthetic breast capsule and the factors which influence its formation and contracture. SEM has been a driving force behind this research, permitting unparalleled direct visualization of some of the subtle architectural and cellular qualities of periprosthetic tissue.

Objectives

Pathological breast periprosthetic capsular formation is a complex process in which mechanical factors play an integral role. SEM is a powerful technology that renders the necessary ultrastructural and elemental assessment of capsular tissue achievable. This thesis consists of two key components.

Firstly, with the relatively recent emergence of ESEM as an attractive tool for the study of biologic tissues, it is crucial to determine its applicability to the analysis of breast periprosthetic capsules. We aimed to compare the performances of Hi-Vac and ESEM for the comprehensive assessment of periprosthetic capsular tissue. In doing so, we hoped to establish a proven, robust protocol for periprosthetic breast capsule SEM analysis that took into account the latest technologies available on the market today.

The findings of the first component established the crucial foundation for our second study component. Using the SEM modality and protocol deemed most optimal for our stated purposes, we aimed to investigate whether differing expansion protocols led to observable modifications in capsular formation around the two most widely used expander implant types in North America, the Allergan BIOCELL® and Mentor SILTEX® devices. This latter portion of the study provided us with important insights into the behaviour of capsular tissue when faced with varying mechanical stresses that are routinely applied in clinical practice by plastic surgeons as part of a two-staged IBR process.

Materials and Methods

Summary of Clinical Study Protocol

All candidate patients included in the studies were breast cancer patients aged over 18 years scheduled to undergo two-stage expander to implant breast reconstruction. Exclusion criteria were as follows: concurrent unrelated cancer diagnosis (except basocellular carcinoma), previous ipsilateral breast surgery (other than mastectomy), patient concurrently included in other medical treatment trial, pregnant or breastfeeding women, and active breast infection. Five plastic surgeons specialized in breast reconstruction from a single tertiary care academic hospital center participated in the studies.

Patients underwent first-stage expander insertion surgery as planned. In general, all participating surgeons employed a standardized surgical approach: 1st generation cephalosporin (or clindamycin, if allergic) IV antibiotic prophylaxis, chlorhexidine skin disinfection, bacitracin implant pocket and prosthesis irrigation, submuscular coverage of expander with pectoralis major and serratus anterior muscle strips, and 1 submuscular Jackson-Pratt drain insertion per reconstructed breast. Postoperative follow-up was performed in accordance with standard practice guidelines; some degree of variation between surgeons was considered normal and expected. The expansion

process was usually initiated at 2 to 6 weeks postoperatively and serial saline expander inflation every 1 to 2 weeks thereafter.

Following completion of the expansion process, patients were scheduled for the usual second-stage expander to implant exchange surgery. Breast periprosthetic capsule tissue biopsy sampling was undertaken intraoperatively during the second-stage surgery for each reconstructed breast. A minimum 1 cm^2 of breast periprosthetic capsular tissue, at the level of the implant dome, was biopsied for each case.

The capsular tissue sample was then placed in a sterile specimen cup. Each specimen cup was labeled with a unique code specific to the corresponding patient and contained a fixation solution of glutaraldehyde 2% (2 mL) and sodium cacodylate 0.1M (48 mL) in order to stabilize the cellular structures at a pH of 7.3. The role of the glutaraldehyde was to provoke rapid tissue death without impacting ultrastructural characteristics while the sodium cacodylate preserved the original tissue composition. All samples were then stored in a refrigerator at 4°C for a minimum of 24 hours. During transport to the external SEM laboratory facilities, all tissue samples were stored on ice in a pathology transport container.

Summary of Electron Microscopy Preparation-Analysis Protocol: Materials

Electron Microscope

All tissue observations were performed using a Quanta 200 FEG Environmental Scanning Electron Microscope (FEI Company, Hillsboro, OR, USA) with EDAX detector. This microscope uses a field-emission gun (FEG) electron source in an exceptionally high chamber pressure environment. It combines 2 main advantages:

- Nanometer resolution and a high signal to noise ratio in both regular high-vacuum and environmental (wet) modes.
- Real "wet" mode (100% humidity in the specimen chamber) and a possibility to examine specimens with a high vapour pressure in the chamber. It is provided by a differential pumping vacuum system and a series of pressure-limiting apertures in addition to a patented gaseous secondary electron detector.

The Quanta 200 FEG ESEM produces enlarged images of a variety of specimens, achieving magnifications of over 300 000X and providing high-resolution imaging in a digital format. This analytical tool provides exceptional depth of field, minimal specimen preparation, and the ability to combine the technique with energy-dispersive X-ray spectroscopy (EDX). The microscope has 3 operating vacuum modes to deal with different types of samples:

- High vacuum (Hi-Vac):
    - Typically 10^{-5} mbar (0.001 Pa)

- Imaging and microanalysis of conductive and/or conventionally prepared specimens

- Low vacuum (Lo-Vac):
  - < 1.3 mbar (130 Pa)
  - Imaging and microanalysis of non-conductive, unprepared specimens (paper, plastics, ceramics, etc.)
- Environmental (ESEM)
  - < 26 mbar (2600 Pa)
  - For wet, unprepared specimens

Preparatory Materials

- Sample stub made of aluminum, SEM specimen mount stubs, Cederlane, Product N° 75510
- Carbon tabs, Conductive carbon adhesive tabs, 12 mm diameter, Cederlane, Product N° 77825-12
- Specimen mount tweezers
- Tweezers, super fine points
- Air compressed Dust-off, Cederlane, Product N° 70837
- Cooling Peltier stage
  - NOTE: The Peltier Cooled Specimen Stage is used to maintain water on samples inside the Quanta specimen chamber. It uses a thermoelectric module to alter temperature, and this, in conjunction with specimen

chamber pressure, creates condensation on the sample. The primary applications of this effect are to produce moisture on the sample or to keep the sample wet.

- Cutting blade

Instruments and Software

- Gold sputter-coater (Agar Manual Sputter Coater, Marivac Inc., Montreal, QC).
- XT Docu software (FEI Company)
    - SEM image processing
- Adobe® Photoshop® CS6 Extended
    - SEM image assembly
- Imagan2 computerized image analysis system (Kompira, Strathclyde, UK)
    - Sample texture three-dimensional relief characterization and measurement

Summary of Electron Microscopy Preparation-Analysis Protocol: Methods

For tissue samples to be analyzed under conventional Hi-Vac SEM, specific preparatory steps were undertaken under an extractor hood:

- Sample division with a cutting blade to obtain a 3 x 3 mm fragment
- Cleansing of 3 X 3 mm sample fragment with distilled water for 10 to 15 seconds (to remove excess glutaraldehyde)
- Placement of sample onto aluminum stub with conductive taping

- Placement of sample onto absorbent paper for a 20 minute air drying process at ambient room temperature
- Gold-coating of sample using an Agar Manual Sputter Coater (Marivac Inc., Montreal, QC), for 30 seconds

Samples to be analyzed under ESEM forewent the gold-coating process and were directly immobilized onto the ESEM-specific stub. Leftover specimen portions were placed back into the original fixation solution for future use, as necessary. Specific parameters were required for sample analysis, depending on the SEM modality employed.

Hi-Vac SEM parameters:

- Hi-Vac mode
- Everhart-Thornley secondary electron detector (ETD)
- Accelerating voltage = 20 kV
- Spot size = 3
- Working distance ~8 mm

ESEM parameters:

- ESEM mode with the cooling Peltier stage
- Gaseous scanning electron detector (GSED)
- Accelerating voltage = 10-20 kV
- Spot size = 3

- Starting pressure: 6.1 Torr (813.3 Pa) and starting temperature: 4 ^{o}C, for an initial relative humidity (RH) of 100%
- After 2 minutes of sample stabilisation, pressure decreased to approximately 3.3 Torr (440.0 Pa) for a final RH of 55-60%.
- Working distance: ~8 mm
    - NOTE: Higher resolution imaging is achieved by moving the sample closer to the pole piece; the final lens performs better when the sample is at about 5mm working distance. At this closer distance, the chamber pressure will need to be higher.

All samples were then studied under magnifications 100X, 200X, 400X, 800X, 1600X and 3000X. EDX microanalysis was also conducted on all samples for full assessment of chemical element composition, including calcium, magnesium and silicon. Images were then analyzed with XT Docu (FEI inc.) software. Texture measurements were performed using Adobe® Photoshop® CS6 Extended; this software enables measurement of distances between two points on an image within a 2% margin of error. All observations were performed with the SEM expert being blinded to the identity of the respective patients.

Results (Part 1) – Is Environmental Scanning Electron Microscopy a Pertinent Tool for the Analysis of Periprosthetic Breast Capsules?

[Is Environmental Scanning Electron Microscopy a Pertinent Tool for the Analysis of Periprosthetic Breast Capsules?]

Le microscope électronique à balayage environnemental est-il un outil pertinent pour l'analyse des capsules periprothétiques mammaires?

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Author Roles:

Laurence S. Paek: Reviewed literature, designed study protocol, coordinated study sample collection, compiled and analyzed data, wrote and compiled manuscript

Jean-Olivier Tétreault-Paquin: Reviewed literature, participated in sample collection and analysis, reviewed medical charts, revised manuscript.

Samuel St-Jacques: Participated in study sample collection and analysis, reviewed medical charts, revised manuscript.

Monica Nelea: Designed SEM laboratory protocol, performed SEM analysis, revised manuscript.

M. Alain Danino: Designed study protocol, supervised overall study, performed surgical cases, revised manuscript.

Abstract

Purpose:

Scanning electron microscopy (SEM) is a powerful analytical tool that allows the study of interactions between commonly used biomaterials and the human body. In conventional SEM (Hi-Vac), hydrated biological samples cannot be analyzed in their natural state and must be dried and metallized.

The primary goal of this study is to present recent developments in SEM, notably Environmental SEM (ESEM). The secondary objective is to define the potential utility of these new technologies in the study of periprosthetic breast capsules.

Materials and Methods:

Our pilot study group prospectively included 10 patients with breast cancer undergoing 2-stage expander to implant reconstruction. Periprosthetic breast capsule specimens were sampled during expander removal. Each sample was analyzed using both Hi-Vac and ESEM modalities. Energy dispersive X-ray (EDX) studies were also conducted in order to assess the chemical composition of the capsular tissue samples. Under each observation mode, comparisons of samples` three-dimensional surface relief, cellular composition and biofilm presence were made. For each image, a score from 1-3 on a Likert scale was attributed by 3 independent experts in electron microscopy.

Results:

Hi-Vac mode was found to be superior to ESEM for the assessment of the 3 main study parameters (surface relief, cellularity, biofilm). The quality of the EDX analysis was equivalent under both SEM modalities.

Conclusion:

Hi-Vac mode was shown to be more appropriate than ESEM for the global analysis of periprosthetic breast capsules. EDX analysis permits the identification of atypical chemical elements in tissue samples.

Key Words:

Scanning electron microscopy (SEM); implants; capsule; High Vacuum; ESEM

Résumé

But de l'étude:

Le microscope électronique à balayage (MEB) est un vieil allié dans notre compréhension des interactions entre les biomatériaux que nous utilisons et le corps humain. Avec le MEB conventionnel (Hi-Vac), les échantillons biologiques ne peuvent être observés directement du fait de leurs fortes hydratations.

Le but principal de cet article est de présenter les révolutions récentes en MEB, notamment le mode environnemental (ESEM). L'objectif secondaire sera de définir les intérêts potentiels de ces technologies dans l'analyse des capsules périprothétiques.

Patients et Méthode :

Il s'agit d'une étude prospective sur 10 patientes atteintes de cancer du sein en cours de reconstruction par expanseurs-prothèses. Lors de l'exérèse de l'expanseur, un échantillon de capsule périprothétique a été prélevé. Chaque échantillon a été examiné en Hi-Vac ainsi qu'en ESEM. Une analyse EDX (spectroscopie X à dispersion d'énergie) a été effectuée afin d'identifier les composants chimiques dans le tissu capsulaire. Pour chaque modalité, nous avons comparé les informations concernant la texturation de la surface capsulaire, le décompte cellulaire et la présence d'un biofilm sur l'interface. Pour

chaque image un score de 1 à 3 selon une échelle de Likert a été attribué par 3 experts en microscopie électronique indépendant.

Résultats:

Le mode Hi-Vac apparaît supérieur au mode ESEM concernant la définition de la texturation, l'identification des cellules et la présence d'un biofilm. L'analyse EDX permet dans les 2 modes une analyse équivalente.

Conclusion:

Le mode Hi-Vac s'avère être plus appropriée que le mode ESEM dans l'analyse des capsules périprothétiques. L'analyse EDX permet de mettre en évidence des éléments chimiques atypiques.

Mots-clés:

microscopie électronique à balayage (MEB); implants; capsule; High Vacuum; ESEM

Introduction

Historiquement les implants mammaires représentent une source de tension constante entre les chirurgiens, les institutions sanitaires et les patientes, ceci ayant amené plusieurs moratoires dans le monde[102, 103]. Cette situation nous oblige à inclure dans notre corpus scientifique des éléments de compréhensions et d'analyses sur les produits que nous mettons en place. L'actualité récente nous montre que nous ne pouvons nous fier totalement aux industriels pour nous donner ces informations[104]. Le microscope électronique à balayage (MEB) permet d'obtenir des images de surfaces de pratiquement tous les matériaux solides, à des échelles allant de celle de la loupe (10X) à celle du microscope électronique en transmission (500 000X ou plus). Ces images frappent d'abord par le rendu très parlant du relief et la grande profondeur de champ. Équipé de détecteurs appropriés, le MEB permet de faire entre autres de la microanalyse spectroscopie X à dispersion d'énergie (EDX), une analyse élémentaire locale[4-6, 105, 106].

Le MEB conventionnel fonctionne dans un vide ordinaire (10-5 à 10-6 mbar [0.001 – 0.0001 Pa]); les échantillons peuvent être massifs, de dimension allant de quelques µm (particules) à une dizaine de cm de diamètre, voire plus (prélèvements industriels). Ils doivent supporter le vide sans le polluer et être conducteurs ; la préparation est en général simple. Le MEB à pression contrôlée, depuis la fin des années 90 (dit environnemental ou « low vacuum ») permet l'observation dans un vide allant jusqu'à 30

mbar (3000 Pa), rendant ainsi possible l'examen d'échantillons humides ou gras (échantillons biologiques), d'isolants sans métallisation préalable (céramiques, métaux corrodés), voire en présence de liquide.

Des observations ont été régulièrement publiées sur des échantillons biologiques dans différentes spécialités : les muqueuses intestinales et pulmonaires, l'os, et plusieurs microorganismes ont ainsi été étudiées avec ce nouvel outil[107-109]. Parallèlement au développement du mode environnemental, le microscope conventionnel bénéficiera de plusieurs avancées permettant notamment de faire un séchage à l'air sans passer par la substitution de l'eau à l'alcool et la déshydratation au point critique. La révolution photographique numérique a bénéficié grandement à la microscopie électronique permettant l'obtention et le traitement d'images beaucoup plus facilement.

L'analyse des capsules périprothétiques est l'application la plus connue du MEB en chirurgie plastique[35, 36]. Le but de ce travail est de comparer pour la première fois en chirurgie plastique les observations faites sur des capsules periprothétiques avec le MEB conventionnel « high vacuum » (Hi-Vac) et le MEB environnemental (ESEM). Les performances de ces deux modes d'observation pour détecter les compositions et modifications structurales des capsules périprothétiques sont évaluées et discutées.

Patients et Méthode

Il s'agit d'une étude d'impact prospective incluant des patientes suivies dans notre centre hospitalier universitaire pour reconstruction du sein post-cancer par technique de prothèse d'expansion mammaire. Ces reconstructions étaient réalisées avec les 2 prothèses les plus répandues sur notre marché nord-américain : le Mentor SILTEX® et Allergan BIOCELL®. L`étude impliquait la participation de 5 chirurgiens plasticiens. Lors de l'intervention de remplacement de la prothèse d'expansion par un implant définitif, 1 cm^2 de capsule périprothétique est prélevée face au dôme de la prothèse. Sont alors colligés la présence d'une double membrane, l'adhérence capsule-prothèse et la présence d'un liquide pseudosynovial. Le prélèvement est immédiatement orienté avec un fil sur la surface capsulaire en contact avec la prothèse. Le prélèvement est ensuite fixé dans du glutaraldehyde 2% et du cacodylate de sodium 0.1 M pour stabiliser les structures cellulaires à un ph de 7.3. Le prélèvement est conservé au réfrigérateur à 4^oC pour au moins 24 heures.

Avant l'observation, chaque échantillon est divisé en deux parties égales d'au minimum 3 x 3 mm. L'une sera examinée en Hi-Vac et l'autre en ESEM.

- Pour l'examen en microscopie électronique conventionnelle Hi-Vac, les échantillons sont lavés à l'eau, séchés à l'air pendant 20 minutes, puis pulvérisés

d'or avec le pulvérisateur manuel AGAR pour une métallisation à faible pression négative.

- L'examen en ESEM ne requiert aucune préparation supplémentaire ; les échantillons sont maintenus à 60 % d'humidité avec une pression à 3.6 torr (480.0 Pa) et une température de 4°C.

Nous avons utilisé un microscope (Quanta 200 FEG, FEI Company, Hillsboro, OR, USA) avec détecteur EDAX. Les observations dans les deux modes ont été faites à grossissement 800X, 1600X et 3000X. Toutes les images Hi-Vac et ESEM sont analysées avec le logiciel XT docu (FEI inc.)

Trois experts indépendants spécialistes en MEB ont d'abord déterminé les éléments observables dans toutes les images sans distinction de mode. Toutes les images étaient revues par les analystes qui listaient les éléments qu'ils observaient sur chaque photo. Seuls les paramètres communs aux 3 observateurs étaient gardés. Ceci permettant l'établissement de paramètres d'observation.

Puis nous avons comparé ces paramètres sur les 2 modes d'observation Hi-Vac et ESEM, soit avec un score de Likert de 1 à 3 (1 = faible qualité, 2 = qualité moyenne et 3 = qualité excellente) déjà utilisé par van Heerden et al.[96] soit avec un décompte quand le paramètre s'y prêtait. Pour chaque échantillon dans chaque mode d'observation les analystes disposaient de 3 photographies; ils donnaient un score global de performance

pour le paramètre d'observation. Nous avons donc eu un total de 30 scores Hi-Vac et 30 ESEM.

La détection d'un biofilm à l'observation des cliches était noté « oui » ou « non » par chaque observateur pour chaque échantillon et chaque mode d'observation. Notons que les caractéristiques principales recherchées étaient une couche pseudoacellulaire ainsi que la présence de cellules coccoïdes compatibles avec le *S. epidermidis.* Les résultats étaient comparés à la détection lors de la chirurgie de pseudomembrane, ou de liquide pseudosynovial.

Une analyse statistique de ces scores a été faite au test T de student.

Pour vérifier la fiabilité de nos évaluations, nos 3 experts indépendants ont relu toutes les images en les classant selon les paramètres choisis. Enfin, un taux de corrélation a été établit entre les différentes mesures et celle de notre équipe.

Une microanalyse Spectroscopie X à dispersion d'énergie (EDX), une technique utilisée en combinaison avec le MEB pour l'analyse chimique élémentaire des échantillons, a été effectuée pour tous les échantillons en mode Hi-Vac et ESEM.

Résultats

Dix patientes ayant un cancer du sein -reconstruit par technique de prothèse d'expansion mammaire- ont été inclus dans cette étude pilote prospective. Dix prélèvements ont été réalisés lors de l'intervention de remplacement de la prothèse d'expansion par un implant définitif. Ce qui a permis de faire 10 observations en Hi-Vac et 10 en ESEM. Dans chaque mode des photographies ont été faites à 800X, 1600X et 3000X de grossissement, ce qui a constitué une banque totale de 60 photographies. Par ailleurs, 20 microanalyses EDX (analyse de composition locale) ont été réalisées.

Détermination des paramètres d'observation :

Tous les observateurs ont stipulés qu'ils pouvaient pour chaque échantillon :

1. déterminer le degré de texturation de la capsule (fig. 12),
2. faire le compte et la caractérisation des cellules sur la capsule (fig. 13),
3. déterminer la présence d'un biofilm sur la capsule (fig. 14)

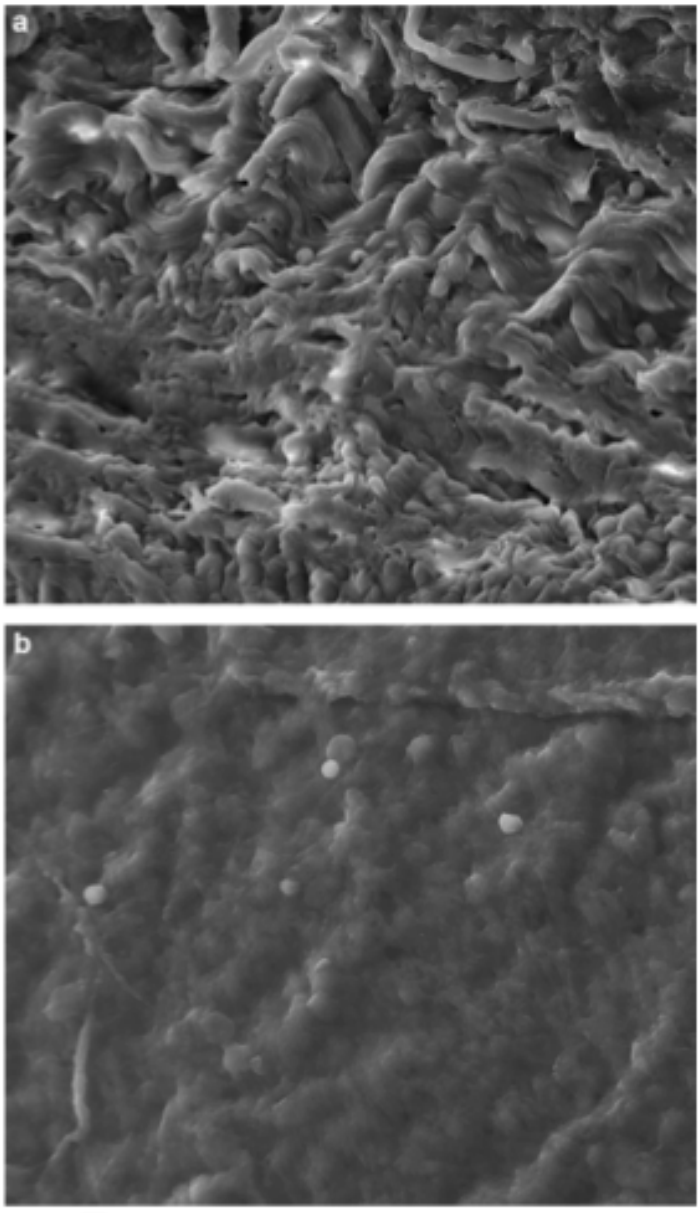

Figure Clichés avec magnification 1600× d'un échantillon de capsule périprothétique en mode microscope électronique à balayage (MEB) conventionnel (HiVac) (a) et MEB environnemental (ESEM) (b). Le mode HiVac permet une meilleure visualisation du relief tridimensionnel ainsi que la possibilité de mesurer les dimensions des aspérités.

Figure 12: SEM images (1600X magnification) of a periprosthetic capsule sample under conventional Hi-VAC (a) and ESEM modes (b). Hi-Vac mode permits better visualisation of 3-D relief as well as measurement of textural dimensions.

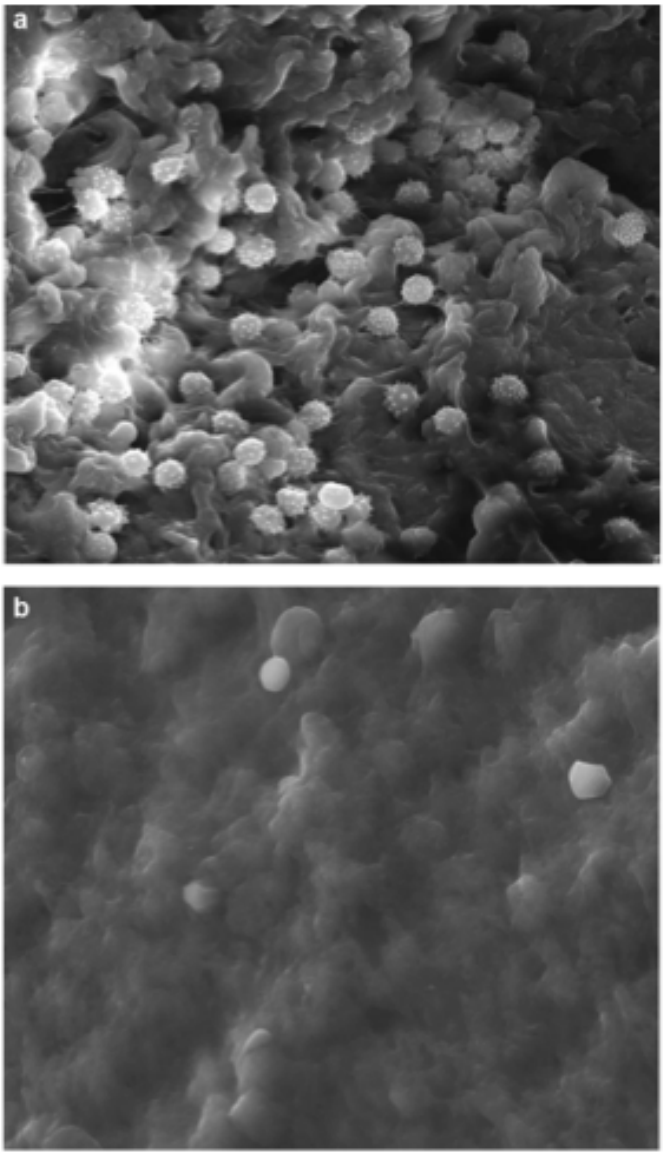

Figure 1 Clichés avec magnification 3000× d'un échantillon de capsule périprothétique en mode microscope électronique à balayage (MEB) conventionnel (HiVac) (a) et MEB environnemental (ESEM) (b). Le mode HiVAC permet un décompte plus précis des cellules et facilite leurs caractérisations ; les échinocytes (érythrocytes dont la membrane cellulaire était modifiée) constituent le type cellulaire majoritaire de cet échantillon.

Figure 13: SEM images (3000X magnification) of a periprosthetic capsule sample under conventional HI-Vac (a) and ESEM modes(b). Hi-Vac mode allows more precise cellular quantification and characterisation: echinocytes (red blood cells with modified cellular membranes) constitute the majority cell type in this sample.

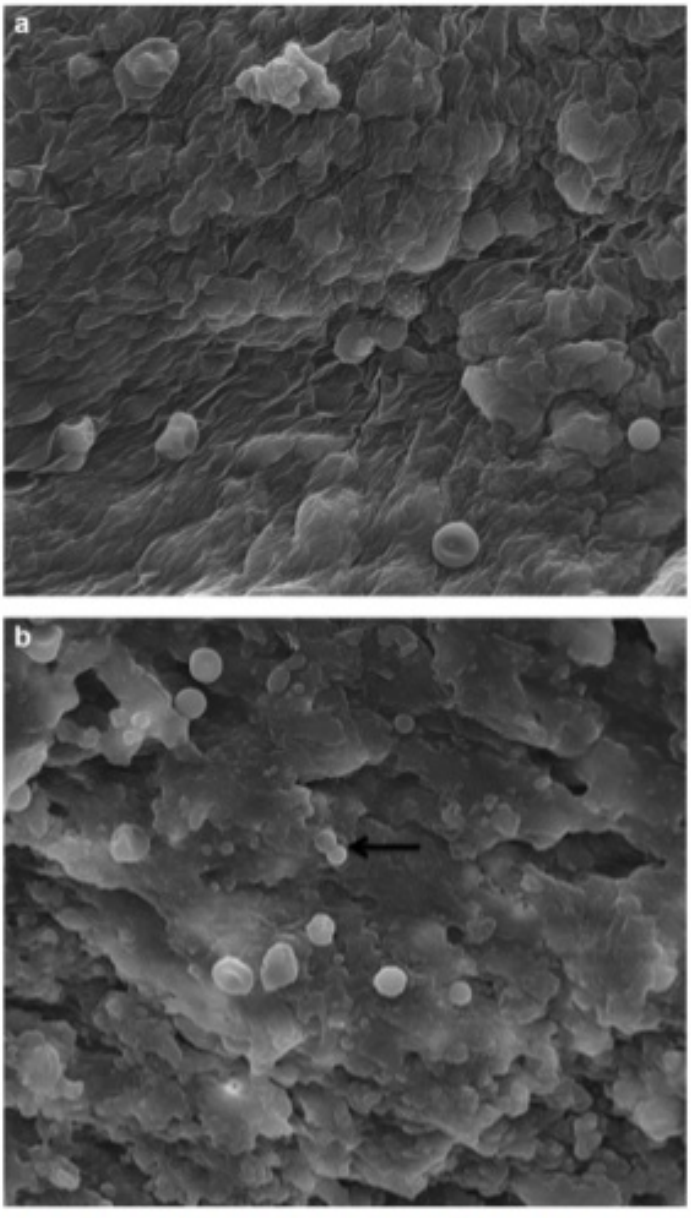

Figure Clichés avec magnification 3000× d'un échantillon de capsule périprothétique avec biofilm en mode microscope électronique à balayage (MEB) conventionnel (HiVac). L'aspect pseudo-acellulaire du biofilm est bien visualisé (a). Des cellules coccoïdes compatibles avec du *Staphylococcus epidermidis* sont indiquées par la flèche (b).

Figure 14: SEM images (3000X magnification) of a periprosthetic capsule sample with biofilm presence under conventional Hi-Vac (a) and ESEM modes (b). The pseudoacellular aspect of biofilm is well visualized (a). Coiccodal cells compatible with S. epidermidis are indicated by the arrow (b).

Comparaison des performances entre Hi-Vac et ESEM (tableaux IV et V) :

1. Détermination du degré de texturation: Le score moyen de performance du mode Hi-Vac était de 30 contre 15 en ESEM. Une supériorité statistiquement significative pour Hi-Vac. Le taux de corrélation entre les observateurs était de 83%.
2. Décompte et caractérisation cellulaire : les cellules retrouvées étaient des globules rouges, des échinocytes (érythrocytes dont la membrane cellulaire était modifiée) et des lymphocytes. Le score moyen de performance du mode Hi-Vac était de 30 contre 12 en ESEM. Une supériorité statistiquement significative pour Hi-Vac. Le taux de corrélation entre les observateurs était de 100%.
3. Biofilm : La présence de biofilm était constatée dans 3 échantillons en mode Hi-Vac. Le biofilm n'a jamais pu être identifié en ESEM. Le taux de corrélation entre les observateurs était de 100%. De plus, la corrélation entre l'identification en MEB Hi-Vac et la clinique était de 100%.

Tableau Sommaire des paramètres d'étude et performances.

Paramètre	Mode d'opération MEB supérieur
Détermination du degré de texturation	HiVac
Décompte et caractérisation cellulaire	HiVac
Présence de biofilm	HiVac
Analyses EDX	HiVac et ESEM

MEB : microscope électronique à balayage ; HiVac : MEB conventionnel ; ESEM : MEB environnemental ; EDX : spectroscopie X à dispersion d'énergie.

Table IV: Summary of study parameters and SEM modality performances.

Tableau Taux de corrélation entre les différents observateurs pour les paramètres d'observation.

Paramètre	Correlation entre observateurs (%)
Détermination du degré de texturation	83,3
Décompte et caractérisation cellulaire	100
Présence de biofilm	100

Table V: Correlation rate between different observers for study parameters.

Spectroscopie X à dispersion d'énergie (EDX) :

La qualité de l'analyse élémentaire de la caractérisation chimique des échantillons était strictement identique dans les deux modes d'observation.

Discussion

MEB dans la recherche en chirurgie plastique: Revue de la littérature des 20 dernières années

Encouragés par les succès initiaux des implants mammaires recouverts d'une couche de polyuréthane texturé à résister à l'évolution de la contracture capsulaire, les implants de silicone à surface texturée ont été développés comme alternative dans les années 1980. On espérait que ces nouveaux produits puissent conserver les avantages du revêtement texturé proposés, tout en évitant les inquiétudes liées à l'hydrolyse de polyuréthane dans l'organisme ainsi que le délaminage potentiel de la surface texturée. Les implants de polyuréthane ont été retirés du marché volontairement par le fabricant en 1991. Parmi les types d'implants de silicone texturée qui ont été initialement popularisés, et continuent d'être utilisés aujourd'hui, sont le SILTEX® de Mentor et le BIOCELL® d'Allergan. La surface SILTEX® est obtenue par le moulage de la couche externe de trempage sur un support rugueux[36]. Allergan utilise la technique « lost-salt » plus agressive ; la surface est posée avec une pression sur un lit de cristaux de sel calibré de

petite taille[36]. De nombreuses études ont démontré que ces implants texturés sont généralement efficaces pour réduire l'incidence de contracture capsulaire[75]

Depuis les années 1990, l'ultrastructure des capsules périprothétiques a généré beaucoup d'intérêt. Par conséquent, le MEB conventionnel est devenu un outil précieux dans la recherche en chirurgie plastique. Les études antérieures sur des échantillons provenant de sujets humains ont fourni quelques détails initiaux concernant la structure et la composition de la capsule[110, 111]. Autour de la même période, Del Rosario et al. ont pu identifier de véritables membranes synoviales entourant 7 des 15 implants mammaires retirés en raison d'une contracture capsulaire. Leurs analyses au MEB de ces capsules ont révélé des concentrations élevées de particules de silicone à l'intérieur de cellules phagocytaires ainsi que dans le stroma collagéneux. Ils ont proposé une fuite de gel de silicone comme un facteur contribuant à cette métaplasie synoviale[112]. Dans une étude antérieure réalisée sur des modèles de lapin, Whalen et al. ont aperçu que la radiothérapie modifiait les propriétés angiogéniques et cellulaires des capsules[113]. À la suite de ces premières études, il s'est avéré que de nombreux facteurs environnementaux, soit internes ou externes, avaient probablement des influences très particulières sur l'évolution de la capsule périprothétique.

En utilisant le MEB, la complexité de l'architecture capsulaire a ensuite été rendue plus évidente par Rubino et al.; ils ont effectué des coupes tangentielles sur des capsules provenant d'implants texturés sous-pectoraux retirés en raison d'une contracture Baker

grade III. Une structure à 5 couches a été observée dans ces échantillons, et celle-ci comprenait une "couche vasculaire" interne ainsi qu'externe. Il est intéressant de noter que cette architecture multicouche n'était pas identifiable dans leurs échantillons de contrôle provenant d'implants sans contracture. Cependant, les modèles d'implants texturés concernés dans l'étude n'étaient pas spécifiés[97].

Au cours de la dernière décennie, l'essentiel de la réflexion semble s'être réorienté vers les subtilités de l'ultrastructure de la surface prothétique et ses effets subséquents sur la réponse capsulaire. Le MEB conventionnel a continué à jouer un rôle clé dans ces études. En 2001, les différences ultrastructurales des surfaces Siltex™ et Biocell® ont été décrites et illustrées en détail. En illustrant la réponse en miroir capsulaire-tissu, ou « l'effet Velcro », l'étude a confirmé la capacité de la surface Biocell® d'accommoder la croissance de tissus. Par ailleurs, un patron de fibrose linéaire a été observé chez les capsules provenant d'implants de gamme SILTEX®[35].

Également par MEB, Barr et al. ont mené une étude observationnelle de la surface des implants Mentor SILTEX®, Allergan BIOCELL®, Allergan lisse, Cereplas CEREFORM® et Polytech Microthane® (micropolyuréthane). Les images présentées ont ajouté de nouvelles informations portant sur les topographies nanométriques variées de ces produits[98]. En créant un milieu de culture comprenant des fibroblastes de souris, Prasad et al. ont étudié la relation entre la topographie superficielle et la cicatrisation afin d'arriver à mieux comprendre la formation capsulaire. Ils ont préparé des

échantillons d'élastomère de silicone avec des rugosités de surface variées; les degrés de rugosité des échantillons ont été analysés qualitativement par le MEB et quantitativement en utilisant des outils associés, soit le microscope à force atomique (MFA). Il est intéressant de noter qu'en utilisant un essai PicoGreen®, la croissance des fibroblastes a diminué face à l'augmentation de la rugosité de surface. De plus, les surfaces lisses ont révélé des concentrations considérablement plus élevées en fibroblastes[99]. Des études menées par Dalby et al. ont démontré que les filopodes cellulaires, des protrusions sensorielles, sont capables de détecter des ilots nanométriques aussi petits que 10 nm[100]. Ces observations soulignent la pertinence de certaines caractéristiques de la surface des implants en ce qui concerne l'évolution naturelle de la capsule adhérente et peuvent avoir des implications pour les futures techniques de fabrication des produits.

D'autres études, tout en utilisant le MEB comme outil d'étude, ont fourni d'importantes nouvelles données dans l'analyse de la capsule avec des implications cliniques plus palpables. Le MEB a joué un rôle important dans l'analyse des biofilms dans les échantillons de capsules mammaires. Pajkos et al. ont démontré l'existence de vastes dépôts amorphes biologiques sur les implants mammaires retirés pour cause de contracture capsulaire. Il est à noter que les images MEB ont été en mesure de révéler un biofilm sur un échantillon de capsule, malgré une culture négative bactérienne. Les auteurs ont postulé que le biofilm bactérien induit et accélère la formation

capsulaire[85]. Tamboto et al. ont exploré cette hypothèse dans leur étude d'inoculation de S. *epidermidis* autour d'implants Biocell® remplis de gel dans les modèles porcins. Les cellules coccoïdes enfermées dans une matrice de glycocalyx ont été observées au MEB sur tous les échantillons de capsules possédant un biofilm; la culture bactérienne était négative dans 19% des biofilms. Les auteurs ont remarqué un risque 4 fois plus élevé de développer une contracture lorsque l'on compare l'inoculation aux groupes de contrôle[86]. Les expériences in vitro de van Heerden et al. sur les effets des différents agents de revêtement antibactériens sur la formation de biofilm, ont produit des résultats intéressants qui pourraient conduire à des changements dans la pratique clinique en chirurgie plastique. En utilisant le MEB afin de mesurer la formation de biofilm sur les disques de silicone texturé et lisse à la fois, ils ont noté que le chloramphénicol, l'acide fusidique et l'oxytétracycline/polymyxine B sulfate étaient supérieurs à la mupirocine, la sulfadiazine d'argent et la néomycine/chlorhexidine en termes de résistance à la formation de biofilm sur une période de 7 jours[96].

Au cours des 20 dernières années, une multitude d'études importantes, provenant de groupes à la fois dans la chirurgie plastique et les sciences fondamentales, ont conduit à une meilleure compréhension de la capsule périprothétique mammaire et les facteurs qui influencent sa formation ainsi que sa contracture. La technologie du MEB conventionnel a été une force motrice de cette recherche, ce qui a permis une

visualisation directe sans précédent de plusieurs des caractéristiques architecturales et cellulaires imperceptibles d'échantillons d'étude.

MEB conventionnel (Hi-Vac) versus environnemental (ESEM)

Jusqu'à présent, les études antérieures sur les prothèses et les capsules, aussi bien dans la chirurgie plastique que dans la littérature des sciences fondamentales, se sont appuyées sur la technologie du MEB conventionnel, qui exige que les échantillons soient déshydratés et conducteurs d'électricité. Comme mentionné auparavant, les échantillons biologiques doivent donc subir un processus de séchage et de revêtement métallique. En évitant les étapes de préparation mentionnées ci-dessus, l'ESEM permet théoriquement une analyse de l'échantillon plus facile, plus rapide tout en réduisant le risque de modifications de surface ou d'artefacts. Au cours de cette étude, nous reconnaissons que l'approche ESEM offre des avantages logistiques en comparaison avec le traditionnel Hi-Vac en ce qui concerne la préparation d'échantillons. Cependant, notre intérêt principal dans cette étude était de comparer directement la qualité et le potentiel des images obtenues afin de déterminer quel mode serait le mieux adapté pour la recherche future sur le surfaçage des prothèses mammaires et la pathologie capsulaire.

En termes d'analyse EDX, le Hi-Vac et l'ESEM se sont avérés être identiques. Cependant, il est important de noter que quelques études dans la littérature biologique environnementale indiquent des potentielles limitations de l'ESEM dans la détection d'éléments[114],[115]. De plus, nos observations comparatives indiquent plusieurs

limitations importantes de l'ESEM à l'égard de l'évaluation des 3 paramètres de notre étude. En ce qui concerne le relief et les aspects cellulaires de la capsule, nous avons constaté que le Hi-Vac offre des images de qualité nettement supérieure, tel que rapporté par nos experts indépendants. Nos taux de corrélation élevés de 83% et 100%, respectivement, nous permettent de juger avec assez d'assurance que le mode de Hi-Vac est préférable pour ces analyses. En revanche, ce qui est encore plus frappant, c'est que nos experts n'ont pas pu identifier les biofilms en utilisant l'ESEM dans les 3 échantillons qui s'étaient révélés positifs à la fois sur la base d'observations de Hi-Vac et d'observations cliniques au moment de l'enlèvement des prothèses d'expansion.

Nos résultats valident certainement les résultats de recherches antérieures effectuées dans ce domaine ; une répétition des études antérieures citées dans notre étude, en utilisant la technologie ESEM, ne serait pas susceptible de produire une analyse de qualité supérieure ou des détails additionnels pertinents. Par conséquent, en ce qui concerne nos paramètres d'étude énumérés, les avantages pratiques de la technologie ESEM actuelle ne justifient pas son utilisation dans des études ultérieures de tissus biologiques périprothétiques. Il est important de noter que selon nos résultats, il n'est pas possible d'extrapoler les conclusions de notre étude à celle d'autres tissus biologiques pertinents pour les chirurgiens plasticiens. Bien que quelques études comparatives MEB conventionnel/ESEM aient attesté la supériorité de l'ESEM en ce qui a trait à la préservation de «l'état naturel» dans la matrice extracellulaire végétale[116] et

dans la muqueuse intestinale de rat[109], cette dernière étude remarque que les 2 modes doivent néanmoins être utilisés conjointement afin d'obtenir une évaluation plus complète des tissus.

Conclusion

Le mode Hi-Vac permet une meilleure appréciation tridimensionnelle de la texturation ainsi qu'une caractérisation et un décompte cellulaire plus précis à la surface des échantillons. De plus, il rend possible l'identification de biofilms bactériens ayant un lien démontré dans l'évolution pathologique des capsules. L'analyse EDX permet de mettre en évidence des éléments chimiques atypiques retrouvés dans le tissu capsulaire, peu importe le mode d'observation utilisé. Il est à noter que, bien qu'il soit primordial de réévaluer de façon intermittente les avantages potentiels de la technologie ESEM alors qu'elle poursuit son évolution, nous concluons que le mode Hi-Vac est actuellement un outil de qualité supérieure pour l'analyse complète des capsules périprothétiques.

References

Please refer to complete list of references

Results (Part 2) – The Impact of Postoperative Expansion Timing on Breast Expander Capsule Characteristics: A Combined Clinical and Scanning Electron Microscopy Study

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

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M. Alain Danino: Designed study protocol, performed surgical cases, analyzed data, revised manuscript, supervised overall study.

Abstract

Background: In the first stage of expander-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 prior to 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 2-staged implant-based breast reconstruction were prospectively enrolled in this study. During expander to implant exchange, clinical presence of Velcro effect, biofilm and double capsule was noted. Periprosthetic capsule samples were also sent for scanning electron microscopy (SEM) observation of 3 parameters: surface relief, cellularity and biofilm. Samples were divided into 4 groups for data analysis (G1: conventional/BIOCELL®, G2: delayed/BIOCELL®, G3: conventional/SILTEX®, G4: delayed/SILTEX®).

Results: Fifty-six breast reconstructions were included. Each group comprised between 13 and 15 breasts. In G1, no cases exhibited the Velcro effect and there was a 53.8% incidence of both biofilm and double capsule. In G2, all cases demonstrated the Velcro effect and there were no incidences of biofilm or double capsule. G3 and G4 cases did not

exhibit a Velcro effect or double capsule formation; however, biofilm was present in up to 20.0%. All G2 samples revealed more pronounced 3-dimensional relief on SEM

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.

Background

Two-stage expander to implant breast reconstruction is the most commonly employed technique for post-mastectomy breast reconstruction. According to the American Society of Plastic Surgeons (ASPS) 2012 statistics report, 70.5% of breast reconstructions were achieved using expanders.

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 the current conventional approach with initiation at 10 to 14 days postoperatively[117, 118]. We hypothesize that there are benefits to waiting at least 6 weeks before initiation of postoperative serial expansion since 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[119], at which point the wound has roughly 70% of the tensile strength of normal skin and net collagen synthesis has ceased[120]. These principles may reasonably be applied to periprosthetic capsule evolution. According to Kronowitz, a mature scar capsule requires at least 4 weeks to form[64]. 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[121]. A

combined clinical and ultrastructural evaluation of periprosthetic capsular characteristics following both conventional and delayed expansion approaches is necessary in order to warrant future clinical outcome comparison studies.

Scanning electron microscopy (SEM) is a powerful tool that has been extensively applied in the study of periprosthetic capsules. Previous studies have employed conventional high-vacuum (Hi-Vac) SEM, which necessitates a drying and metallization process, in order 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[35, 36, 98]. Our group has recently demonstrated that conventional Hi-Vac SEM is superior to newer environmental scanning electron microscopy (ESEM) technology for the assessment of breast periprosthetic capsules, despite the ability to directly examine wet, nonconductive biological tissue samples with the latter. Hi-Vac SEM allows excellent assessment of capsule 3-dimensional relief, cellularity and biofilm presence[122].

In this study, we aim to prospectively investigate, using conventional Hi-Vac SEM, whether differing expansion protocols lead to observable modifications in capsular formation around both Allergan BIOCELL® and Mentor SILTEX® expander prostheses. Intraoperative observations regarding expander periprosthetic capsular adhesiveness and biofilm presence will equally be considered.

Methods and Materials

Patients with breast cancer undergoing 2-stage implant-based breast reconstruction were prospectively included in this study prior to second-stage expander to permanent implant exchange surgery. All included patients were treated at the same university hospital center by 1 of 5 plastic surgeons specialized in breast reconstruction. Each surgeon adhered to his or her usual standard surgical technique. Prophylactic antibiotics were administered at induction (1st generation cephalosporin, or if allergic, clindamycin). Skin prepping 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 in order to provide lower pole coverage and, thereby, total submuscular coverage. Prior to 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 2 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 was 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 surgery, the surgeon documented the presence/absence of 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 in order to peel it off, hence simulating the feel of separating 2 actual Velcro surfaces apart. Double capsules were characterized as 2 distinct capsular layers: an 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 cm^2 sample of periprosthetic capsule adjacent to the prosthesis dome was then biopsied; 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 biopsied; 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 0.1M in order 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 SEM in Hi-Vac mode, a 3 x 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. Sample analysis under SEM was performed in blinded fashion on the tagged implant-side of the capsule specimens and the following 3 parameters were assessed: texture (surface relief characterization), cellularity (cell count and characterization) and biofilm (presence/absence), as previously described by our team[122]. EDX microanalysis was conducted in all samples for chemical element composition measurements. All observations were done using a Quanta 200 FEG microscope (FEI Company, Hillsboro, OR) with EDAX detector and XT Docu (FEI inc.) image acquisition software. All samples were studied under Hi-Vac modality with magnifications of 100X, 400X, 1600X and 3000X and collected images were subsequently assembled in Adobe® Photoshop® CS6 Extended.

For sample texture three-dimensional relief analysis, capsular peak and trough dimensions were measured using the Imagan2 computerized image analysis system (Kompira, Strathclyde, UK). Microscopic fields were examined with a 100X phase contrast objective for capsule structural peaks and troughs and 50X for density. The fields were then observed with a video camera and corresponding images 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, either as minimal texture (flat/linear fibrotic) or high texture (rough/non-linear) pattern. All cell counts were performed manually at 100X magnification and densities 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 4 distinct groups, constituted on the basis of expander prosthesis type and time delay until first postoperative saline inflation:

Group 1 (G1): Allergan BIOCELL® - first postoperative expansion at 2 weeks or less (conventional approach)

Group 2 (G2): Allergan BIOCELL® - first postoperative expansion at 6 weeks or more (delayed approach)

Group 3 (G3): Mentor SILTEX®, first postoperative expansion at 2 weeks or less (conventional approach)

Group 4 (G4): 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 8 bilateral reconstructions. Each of the 4 study groups was comprised of between 13 and 15 breast reconstructions. Patient demographic and clinical data were comparable between groups (table VI). All radiotherapy treatment, when applicable, occurred prior to 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 utilized in this study. Other pertinent clinical data regarding first-stage operative and postoperative management details are summarized in table VII.

Group	Number of breast reconstructions	Mean age (years)	Diabetes (%)	Active smokers (%)	Past chemotherapy (%)	Past radiotherapy (%)
G1	13	55.6	7.7	23.1	23.1	23.1
G2	15	55.1	6.7	26.7	20.0	26.7
G3	15	57.3	0.0	13.3	20.0	20.0
G4	13	55.5	0.0	15.4	23.1	23.1

Table VI: Demographic and clinical data, by group.

(G1, BIOCELL® - first postoperative expansion at 2 weeks or less; G2, BIOCELL® - first postoperative expansion at 6 weeks or more; G3, SILTEX® - first postoperative expansion at 2 weeks or less; G4, SILTEX® - first postoperative expansion 6 weeks or more)

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 (%)
G1	444	84.6	28.3	112.4	13.0	81.8	7.7
G2	400	86.7	28.5	103.4	52.0	80.0	13.3
G3	390	86.7	30.0	102.7	13.5	72.3	13.3
G4	450	84.6	31.8	101.1	51.2	69.9	7.7

Table VII: First stage and expansion process clinical data, by group.

(G1, BIOCELL® - first postoperative expansion at 2 weeks or less; G2, BIOCELL® - first postoperative expansion at 6 weeks or more; G3, SILTEX® - first postoperative expansion at 2 weeks or less; G4, SILTEX® - first postoperative expansion 6 weeks or more)

Thorough SEM analysis of each group of samples revealed observable differences with regards to the aforementioned study parameters. SEM observations were correlated with clinical findings made during the second-stage expander to implant exchange surgery.

In G1, there were no clinically observable cases of the Velcro effect. This clinical finding was well corroborated by SEM observations; there was minimal 3-dimensional texture in G1 samples (figure 15a) compared to G2 samples (figure 15b). Furthermore, cellularity was minimal and EDX analysis revealed silicon in the capsular tissue. In 53.8% of breast capsules, a biofilm was clinically identifiable and confirmed under SEM (figure 16). Importantly, 7 cases of double capsule (53.8%) were noted intraoperatively, associated with the same cases possessing a biofilm (figure 17).

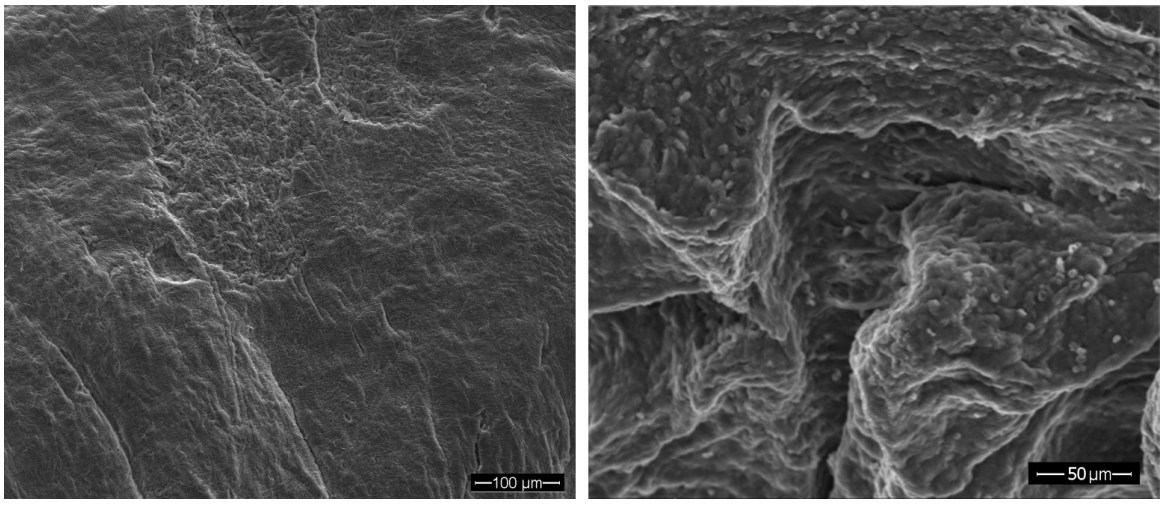

Figure 15: Example of minimal 3-D capsule relief in a conventional BIOCELL® (G1) sample, (a). Example of pronounced 3-D capsule relief in a delayed BIOCELL® (G2) sample (b).

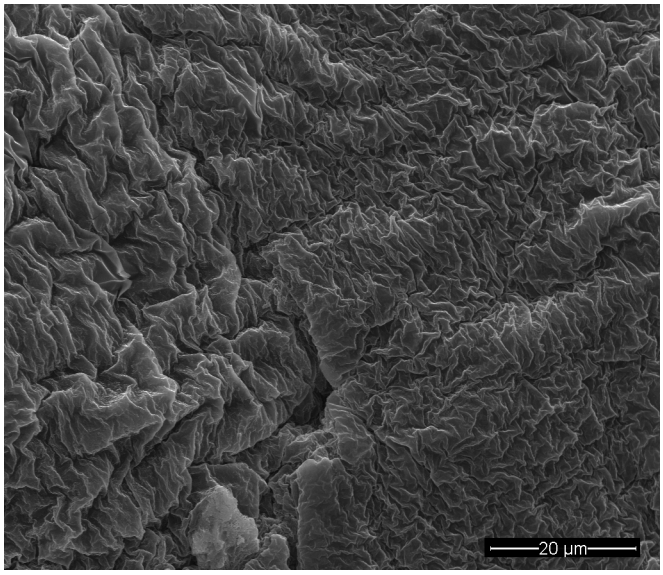

Figure 16: Biofilm presence on the inner surface of capsule tissue in a conventional BIOCELL® (G1) sample.

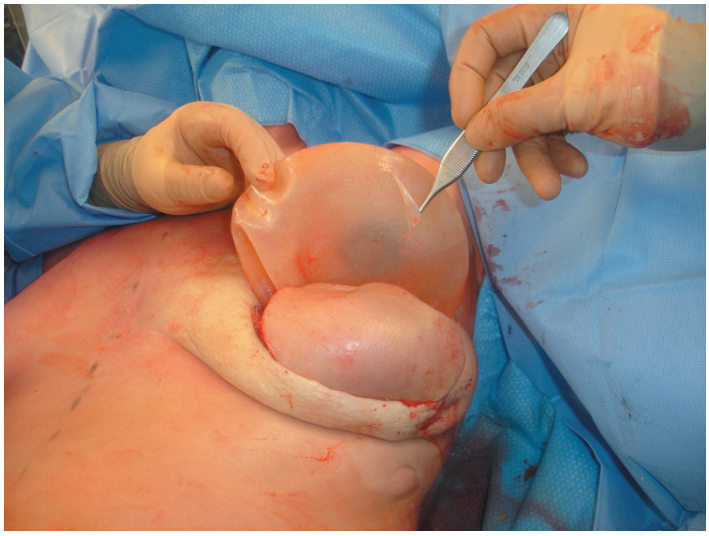

Figure 17: Demonstration of double capsule around BIOCELL® implant from conventional group (G1). The inner adherent capsule is being grasped with the forceps.

In G2, all cases exhibited a clinical Velcro effect and a high level of 3-dimensional texture was noted in the corresponding capsular tissue samples under SEM (figure 15b). A high cellularity was observed in G2 samples with over 40 cells per 40 μm^2; up to 45% 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 expander to implant exchange in these cases; the subsequent SEM analyses confirmed this lack of biofilm. No cases of clinical double capsule were observed intraoperatively.

In G3, no cases of clinical Velcro effect were noted. On SEM, texture was minimal and revealed linear fibrotic patterns (figure 18a). SEM observations also revealed low cellularity with less than 5 cells per 40 μm^2. There were 3 cases of clinical biofilm in G3 samples (20.0%) and this was consistent with parallel SEM observations. No cases of clinical double capsule were observed intraoperatively.

In G4, there continued to be an absence of clinically observable Velcro effect in the samples. On SEM, texture level and cellularity remained low (less than 5 cells per 40 μm^2) and were comparable to G3 samples in these respects (figure 18b). Biofilm was observed in 2 breast capsules intraoperatively (15.4%); again, these findings were corroborated by subsequent SEM analyses (figure 19). No cases of clinical double capsule were observed intraoperatively. Clinical intraoperative observations for all groups are summarized in table VIII.

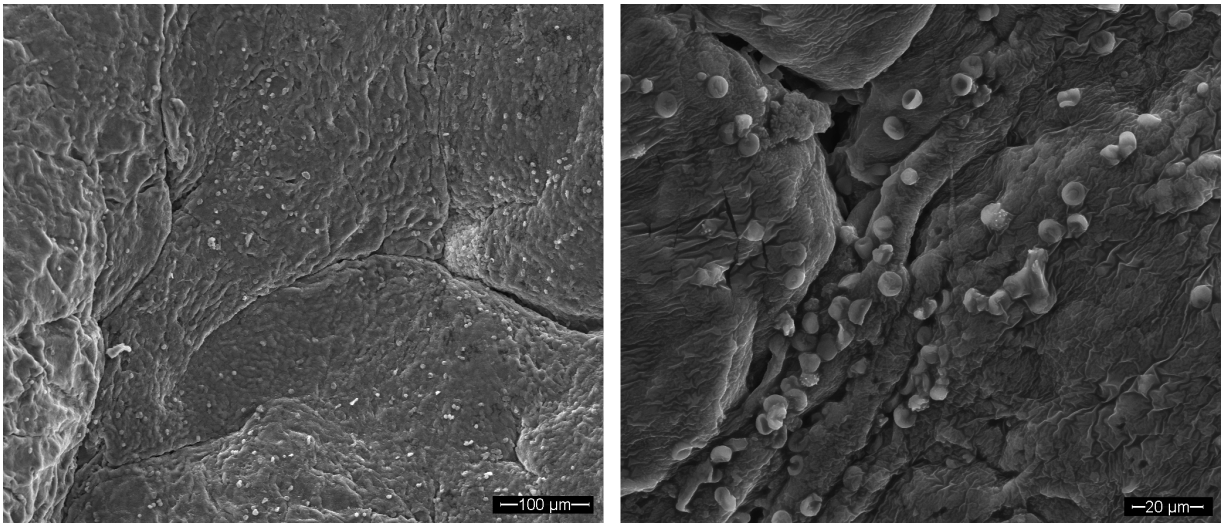

Figure 18: Example of linear fibrotic pattern in a conventional SILTEX® (G3) sample (a). Example of a periprosthetic capsule in a delayed SILTEX® (G4) sample (b). Three-dimensional capsule relief was comparable to that observed in G3 samples.

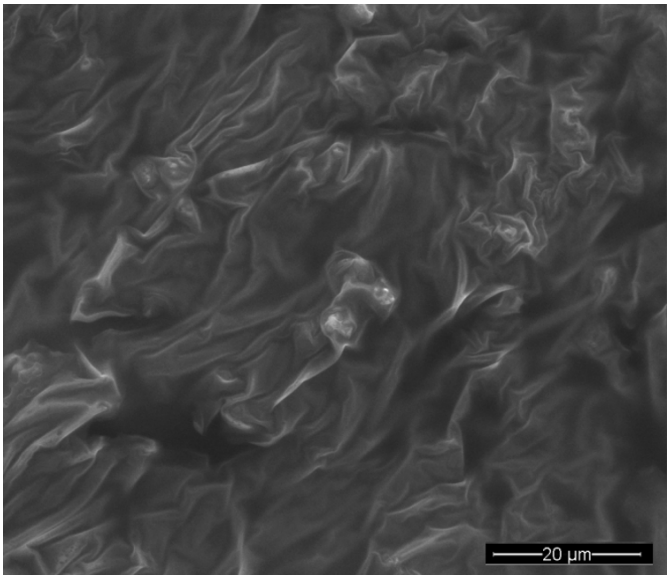

Figure 19: Example of biofilm in a delayed SILTEX® (G4) sample.

Group	Velcro effect (%)	Biofilm (%)	Double capsule (%)
G1	0.0	53.8	53.8
G2	100.0	0.0	0.0
G3	0.0	20.0	0.0
G4	0.0	15.4	0.0

Table VIII: Summary of intraoperative clinical observations, by group.

(G1, BIOCELL® - first postoperative expansion at 2 weeks or less; G2, BIOCELL® - first postoperative expansion at 6 weeks or more; G3, SILTEX® - first postoperative expansion at 2 weeks or less; G4, SILTEX® - first postoperative expansion 6 weeks or more)

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

Discussion

The Mentor SILTEX® texturing is a patterned surface created as a negative contact imprint off of a texturing foam. On the other hand, 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 IX). The SEM analyses reveal noteworthy ultrastructural differences between the conventional and delayed BIOCELL® groups (G1 and G2). In the delayed group (G2), the capsule texture 3-D surface relief was notably more pronounced (rough/non-linear); 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 G2 capsules exhibited the Velcro effect). This may imply that a longer healing and evolution period for the periprosthetic capsule prior to 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 (G3 and G4), no discernable 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 [123-126]. 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[35, 36, 98, 127]. Since 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 3-D texture relief observed in G2 (delayed BIOCELL®) samples with respect to complication rates

and aesthetic outcomes. However, our overall first-stage complication rates were similar between the 4 groups studied and comparable to rates of 8.5 to 11% cited in the literature[16, 17].

	Ultrastructural Texture Feature	Diameter (µm)	Height [H] or Depth [D](µm)	Density/1.5 mm^2
Biocell®	**Depressions**	**600-800**	**[D] 150-200**	**8**
Siltex®	**Nodules**	**70-150**	**[H] 40-100**	**15**

Table IX: Overview of textured implant surface ultrastructural topography.

The finding of double capsules in the conventional BIOCELL® group (G1) deserves special mention. Maxwell et al. define a "double capsule" to be capsular adherence in 2 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[128]. In a large complications review of breast augmentation and mastopexy-augmentation cases, Hall-Findlay reports findings of double capsule in 14 re-operated patients with BIOCELL® textured permanent implants, with 3 cases presenting in the context of late seromas. Hall-Findlay proposes a mechanical etiology, 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[129].

The senior author's belief is that in order to truly optimize the use of the aggressively-textured BIOCELL® expanders in breast reconstruction, a longer delay before initiating postoperative saline inflation is desirable since it allows sufficient maturation and adherence of the developing capsule, as demonstrated clinically and ultrastructurally in our study. We observed 7 cases of double capsule in the conventional BIOCELL® expansion group (G1); we believe that this finding indicates that the immature capsule, which we were able to appreciate under SEM 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 due to 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 SEM 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*[130]. 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 (G1).

While a total of 5 cases of biofilms were found in the SILTEX® groups (G3 and G4), 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 since, in our experience, the periprosthetic capsule never truly adheres to this textured shell. Even if consequential quantities of periprosthetic fluid were to be produced due to shear forces, the nature of the SILTEX® surface would be unlikely to accommodate seeding of cells and eventual inner capsule development.

Conclusion

Our SEM analyses demonstrate that variations in expansion protocols can lead to modifications in periprosthetic capsular architecture. The more pronounced 3-D texture

of capsules in the delayed BIOCELL® (G2) group suggest that there may be real benefits to delaying the first postoperative saline inflation until 6 weeks after BIOCELL® expander implant insertion. Employing 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 in order to determine the ultimate clinical impact of our findings to patients undergoing 2-stage expander to implant breast reconstruction.

References

Please refer to complete list of references

General Discussion

High-Vacuum vs. Environmental Scanning Electron Microscopy

To the best of our knowledge, no previous studies on breast periprosthetic capsules have employed the ESEM mode for sample observation. As stated, ESEM offers the possibility to study biological samples while largely averting complex and artifact-generating manipulations. As it currently stands, ESEM is best applicable to biomaterial and tissue-engineering research. It has served as an important tool for the study of interactions between mammalian cells and biomaterials under development[131, 132]. Therefore, a test of ESEM's applicability to the study of periprosthetic tissue at the implant-capsule interface was deemed necessary. However, as established in the first article, Hi-Vac should remain the gold standard for such analyses. Hi-Vac permits superior 3-D architecture visualization and, perhaps most importantly, overall better image definition. Furthermore, cell and biofilm assessments were also superior with Hi-Vac.

While the metallic coating of samples for Hi-Vac SEM may obscure some fine details on specimen surfaces, FEGs largely obviate this potential drawback[133]. Theoretically, our capsular tissue samples could have been directly observed under ESEM without fixation; however, this presented logistical problems, as the electron microscope was located at an external facility. Furthermore, it is not unusual to apply fixation and drying processes to samples prior to ESEM observation. Schmidt et al. studied neurite outgrowth

stimulation using an electrically conductive polymer (oxidized polypyrole) as a nerve guidance channel between severed nerve ends. The study samples underwent 1% glutaraldehyde fixation, alcohol-dehydration and an overnight air-drying process prior to ESEM observation[134]. Importantly, fixed specimens show cell morphologies similar to those of specimens that are neither dried nor coated[135].

Some authors have emphasized the difficulties of conducting SEM manipulations in environmental or "wet" mode, noting peculiar contrast effects that impede clear imaging of cells and tissues[136]. Our experiments indeed demonstrated that adequate image resolution was difficult to achieve. Additionally, the water vapour environment of the ESEM puts uncoated specimens at potential risk for radiation damage. Water molecules, ionized by the electron beam, may produce free radicals that can attack the organic material of study samples. Kitching and Donald documented this phenomenon in their experiments on polypropylene specimens; they note that the extent of the damage depends principally on operating parameters, including but not limited to accelerating voltage, magnification and spot size[137]. While we did not note any such radiation-induced modifications in our observations, these alterations may skew the evaluation of biomaterials such as silicone implant shells.

While the contributions of ESEM technology to biomedical research are certainly important, its universal application for the study of various biological tissues is far from established. The particular intricacies of specimen-specific operating parameters required with ESEM highlight the need for further studies on methodology

standardization. The published data seems to support our experience; current ESEM technology cannot replace conventional SEM for the study of biological tissues, including periprosthetic capsular tissue. There seems to be a consensus that for the time being, the ESEM should rather serve as a complement to conventional Hi-Vac SEM for a wide array of applications[109, 133]

Postoperative Expansion Timing and Effects on Capsular Architecture: Implications on Capsular Adherence, Double Capsules, Seroma and Biofilm

The first article established the reproducibility of our study protocol and justified the usage of conventional Hi-Vac SEM for subsequent studies of periprosthetic capsular tissue. The second article, as discussed, demonstrates that there are observable modifications in capsular structure, notably in terms of 3-D relief, when a delayed approach to postoperative expander saline inflation is employed for BIOCELL® textured expanders (G2). These capsules showed ultrastructural features compatible with superior tissue adherence than those in the early approach group (G1), which was corroborated clinically by the surgeons during the second-stage expander to implant exchange surgery. These SEM findings suggest that a delayed strategy with BIOCELL® textured expanders could potentially lower incidences of expander prosthesis instability/malposition, wound dehiscence, infection, hematoma and seroma, among others. As noted by Maxwell and Falcone, a more mature and adherent periprosthetic

capsule leads to a more relaxed, softened breast tissue envelope[121]. Based on this logic, a delayed approach with BIOCELL® expanders could also reasonably help improve final aesthetic results in women undergoing two-staged IBR. Needless to say, more extensive clinically focused trials are required before being able to truly extrapolate our SEM observations to clinical complication rates and outcomes in the patient population in question. At the very least, our study provides a robust foundation that can justify further clinical research in breast expansion strategies and protocol standardization. There were no discernable differences in capsular architecture between the two SILTEX® groups (G3 and G4). However, theoretically, there could still be benefits to a delayed approach at least in terms of wound dehiscence and infection by allowing sufficient strengthening of the breast incision scar, which requires about 6 weeks[119]. However, clinical trials would be necessary in order to support this prospective benefit.

While the main focus of the second article was to compare and contrast capsule ultrastructural characteristics between the four groups under SEM, there were additional noteworthy findings, namely the clinical presence of double capsules in 53.8% of early BIOCELL® group (G1) samples. No double capsules were noted on the BIOCELL® capsules sampled from patients undergoing the delayed protocol (G2). Furthermore, none were observed in both SILTEX® groups (G3 and G4).

Maxwell et al. define a “double capsule” to be capsular adherence in two layers (inner adherent to device, and outer adherent to surrounding tissue). There are numerous proposed causes of this presentation: foreign body reactions (FBR), oversized breast

implant pockets, implant micromotions, mechanical shear forces, trauma and infection/biofilm. The authors emphasize that oversized subglandular pockets, lack of perioperative pocket fluid drainage and failure to limit patient postoperative physical activity are the main factors contributing to potential suboptimal implant-capsule adherence and, therefore, double capsule formation[128].

A detailed literature review of the double capsule phenomenon in IBR revealed some interesting information. There were a total of 15 relevant publications describing cases exhibiting double capsules: 2 original articles[129, 138], 1 continuing medical education paper[139], 4 case reports[140-143], and 8 letters[144-151]. Due to the high proportion of letters, important details were often missing; authors were contacted in order to render this review as complete as possible. There were a total of 44 patients reported as having double capsules. Ages ranged from 23 to 62 years. Of the 40 cases in which the original surgery was described, there were 23 bilateral breast augmentations (57.5%), 6 bilateral breast augmentation-mastopexies (15%) and 11 IBRs (27.5%). The plane of implant insertion was described in 39 of these surgeries: 16 were subglandular, 2 subfascial, 18 submuscular, and 3 dual-plane (partial submuscular). Overall, implant types were as follows: 39 BIOCELL® (88.6%); 1 SILTEX® (2.3%); 2 Lipomatrix Inc./AEI Inc. Trilucent™ (4.5%); and 2 unspecified (4.5%). Finally, indication for revision surgery was noted for all 44 patients. There were 10 cases of seroma (22.7%), 2 hematomas (4.5%), 12 capsular contractures (27.3%), and 10 implant rotation/dislocations (22.7%). Lastly, 10 cases (22.7%) were operated for various aesthetic reasons, including ptosis

and size; the double capsules were found incidentally in these cases. For an overview of the reviewed publications, please refer to the appendix.

The most striking information that can be drawn from the reviewed double capsule publications is the implant type implicated. The great majority of double capsules developed with BIOCELL® type implants[129, 138, 140-144, 146-149]. One of the 3 cases reported by Matteucci and Fourie was actually with a BIOCELL® expander, the only double capsule case in an expander documented in the literature to date[146]. Maxwell et al., based on Allergan internal company data and studies conducted by Hedén et al. (unpublished), estimate the incidence of double capsule with BIOCELL® implants as being between 0.019 to 1 percent[128].

The phenomenon of double capsule is relatively new and its exact pathophysiologic mechanisms have yet to be elucidated. In a large complications review of breast augmentation and mastopexy-augmentation cases, Hall-Findlay reports findings of double capsule in 14 re-operated patients. This double capsule was only found with BIOCELL® textured permanent implants in her series; the author suggests a mechanical etiology[129]. Hall-Findlay believes that the double capsule results from incomplete adherence of the capsule to the corresponding textured surface, with subsequent detachment of the implant-capsule complex and seroma development due to shear forces at this interface; seeding of cells from the serous fluid then leads to a new adherent inner capsule. Past SEM studies by Danino et al. comparing BIOCELL® and SILTEX® implant surface features confirmed that only the former possesses the critical

pore size necessary to allow some degree of tissue ingrowth (table IX). As such, the BIOCELL® surface would be most likely to accommodate seeding from seroma fluid as proposed by Hall-Findlay.

In a letter replying to Hall-Findlay's artcle, Dini et al. reported their own experience with BIOCELL® implants and double capsules and agreed with her version of the mechanical theory. In their 10 cases of double capsules, which were discovered either incidentally or in the context of implant rotation, they replaced the BIOCELL® implants with polyurethane-covered ones, noting that capsules around the latter actually exhibit greater adherence and genuine tissue ingrowth; over 5 years of follow-up, no complications have been reported in these patients[149]. Toscani et al. further noted significant calcifications, an indication of long-standing foreign-body inflammatory response, only on the inner capsule of their reported case with an unspecified textured implant; the outer capsule remained collagenous. This led them to believe that the inner capsule is the second to form, which is also consistent with Hall-Findlay's proposed mechanism[151]. Other authors that support the mechanical theory of the double capsule phenomenon in BIOCELL® implants suggest that the initial detachment actually occurs between the implant-capsule complex and the surrounding breast tissue[144, 146, 148]. Pandya and Dickson suggest that a hematoma subsequently forms and develops a new outer capsule layer[144]. Robinson believes that normal periprosthetic pocket fluid prevents reattachment of this new implant-capsule complex to the breast tissue, leading to the formation of a second outer capsule that may cause subsequent

contracture[148]. Regardless of the level at which the separation occurs, the implant subsequently becomes prone to dynamic malrotation because of the new, smoother interface between the 2 capsular leaflets[146]. Only one case of SILTEX® type textured implants has been described in the literature[139]*;* this is unsurprising; in our experience, the capsule doesn't truly adhere to the SILTEX® surface to begin with. Other authors with extensive experience in breast surgery also attest to this lack of adherence[152]. To date, no cases of double capsule with polyurethane foam-covered breast implants have been documented in the literature. The implant-capsule complex of these polyurethane implants have been found to be extremely robust, thereby minimizing any possibility of detachment from minor trauma or shear stress.

The report by Colville et al. of 2 cases of double capsule formation around the now discontinued oil-based Trilucent™ implants deserves specific mention. They postulated that a second outer capsule was triggered by a new FBR secondary to implant bleed through the initial inner capsule. Their histopathological analyses demonstrated implant material and inflammation in both capsules[145, 153]. Pandya and Dickson note that they have found partial Trilucent™ double capsules in the past as well[144]. The fragility and bleed tendency of these discontinued implants are well recognized; however, a mechanical cause is still most likely. The textured surface of these Trilucent™ implants was known to exhibit the Velcro effect much like its BIOCELL® counterpart[154]. As such, incomplete adherence and frank detachment of the immature capsule likely contributed in the cases of double capsule formation in these Trilucent™ implant devices

as has been suggested with BIOCELL® textured devices. Implant bleed and FBR would explain a thickened contracted capsule as has been extensively documented with older generation implants. It is hard to imagine that such an inflammatory reaction would lead to the formation of two distinct capsule layers. FBR-type inflammation has been documented in a BIOCELL® double capsule accompanied by periprosthetic seroma between the two capsular leaflets, as described in the Hall-Findlay paper[129, 141]. In fact, presence of FBR inflammatory remnants in the outer capsule are consistent with the aforementioned theory that the initial detachment takes place at the implant-capsule interface, since it indicates that both capsule layers were in contact with the implant device at some point. Capsule samples in our lab demonstrate that implant particles accompanied by reactive inflammation are not uncommon, even with the thick, "low bleed" shells of modern expanders and permanent implants (figure 22).

Based on the body of evidence available in the literature, the underlying cause of double capsule formation is almost certainly mechanical. Our findings further support the mechanical theory and suggest that the detachment occurs at the level of the initial capsule-implant interface. It is our belief that in order to truly take advantage of BIOCELL® texture features in breast tissue expansion, a longer delay before initiating postoperative saline inflation is necessary to allow sufficient maturation and adherence of the developing capsule. In the 7 double capsule cases (53.8%) from the early BIOCELL® expansion group (G1), the immature capsule likely partially separated from the implant expander shell, thereby creating a potential space with fluid production due

to frictional forces, followed by formation of a new inner capsule. Minor traumas to the breast and, more importantly, the shearing force caused by expander inflation most probably provoked this separation.

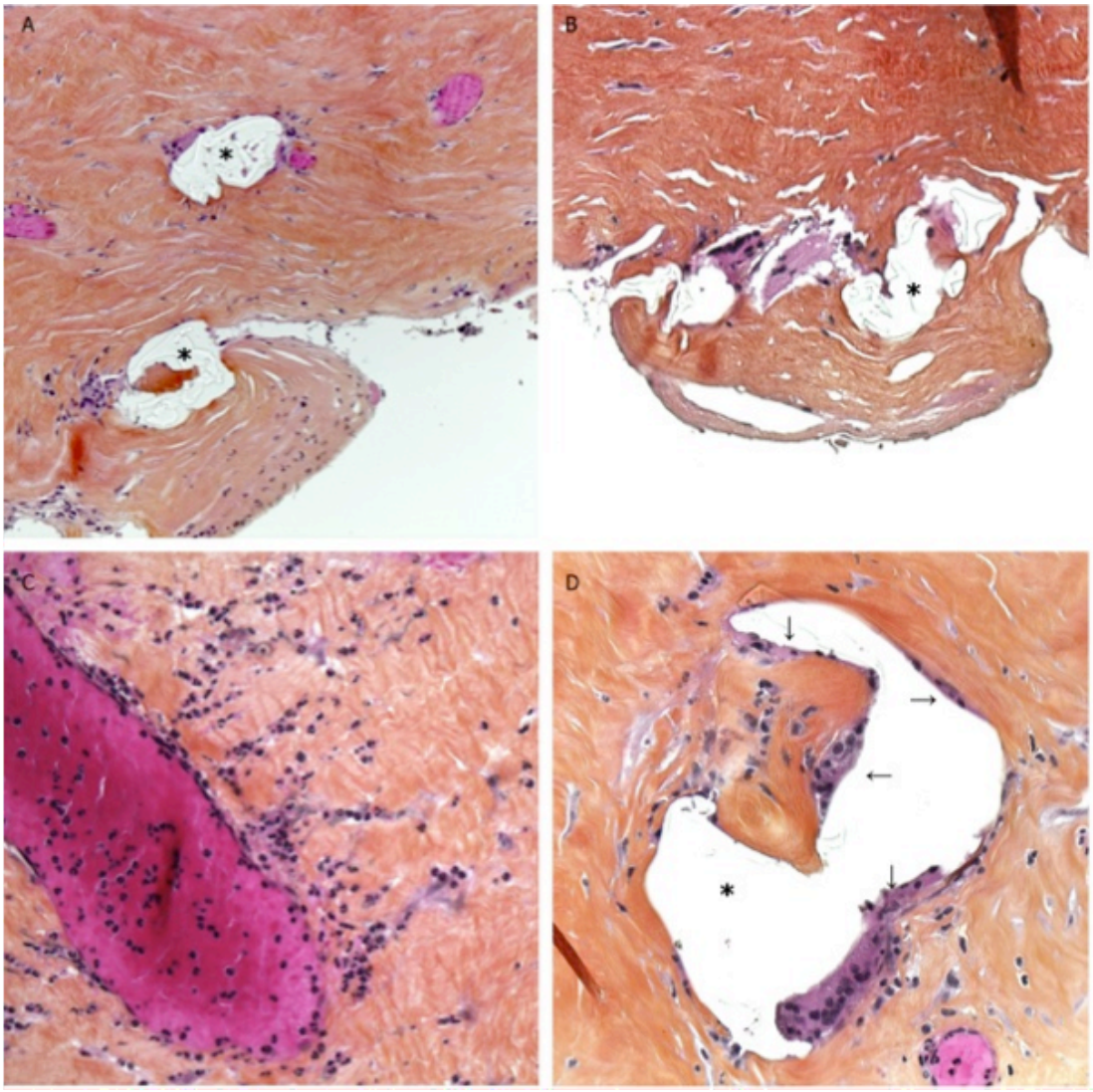

*Figure 20: Hematoxylin & Eosin staining of breast capsule surrounding Allergan BIOCELL® textured expander. Silicone deposits denoted by *, (A, B). Perivascular infiltration of neutrophils and lymphocytes, signifying acute inflammation, (C). Giant multinucleated cells in contact with the microscopic silicone deposits denoted by arrows, (D)*

On another note, textured implants, especially aggressively textured ones such as the BIOCELL® and CUI MicroCell™ surfaces, appear to be associated with both double capsule and late periprosthetic fluid collections, often referred to as late seroma[139, 155, 156]. The concept of late seroma has been defined by experts in implant-based breast surgery as significant periprosthetic fluid collections arising one year or more following implant insertion. Reported late seroma incidences range from 0.88 to 1.59% in breast augmentation[44, 73, 138, 157] and are slightly higher in breast reconstruction (1.84%)[157]. In Spear et al.'s five year retrospective series, 27 of 28 (96.4%) of late seromas occurred with textured devices, which were all of the BIOCELL® type; only 1 case (3.6%) occurred with a smooth implant[158]. Park et al.'s literature review of late seroma documented 57 cases where the implant surface type was reported. Fifty-five cases (96.5%) were associated with textured implants. Of these, 90.7% were BIOCELL®, 5.6% SILTEX® and 3.7% Microthane® (polyurethane foam-covered). They found only 2 documented cases of smooth implant-related late seromas [143].

Evidently, the interlinked relationship between aggressively textured breast implants, late seromas and double capsules obliges additional, in-depth reflection. Three of the patients in the Hall-Findlay series presented with late seromas forming within the two capsular layers and 2 of the cases required urgent revision surgery and drainage[129]. Pinchuk and Tymofii described 6 cases of late seroma in breast augmentation patients with BIOCELL® (n=5) and Silimed (n=1) textured implants; they evoke synovial metaplasia as part of the cause of these late fluid accumulations. Although the specific

term “double capsule” is never employed in the paper, 3 of the 4 cases that had implant removal had some form of double capsule formation; all 3 double capsules occurred with BIOCELL® type implants[138]. The development of synovial metaplasia on breast periprosthetic capsule surfaces in contact with the implant shell is well documented and the phenomenon is thought to be evidence of repeated frictional forces at the implant-capsule interface[112, 159-164]. The cells associated with synovial metaplasia have potential fluid secretory functions[165, 166] and this has led some to propose a link with seroma formation[144, 159]. Interestingly, Hasham et al. report a case of persistent right breast seroma within residual capsule tissue 3 years after bilateral implant explantation for recurrent capsular contracture. During a previous revision surgery, a double capsule had been discovered on the right BIOCELL® implant. Furthermore, evidence of synovial metaplasia had been found on a left breast capsule sample. The authors suggest that the residual capsule in the right breast may have been the cause of the fluid accumulation due to synovial metaplasia[140]. Given the ensemble of experiences conveyed in the literature, it is very possible that this was indeed the case. Roth et al. also found synovial metaplasia in their BIOCELL®-related double capsule and seroma case[141].

In a study by Ahn et al., 15% of breast implants removed electively for either breast/axillary pain, upper extremity paresthesia, capsular contracture, or unsatisfactory breast shape revealed some fluid in the periprosthetic cavity; furthermore, there was a positive trend toward the presence of fluid with those implants that were textured[167]. But what explains the development of clinically significant

seroma in only a small portion of double capsule cases? Hall-Findlay believes that many late seromas go unnoticed and settle on their own. There are at least 3 pertinent cases of breast periprosthetic seroma resolution without surgical or percutaneous drainage[129, 138, 168]. The case reported by Farina et al. points to a mechanical cause of seroma, notably due to micromotions. Nineteen months following bilateral breast augmentation with Silimed anatomic-profile textured silicone gel implants, an 18 year-old patient developed sudden left breast swelling with seroma-like periprosthetic fluid collection visualized on ultrasound thereafter. The patient had been doing gymnastics and jogging regularly just prior to the swelling. The swelling subsided over a month of rest but recurred thereafter once the patient resumed her jogging routine. The swelling again disappeared after a week of rest. Since no surgical intervention was required, it is not possible to know whether a double capsule and/or synovial metaplasia was present; however, the patient eventually developed bilateral Baker grade II contractures 4 years later[168]. The incidence of double capsule in the context of late seromas is likely underreported.

Pinchuk and Tymofii note that the majority of their seroma cases had been recovering from viral illnesses at the time of presentation, and showed some evidence of systemic inflammation and/or infection, as evidenced by elevated temperatures, leukocytic left shift or elevated ESR. Moreover, serous fluid analysis revealed *S. epidermidis* in 2 patients (only one of those patients had a double capsule), although it is unclear whether they were contaminants[138]. The authors postulate that a temporarily weakened immune

system may contribute to seroma formation, or type of inflammatory synovitis, due to the consequent activation of infectious organisms dormant within the periprosthetic capsular tissue. No mention is made of biofilm presence or absence in the studied cases. Is it possible that some quantity of biofilm had been present in these seroma cases, thereby serving as a nidus for this suspected inflammatory process? Many of the reviewed double capsule cases included seroma fluid[141, 143, 144] and prosthesis or capsular tissue [129, 140] which yielded negative results. However, it is important to note that many of the slow-growth pathogens implicated in prosthetic device biofilm formation are often unreliably isolated by traditional methods. Rieger et al. used sonication (ultrasonic frequencies to agitate particles in a sample) cultures to isolate bacteria from removed breast implants without overt signs of clinical infection: *P. acnes* were isolated in 22.3% of implants, coagulase-negative staphylococci in 18.8% and Bacillus species in 2.7%. Overall, 46,4% of implants had positive sonication cultures[89]. Tunney et al. also used sonication techniques to dislodge bacteria growing within adherent biofilms on surfaces of removed hip prostheses; 62% of implants were positive for either *P. acnes* or gram-positive cocci[169]. Hence, it is reasonable to believe that routine microbiological analyses presented in many of the double capsule cases grossly underestimate the presence of bacterial organisms. The difficulty of isolating pathogens involved in prosthetic device colonization and the fact that biofilms are not always visible to the naked eye further reinforces the relevance of SEM for prosthetic device and periprosthetic capsular studies[86, 122, 170].

Interestingly, Hall-Findlay reported the finding of a *S. epidermidis* biofilm within a double capsule sample sent for SEM analysis. Allan et al. further reported a case of bilateral double capsule post-augmentation with *icaA* gene-positive *S. epidermidis* biofilm documented via "enhanced" cultures and SEM imaging. The type of implant was not specified. They also describe two cases of double capsules around custom-made smooth implants inserted into pigs that had their implant pockets inoculated with *S. epidermidis*. Again, these double capsules were associated with *icaA* gene-positive *S. epidermidis* biofilm[150]. As an aside, the *ica* locus confers biofilm production abilities to *S. epidermidis* strains. What role, if any, does biofilm play in the development of double capsules? In our series, biofilms were observed in all the double capsule cases, which were limited to the early BIOCELL® group (G1). We did not conduct microbiological tests in our series since the study focused purely on capsule ultrastructural characteristics; therefore, we are not able to comment on the specific pathogens responsible for this biofilm formation. However, we have previously identified *S. epidermidis* cells within breast implant biofilms, as demonstrated in our first article[122]. None of our double capsule patients had any evidence of either active infection or seroma intraoperatively during expander to implant exchange. In any case, periprosthetic seromas would be unlikely with expanders due to the significant pressure induced by serial saline inflations against the adjacent tissues. We hypothesize that, in fact, biofilms formation may be simply associated with double capsule formation and seroma, rather than being an actual trigger. Weaver et al. conducted a thought-provoking

in-vitro study of biofilms in a model meant to replicate the catheter microenvironment; they demonstrated that fluid shear stress induced biofilm formation in strains of *ica*$^{+}$ *S. epidermidis* that do not constitutively secrete polysaccharide intercellular adhesion (PIA), which is one of the main components of biofilms[130]. The extensive published descriptions of synovial metaplasia and periprosthetic fluid collections around textured breast implants may be considered signs of such shear stresses and forces. Furthermore, the fluid in the periprosthetic space creates a favourable environment for biofilm formation[171]. The prospect that Weaver et al.'s in-vitro findings could be extrapolated to breast implant biofilms is appealing, and may help consolidate the theories put forward by various authors as well as clarify the precise relationship, if any, of biofilms with double capsule formation.

While a total of 5 analyzed samples with biofilms were found in the SILTEX® groups (G3 and G4), 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 since, in our experience, the periprosthetic capsule never truly adheres to this textured shell. Even if consequential quantities of periprosthetic fluid were to be produced due to shear forces, the nature of the SILTEX® surface would be less likely to accommodate seeding of cells and eventual inner capsule development.

Conclusion

SEM is a powerful tool that plays a key role in the study of implant surface and periprosthetic capsule ultrastructural characteristics. It can also complement advanced microbiological techniques aimed at identifying bacteria residing within biofilms around implants. Our first article demonstrates that conventional Hi-Vac mode is superior to ESEM for the comprehensive analysis of breast periprosthetic capsular tissue.

Furthermore, The initial analyses presented in the second article indicate that there may be real benefits to delaying the first postoperative expansion until 6 weeks following the insertion of aggressively textured BIOCELL® expander implants. More pronounced 3-D relief on SEM and enhanced clinical adherence was observed in these more maturated capsules. Furthermore, there appears to be a reduction in the incidence of biofilm formation in patients that underwent delayed expansion with BIOCELL® expander prosthesis. Based on our series, benefits of the delayed approach do not seem to extend to the SILTEX® type expanders.

Double capsule formation, which has been described only recently in the literature and was observed exclusively in BIOCELL® textured implants when the early approach was employed, may be attributed to the incomplete adherence of an immature periprosthetic capsule combined with shear forces caused by expander saline inflation. Implants which accommodate some degree of tissue adherence may lead to implant-capsule complex

detachment due to shear forces or trauma. Synovial metaplasia may subsequently result from frictional forces with associated periprosthetic fluid or seroma production, which provides a favourable milieu for biofilm formation. A new second inner capsule may form due to the expected foreign-body reaction and seeding of cells from the seroma. Further clinical trials are required to determine the real impact of a delayed approach to postoperative expansion with aggressively textured expander implants on patient outcomes.

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Appendix

Overview of published double capsule cases

Year	Authors	Journal & Article Type	Cases (n)	Double Capsule Side	Age	Initial Surgery	Implant	Plane	Presenting Problem	Delay until Present	Lab and Pathology Findings	Proposed Double Capsule etiology	Additional Notes
2002	Pandya and Dickson	PRS (Ltr)	1										Authors have observed partial double capsules with Trilucent™ implants as well
				R	23	BBA	270 cc McGhan 410 (BIOCELL®)	SG	Seroma	10 mo	Fluid culture: negative	Mechanical[1]	Seroma recurred despite 2 aspirations prior to surgery
2002	Colville et al.	BJPS (Ltr)	2										
				L	46	BBA	Trilucent™	SM	Capsular Contracture	18 mo	Capsule Path: FBR with inflammation	Implant bleed	-
				R	23	BBA	Trilucent™	SM	Capsular Contracture	23 mo	Capsule Path: FBR with inflammation	Implant bleed	-

Legend: *APS – Annals of Plastic Surgery; BBA – Bilateral breast augmentation; BBA-M – Bilateral breast augmentation-mastopexy; BJPS - British Journal of Plastic Surgery; CR – Case report; DP – Dual plane; EJPS- European Journal of Plastic Surgery; IBR- Implant-based breast reconstruction; JPRAS – Journal of Plastic, Reconstructive and Aesthetic Surgery; LOC – Loss of consciousness; Ltr – Letter; OA – Original article; PRS – Plastic Reconstructive Surgery; SG – Subglandular; SM – Submuscular; SF – Subfascial; [1] - Inner capsule layer forms first; [2] – Inner capsule layer forms second.*

Year	Authors	Journal & Article Type	Cases (n)	Double Capsule Side	Age	Initial Surgery	Implant	Plane	Presenting Problem	Delay until Present	Lab and Pathology Findings	Proposed Double Capsule etiology	Additional Notes
2004	Matteucci and Fourie	BJPS (Ltr)	3										All 3 patients reported episodes of "minor trauma"
				Uni	-	-	Allergan 150 cc SH expander (BIOCELL®)	-	Implant rotation/ dislocation	-	-	Mechanical[1]	Minimal periprosthetic fluid presence
				Uni	-	-	McGhan 410 (BIOCELL®)	-	Implant rotation/ dislocation	-	-	Mechanical[1]	Minimal periprosthetic fluid presence
				Uni	-	-	McGhan 410 (BIOCELL®)		Implant rotation/ dislocation	-	-	Mechanical[1]	Minimal periprosthetic fluid presence
2006	Hasham et al.	EJPS (CR)	1										
				R	69	BBA	270 cc McGhan cohesive gel (BIOCELL®)	SM	Capsular Contracture	6 mo	Prosthesis culture: negative	-	- Capsular contracture with double capsule recurred with asymmetry (same implant type) - Persistent right seroma despite implant removal

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Year	Authors	Journal & Article Type	Cases (n)	Double Capsule Side	Age	Initial Surgery	Implant	Plane	Presenting Problem	Delay until Present	Lab and Pathology Findings	Proposed Double Capsule etiology	Additional Notes
2007	Cagli et al.	PRS (Ltr)	1										
				R	40	BBA	Anatomic cohesive-gel (BIOCELL®)	DP	Hematoma	4 mo	Capsule culture: negative Capsule path: normal	-	Right upper arm myoelectro-stimulation day prior to swelling
2011	Pinchuk and Tymofii	APS (OA)	3										Author suggests seroma due to implant micromotions, synovial metaplasia and triggered by infection
				L	26	BBA	240cc McGhan style 110 (BIOCELL®)	SF	Seroma	118 mo	Labs: left shift Fluid culture: negative	-	Paracenthesis initially for serous fluid, revision surgery 1 mo after
				L	33	BBA	235cc McGhan 410 FM (BIOCELL®)	DP	Seroma	47 mo	Labs: left shift, ↑ ESR Fluid culture: *S.epidermidis* Fluid cytology: + neutrophils	-	Bilateral Swelling appeared with T 38°C, general weakness, transient LOC (Right breast subsided)

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Year	Authors	Journal & Article Type	Cases (n)	Double Capsule Side	Age	Initial Surgery	Implant	Plane	Presenting Problem	Delay until Present	Lab and Pathology Findings	Proposed Double Capsule etiology	Additional Notes
				L	36	BBA-M	310cc McGhan 410 FM (BIOCELL®)	DP	Seroma	43 mo	Labs: normal Fluid culture: negative	-	-Swelling appeared with T 37.5°C and general weakness (subsided prior to surgery) -Implant had small rupture point, no gel bleed
2011	Hall-Findlay	PRS (OA)	14										1 double capsule was sent for SEM: positive for biofilm
				L	-	BBA	410FX-460g (BIOCELL®)	SG	Seroma	19 mo	Inner capsule path: normal Inner capsule culture: negative (including mycobacteria)	Mechanical[2]	
				R	-	BBA	410FX-360g (BIOCELL®)	SG	Shape	6 mo	-	Mechanical[2]	-
				B	-	BBA-M	115-700cc (BIOCELL®)	SM	Size	96 mo	-	Mechanical[2]	-
				L	-	BBA-M	115-213cc (BIOCELL®)	SG	Ptosis	9 mo	-	Mechanical[2]	-
				R	-	BBA	115-354cc (BIOCELL®)	SG	Asymmetry	9 mo	-	Mechanical[2]	-

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Year	Authors	Journal & Article Type	Cases (n)	Double Capsule Side	Age	Initial Surgery	Implant	Plane	Presenting Problem	Delay until Present	Lab and Pathology Findings	Proposed Double Capsule etiology	Additional Notes
				L	-	BBA-M	115-322cc (BIOCELL®)	SG	Capsular contracture	11 mo	-	Mechanical[2]	-
				L	-	BBA	115-222cc (BIOCELL®)	SM	Capsular contracture	15 mo	-	Mechanical[2]	-
				L	-	BBA	115-290cc (BIOCELL®)	SM	Distortion	7 mo	-	Mechanical[2]	-
				B	-	BBA	115-290cc (BIOCELL®)	SG	Bottoming out	16 mo	-	Mechanical[2]	-
				L	-	BBA	115-354cc (BIOCELL®)	SG	Bottoming out	16 mo	-	Mechanical[2]	-
				B	-	BBA	115-354cc(L) 115-322 (R) (BIOCELL®)	SG	Capsular contracture	17 mo	-	Mechanical[2]	-
				L	-	BBA-M	115-378cc (BIOCELL®)	SG	Seroma	16 mo	-	Mechanical[2]	-
				L	-	BBA	168-210cc (BIOCELL®)	SG	Capsular contracture	42 mo	-	Mechanical[2]	
				B	-	BBA	115-322cc (BIOCELL®)	SF	Capsular contracture	24 yr	-	Mechanical[2]	Left breast swelling (suspected seroma) had settled 6 mo prior to revision surgery

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Year	Authors	Journal & Article Type	Cases (n)	Double Capsule Side	Age	Initial Surgery	Implant	Plane	Presenting Problem	Delay until Present	Lab and Pathology Findings	Proposed Double Capsule etiology	Additional Notes
2011	Robinson	PRS (Ltr)	2										
				Uni	-	BBA	McGhan style 100 gel (BIOCELL®)	SG	Capsular contracture	-	-	Mechanical[1]	95% DC
				B	-	BBA	McGhan style 100 gel (BIOCELL®)	SG	Capsular contracture	-	Inner capsule path: irregular collagen inner surface	Mechanical[1]	Small amount of fluid b/w capsule layers
2011	Dini et al.	PRS (Ltr)	10										All implants replaced by polyurethane-covered (SM plane); no complications after 5 yr follow-up
				-	-	IBR	(BIOCELL®)	SM	Incidental (n=3)	-	-	Mechanical[2]	-
						IBR	(BIOCELL®)	SM	Implant Rotation/ dislocation (n=7)	-	-	Mechanical[2]	-

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Year	Authors	Journal & Article Type	Cases (n)	Double Capsule Side	Age	Initial Surgery	Implant	Plane	Presenting Problem	Delay until Present	Lab and Pathology Findings	Proposed Double Capsule etiology	Additional Notes
2012	Roth et al.	JPRAS (CR)	1										
				R	29	BBA	500cc textured (BIOCELL®)		Seroma	18 mo	Fluid aspiration culture neg Path: inner capsule-synovial metaplasis, FBR	-	Patient had noted right breast swelling during pregnancy (~3 mo after BBA) and seroma was persistent despite percutaneous drainage
2012	Allan et al.	PRS (Ltr)	1										
				B	30	-	Not specified	--	Seroma	-	Capsule culture: *S. epidermidis* PCR bacteria: *icaA* gene + SEM: Biofilm	Chronic infection with biofilm	Patient also had capsular contracture
2013	Toscani et al.	PRS (Ltr)	1										
				B	-	BBA	Textured (not specified)	SM	Capsular contracture	15 yr	Capsule path: calcifications (inner), collagenous (outer)	Mechanical[2]	Calcifications represent long-standing FBR

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Year	Authors	Journal & Article Type	Cases (n)	Double Capsule Side	Age	Initial Surgery	Implant	Plane	Presenting Problem	Delay until Present	Lab and Pathology Findings	Proposed Double Capsule etiology	Additional Notes
2013	Grippaudo et al.	APS (CR)	1										
				R	61	IBR	Anatomic (BIOCELL®)	SM	Hematoma	21 mo	Capsule path: hyaline fibrous capsule (outer) non-specific inflammation (inner)	-	Small-sized bleeding vessel found intra-operatively between capsule layers
2013	Park et al.	APS (CR)	1										
				L	62	BBA	240cc style 110 (BIOCELL®)	SG	Seroma	48 mo	Fluid culture and cytology: negative	-	Implant had multiple rupture sites (Note: patient had 3 prior revision surgeries due to implant ruptures)
2013	Lista and Ahmad	PRS (CME)	2										
				Uni	-	BBA	(BIOCELL®)	SG	Seroma	-	-	-	-
				Uni	-	BBA-M	(SILTEX®)	SG	Capsular contracture	-	-	-	-

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