Exhibit F

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Protocol for Retrieval and Analysis of Breast Implants

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ABSTRACT: The Center for Implant Retrieval and Analysis has been established at Washington University's Division of Plastic and Reconstructive Surgery for the purpose of studying implantable devices retrieved after surgery or autopsy and assessing their condition after implantation. Since the early 1990s, significant experience has been gained in testing and analyzing silicone gel breast implants and, to a lesser extent, saline-filled devices. However, there has been no systematic method reported for collecting and evaluating these implants in a way that would permit different laboratories to compare their data. This article offers the plastic and reconstructive surgery community a standardized protocol for analyzing explanted silicone gel and saline-filled breast implants. The protocol gives surgeons a clearly defined approach for removing, handling, documenting, and shipping explanted breast implants. At the same time, biomaterials researchers can use the protocol to acquire implant data with reliable and reproducible methods. Because the study of saline implants has lagged behind the study of silicone gel implants, the article concludes with a demonstration of how this protocol can be applied to obtain mechanical properties data and use scanning electron microscopy to illuminate failure mechanisms of saline devices, including three explants removed after 20+ years in vivo.

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I. INTRODUCTION

Over the last decade, the durability of breast implants has become an important issue to plastic and reconstructive surgeons, as have the incidence and mechanisms of implant failure. Arising from concerns about durability and failure, the Center for Implant Retrieval and Analysis (CIRA) at Washington University established a structured study of explanted silicone gel and saline breast implants. Major goals of CIRA include identifying the effect(s) the body has on breast implant materials and the modes, causes, and prevalence of device failure. Analysis of failed breast implants is so complex and multidimensional that a variety of scientific specialties is required to address the problem. For this reason, CIRA is made up of researchers encompassing a range of technical disciplines, including clinical medicine, engineering, chemistry, materials analysis, statistics, and basic science. Since the early 1990s, our research group has tested large numbers of explants and never-implanted controls filled with silicone gel or saline. As the testing and analysis have proceeded, a growing database has been maintained to compare the ultimate strength properties of different types of breast implants and identify failure characteristics. At the same time, procedures for collecting, processing, and studying explants have been developed and refined.

What follows is a description of the protocols CIRA has developed during many years of analyzing removed breast implants. It is hoped this article will give surgeons a better understanding of how breast implants are tested and why specific procedures are required, and also allow other researchers involved in implant analysis to evaluate our methods. In addition, this presentation proposes a standardized protocol for breast implant analysis, which does not currently exist. At present, different laboratories and implant manufacturers may use different approaches, conduct fewer or different tests, or collect less data regarding implant history. With modifications, this basic protocol could also be adapted for testing other plastic and reconstructive implantable devices. Although the procedures described herein produce reliable and reproducible data, we welcome feedback from other researchers in this field who have successfully adopted additional or different testing techniques. Their feedback will be incorporated into an updated protocol as significant new testing and analysis procedures are developed in the future.

During the 1990s, saline-filled implants received less analytical attention than those filled with silicone gel. In the last few years, CIRA has shifted some of its attention to saline implants in an effort to determine how and why these devices fail. The last section this article illustrates how the described protocol can be applied to gain a better understanding of what happens to saline implants over time. Included are explants with implantation durations of 21, 22, and 23 years, which we believe are the oldest saline devices ever analyzed.

II. RETRIEVING AND SHIPPING REMOVED IMPLANTS

Because testing intact implants can be as important as testing failed devices, both types need to be analyzed. Surgeons removing intact and failed silicone gel or saline breast implants should adopt the following procedures:

- Remove silicone gel and saline-filled implants with whatever filler material remains inside, regardless of implant status (intact or ruptured).
- If a small or remote incision is used for explantation, deflate saline-filled implants with a 20-gauge needle before removal. Note the position of the needle puncture so the testing lab will know if a hole is related to intentional deflation.
- When sending an explant for testing to any lab or manufacturer, first request (via mail or fax) a schematic drawing (sketch) of an implant from CIRA. This schematic illustrates the anterior and posterior sides of round or anatomical implants. Represent the location(s) of any implant damage on the drawings, including damage induced during removal, such as needle holes. Another example might be the type of instrument used to grasp an implant and the location of its application. Also sketch the location and approximate size of obvious failure sites.
- Clearly label right and left implants. When sending explants from more than one patient, make sure they are clearly identified. Any coding system is acceptable, and names are kept confidential at CIRA.
- An explant may be rinsed with saline, but leave any adherent material—such as silicone gel, capsular tissue, or calcifications—on the explant so it can be examined. Do not place explants in formalin or other preservatives, and do not autoclave.
- Place the explant in a container suitable for shipping and enclose it in a box that will protect the device from damage. Any packaging appropriate for the shipment of biohazardous material is acceptable. The box should also contain the schematic drawing and history form (described below).

III. DATA COLLECTION WITH PATIENT AND EXPLANT HISTORY FORM

Important historical information is needed for analysis of explanted silicone gel or saline implants. Along with the schematic drawing, CIRA provides its Patient and Explant History Form so specific details can be recorded as completely and quickly as possible. Copies of patient records, especially operative reports, may also be included. The information needed regarding patients and explants includes:

1. dates of implantation and explantation
2. reason for implantation (augmentation, reconstruction, correction of deformity, etc.)
3. reason for explantation (device failure, size change, capsular contracture, patient request, asymmetry, etc.)
4. integrity status of the explant (intact, pinhole defect, rupture, deflation)
5. suspected mode of explant failure, if known and applicable
6. incision used for implantation (periareolar, inframammary, transaxillary, transumbilical, mastectomy, mastopexy, etc.)
7. incision used for explantation and approximate length
8. tissue plane the explant was in (subglandular, submuscular, subcutaneous)
9. right or left implant
10. manufacturer of explanted implant, if known
11. lot number, style, and/or catalog number of the explant, if available
12. original volume of the explant (include nominal and actual fill volume for saline implants, if possible)
13. any pre-explantation complications experienced by the patient and how the complication was addressed (deep or superficial infection, hematoma, delayed wound healing, necrosis, etc.)
14. number and type of other implant-related surgery (previous implant exchange, previous revision without exchange, breast biopsy, etc.)
15. history of any potential trauma or stress to the breast or implant, such as accident or injury, closed capsulotomy, breast size change following mammogram, additional surgery without implant exchange (open capsulotomy, inframammary crease or pocket revision, cyst aspiration, biopsy, etc.)
16. other explantation surgery procedures performed (removal with no replacement, replacement with another implant, planned replacement in future, mastopexy, open capsulotomy, total or partial capsulectomy, other capsule revision, etc.)
17. relevant clinical observations at time of explantation (appearance of gross shell defects, condition of patches and valves, presence of explant discoloration, approximation of any extruded/leaking fill volume, condition and appearance of surrounding capsule, etc.)
18. number of mammograms the patient had with the explanted implant in place
19. patient use of breast massage (yes or no)
20. grade of capsular contracture (I, II, III, or IV)
21. grade of skin wrinkling (I, II, or III)
22. grade of implant palpability (I, II, or III)
23. notation of tissue ingrowth into textured surface, if applicable
24. notation of tissue ingrowth into a saline fill valve, if applicable
25. notation of permanent creases in the implant shell (should be evident after removal)
26. reports from relevant histological examinations of tissue surrounding an explant

IV. LOGGING AND MACROSCOPIC EXAMINATION OF RETRIEVED EXPLANTS

The following protocols are used by CIRA when explants are received from surgeons:

- Treat all explants as though they could be contaminated and handle with gloves. Keep explants in a designated storage space that has secured access as

well as controlled temperature and humidity to avoid extremes.

- Log retrieved explants into the testing inventory and assign each a coded identification number to ensure patient anonymity. Enter ID codes into the database, along with information provided by the operating surgeon from the Patient and Explant History Form and schematic drawing. Write the ID number on the schematic and history form and securely attach it to the explant container so the code can be used to trace an explant throughout testing and data analysis.

- If the manufacturer of an explant is not obvious, make every attempt to identify it. Rely on experience as well as descriptions from manufacturers and documents that identify various types of implants.[1]

- Superficially clean the explant with isopropyl alcohol. Do not remove areas of adherent calcification or tissue so any particulate matter and the underlying shell can be inspected more closely. If needed, transfer the explant to a new clean storage container.

- Document the integrity status of the explant (intact, pinhole defect, rupture/deflation) through macroscopic inspection. Based on patient history and initial inspection, note on the schematic any unusual features or areas of the explant that should be studied microscopically. Keep the drawing with the implant storage container.

- Throughout the analysis, enter and update testing measurements and observations in the database to aid in identifying patterns of failure and determining the most common causes.

After an explant is logged in, an Explant Inspection Report is filled out to document the following types of information:

- Description of explant as received.

- Explant status (intact, pinhole defect, rupture/deflation).

- Shell type (smooth, textured, polyurethane) and filler material (silicone gel, saline, double lumen).

- Shell and filler color.

- Presence of particulates on inner or outer surfaces.

- Explant weight.

- Shape (round, oval, high/low profile) and dimensions of explant.

- Seal patch description (size, logo, volume imprint).

- Valve description and location (if applicable).

- Mandrel size and number imprinted on shell by the manufacturer.

- Description of failure site (if present), including dimensions (length, width, or diameter in cm), quadrant, and location (anterior, posterior, perimeter, within a crease, along a seam, at the shell/patch interface, etc.). If an explant has more than one failure site, number each site so it can be correctly identified in the database and subsequent analysis. Also describe any unusual features. Represent all regions of interest on the schematic.

V. PROCEDURES FOR ANALYSIS OF BREAST IMPLANT SHELLS

V.A. Shell Preparation and Cutting

Procedures for cleaning and preparing explants for analysis are as follows:

- Record dates and times when an implant is received in the lab, when it is photographed, when the testing specimens are cut, and when the various tests are performed.

- In the testing laboratory, visually inspect the explant to document its general condition. Areas of particular interest are the shell as a whole, any failure sites, the fill valve, the sealing patch, the seams, and special features such as fixation devices. Look for small holes, frank rents, folds or permanent creases, areas where texturing (if applicable) is worn away, and anything unusual.

- If failure of a silicone gel implant is suspected but its location is not evident, gently squeeze the explant to produce gel extrusion at the failure site.

- If the failure site is not evident in a saline explant, fill it with air through a fill tube, close the valve, and place the explant in a container of water. Gently squeeze the implant to produce bubbling at the failure location.

- Photograph the anterior and posterior sides of the explant as a whole. Document areas of failure or special interest photographically with appropriate magnification.

- Before an explant is cleaned for testing, cut out any failed region (and a surrounding margin of "normal" shell). This prevents tearing or stretching of the shell during cleaning, which could cause additional damage or alter/extend the failure site. Clean the failed region very cautiously.

- Cut the explant shell around the perimeter with a sharp instrument to separate the anterior and posterior sides. If there is a hole or tear along the perimeter, carefully remove that section first.

- Gently clean the inside and outside surfaces of both halves (posterior and anterior) of the explant with isopropyl alcohol-moistened Kimwipes (Kimberly-Clark, Roswell, Georgia). Take care not to damage or abrade the shell during cleaning.

- For a silicone gel explant, store the gel filler in a separate container for possible later analysis. If a saline-filled explant still contains saline, aspirate it through the valve with a fill tube and store the saline in a separate container. Mark filler containers with the implant ID code.

- When testing specimens are cut from the shell, record the locations from which each specimen is cut on another schematic of the implant.

- Cut shell specimens for mechanical testing using a standard cutting die attached to a hand press.

V.B. Microscopic Analysis of Failure Sites

After the shell is cleaned, small specimens cut from the explant undergo optical and scanning electron microscopy (SEM) examination of both the outer and inner surfaces. SEM can reveal the surface morphology of explant shell specimens; identify holes, tears, abrasion, or deposits of physiological origin; and document any changes, deformations, or irregularities, such as pitting patterns or evidence of wear. Irregularities that might be related to folds or creases in the shell, as well as related signs of abrasion, are also observable. Areas of failure, especially the defect and associated shell surface features, are documented with micrograph hard copies.

The shell specimen containing the failed region (and a surrounding margin of normal shell) is extensively analyzed with a field emission scanning electron microscope such as an Hitachi S-4500 (Tokyo). Use of field emission SEM is preferable to a standard SEM because it has higher sensitivity and permits viewing of nonconducting polymers without a coating, which can create artifacts or distort failure site features. The SEM permits highly detailed microscopic examination of surface contours, textures, and structural changes that have developed in the shell.

SEM is the best method for identifying defects that have been surgically induced with instruments, either during implantation or explantation, and therefore distinguishes between in vivo and induced failure. We have developed a catalog of such defects caused by a variety of surgical instruments and can compare what is seen with SEM to the micrographs in our catalogue.[2]

Other methods are available to characterize implant materials and failure sites, such as laser Raman microprobe (LRM) spectroscopy.[3] Using a visible monochromatic laser as an excitation source, LRM permits examination of micrometer-sized areas of implant shells. Before this method is employed to study elastomer samples, the specimens are visually examined for inhomogeneities and impurities. This is done using optical, polarized-light microscopy with a research-grade microscope such as an Olympus BH-2 (Olympus America, Melville, New York), which permits viewing at high magnification (up to 1200×) in either reflected or transmitted white light.[4]

Raman spectra of microscopically small regions in explant shells are obtained with a Jobin-Yvon S-3000 laser Raman microprobe system (Jobin Yvon/Horiba, Edison, New Jersey). LRM technology uses an optical microscope (operating in either the reflection or transmission mode, with or without crossed polarizers) to identify a particular region of interest in a shell sample and then characterize its molecular structure through spectral analysis. Our system consists of an argon ion laser coupled to the Olympus BH-2 optical microscope outfitted with an 80× ultra-long-working-distance objective. Raman spectra of the microscopically small regions (with a spatial resolution of ~1 μm) are obtained by irradiating the sample with the 514.5 nm line of an argon ion laser focused through the microscope objective. This optical unit is coupled to a spectrometer system that records the Raman spectra of the irradiated area. The spectra are processed with specialized software, and photomicrographs of failure sites are obtained with an NEC NC-15 CCD color camera (NEC USA, Melville, New York). LRM was used for the analysis of silicone in human tissues as early as 1979,[5] and it is particularly effective in identifying calcium species and crystalline entities. The specific instrument configuration

and operating conditions used by CIRA are described by Pasteris and Chou.[6]

V.C. Analysis of Shell Mechanical Properties

The advantage of having the lot number of any retrieved explant cannot be overemphasized. Whenever possible, we try to identify an implant's lot number, which sometimes necessitates requesting the OR nurse records from the implantation surgery. With a lot number, a lot-matched control can often be obtained from the manufacturer. Assuming control properties are independent of time, testing lot-matched controls in a manner identical to explants is indispensable when trying to determine whether the mechanical, physical, or chemical properties of a breast implant change over time because of exposure to a biological environment.[7] If lot-matched controls are not available, we attempt to test numerous controls of the same implant type manufactured in the same general time period to learn the range of properties values (minimum and maximum) that can be expected.[8] Inherent in our analysis is the assumption that control properties are independent of time.

A large majority of augmentation patients and some reconstruction patients have similar or identical implants placed bilaterally at the same time. Because many surgeons commonly replace both breast implants when a failed device is exchanged, we prefer to retrieve and analyze both. Mechanical testing and SEM evaluation of bilateral devices is extremely important, especially when the two implants are alike. In a case where one implant has failed and the other is intact, it is useful to test the two implants in an identical manner to look for any differences in the material or identify other factors (such as trauma) that might have caused failure. If both implants have failed, we can establish whether they failed in the same way.

Mechanical testing is performed with an Instron 5583 (Instron Corp., Canton, Massachusetts) that is computer controlled and equipped with an automatic data acquisition system. To measure strain in the gauge section of test specimens, we use a video extensometer. (An alternative instrument is a mechanical extensometer.) The force and strain of the Instron are calibrated at least once daily.

Prior to testing, all tensile and tear specimens are weighed individually and their thickness measured with a digital thickness gauge that has a resolution of 0.000254 cm. Thickness measurements are also obtained for shell material that is not cut for mechanical properties analysis. In addition, the shell thickness around any failure site is measured to determine if the failure occurred in a region thinner than the rest of the shell.

All tensile specimens are prepared and tested following ASTM D412-98a protocols as closely as possible.[9] The specimens are cut with a die and hand press from various "non-failed" regions of both the posterior and anterior halves of a shell. If there is ample material in the shell of a failed explant, we test additional specimens from the area directly adjacent to the failure site. Through experience, we have learned that the ASTM Die C half-scale is preferable to the Die C full-scale for cutting test specimens. A breast implant shell has a relatively small surface area; only the Die C half-scale allows us to obtain the required number of testing specimens. A minimum of five dogbone-shaped specimens are needed for tensile testing and at least three specimens are required for tear resistance. These specimens are tested "as is" (after cleaning) without extraction of the elastomer.

The five dogbone-shaped specimens for tensile testing have a cross-section sufficiently large for the extensional forces imposed by the Instron to be measured by a load cell. Both ends of the specimen are clamped in the pneumatic grips of the Instron. As the grips are moved apart at a constant velocity of 10 in (25.4 cm)/min, a uniform pressure is exerted across the gripping surface and the specimen is elongated until it fails. Force is measured as a function of separation. The measured data are converted to engineering-stress versus engineering-strain curves. These stress–strain curves are used to calculate several mechanical properties of the elastomer: tensile strength, elongation, force-to-break (breaking energy), and moduli. Force-to-break and the distance a specimen has stretched at break are used to calculate tensile strength and percent of elongation, which indicate the largest sustainable stress and stretching deformation the elastomer can withstand before breaking. According to ASTM standards, the breaking force in tension should be at least 2.5 pounds (1.125 kg) and the percent elongation at least 350% for Die C full-scale. For each mechanical property, the measurements obtained from all specimens of a single shell are averaged.

To measure the resistance of shell elastomer to tearing action, three tear specimens are cut with a Die C half-scale and tested according to the ASTM D624-00e1 protocol.[10] After a tear specimen is clamped in the Instron grips, the grips are separated at a steady rate of 10 in (25.4 cm)/min until the specimen tears. Tear resistance is calculated as the amount of force applied at tearing divided by a specimen's thickness. Again, measurements from a single shell are averaged.

If there is sufficient shell material, three additional tensile specimens are cut and extracted before testing. We extract smooth and textured shells in chromographic grade hexane (Fisher Scientific, Pittsburgh, Pennsylvania) that is refluxed at 60°C over a period of 96 hours (48 h, 24 h, 24 h) and then dried to constant weight.[7,8,11-15] Hexane extrac-

tion removes the non-crosslinked low-molecular-weight silicones from elastomer and thereby yields a specimen with properties much closer to those of the original elastomer before implantation. The extracted specimens are tested with the same procedures used for the unextracted specimens. The tensile strength and elongation properties for extracted and unextracted shells are then compared.

If enough material is available, we also test the sealing patch bond strength according to the ASTM F703-96 protocol.[16] Patch bond strength measurements are obtained by testing three specimens from the shell, with the patch–shell interface centered in the cut specimens.

Because fewer specimens can be cut from small implants and those with large failure sites, not all mechanical tests can be performed on every implant. At the very least, explant failure sites are examined with SEM, and the ultimate strength properties (tensile strength, percent elongation, and tear resistance) are determined for the non-failed regions. Beyond this, we must decide what other tests would be most valuable and allocate the material accordingly. For example, if implant failure occurred in a crease or fold, we try to obtain a tensile specimen from this area to see if there is any local weakening in the shell properties. If the amount of shell material available is limited, tensile testing of extracted shell samples and patch bond strength are omitted. No reliable test for studying shell abrasion has been developed, but if one becomes available it will be added to this protocol.

A final note on the use of Die C half-scale in the analysis of breast implant mechanical properties is in order. To date, no study has investigated the relationship between results obtained for tear resistance conducted with Die C and Die C half-scale. Hence, at this point, Die C half-scale tear data should not be compared with Die C full-scale tear data. For tensile testing, however, CIRA has compared the Die C full-scale and Die C half-scale. In 1986, Dow Corning silicone gel control implants were tested by Battelle using Die C at 20 in (50.8 cm)/min; in 1997, controls from the same manufacturing lots were tested at Washington University using Die C half-scale at 10 in (25.4 cm)/min.[17] The ratios of the Die C half-scale to Die C values of tensile strength and elongation were 0.87 and 0.79 for Silastic I controls and 0.97 and 0.94 for Silastic II controls. Some of the differences in the tensile data obtained with the two dies were probably a result of variability within a lot.

In another study, CIRA conducted tensile tests on 20 control saline implant shells from six manufacturing lots fabricated under tight quality control procedures by the same manufacturer.[18] Five specimens from each of the twenty implants were tested, with ten shells cut using Die C full-scale and the other 10 cut with Die C half-scale. The full-scale specimens were tested at a displacement rate of 20 in (50.8 cm)/min, and the half-scale specimens at a rate of 10 in (25.4 cm)/min. The average tensile strength and elongation for the two dies are given in Table 1. The ratios of the Die C half-scale to the Die C average values of tensile strength and elongation were 1.14 and 0.97, respectively. There was probably some lot-to-lot variability effect in the study.

TABLE 1. Average Tensile Strength and Elongation for 20 Saline Implant Controls From 6 Lots Tested with Die C and Die C Half-Scale

Die	Tensile strength (PSI)	Elongation (%)
Die C half-scale	1453 (SD = 90)	851 (SD = 53)
Die C	1280 (SD = 58)	875 (SD = 24)

PSI = pounds force per square inch (1 psi = 6.895 kPa)
SD = standard deviation

Our third investigation of die effects was recently completed, and the previously unpublished results are presented herein. A total of 22 implants were tested with Die C and Die C half-scale. Thirteen were explanted silicone gel devices (7 Silastic I and 6 Silastic II) with unknown implantation times and clinical histories. The remaining 9 implants were saline control shells, 3 each from manufacturers X, Y, and Z. A nominal value of 5 Die C half-scale specimens from all 22 implants were tested at 10 in (25.4 cm)/min, and additional Die C specimens were cut from the remaining shell material for each implant and tested at 20 in (50.8 cm)/min. Table 2 presents the ratios of the Die C half-scale to the Die C average values of tensile strength and elongation; the number of Die C full-scale specimens tested from each implant is also listed. (It should be noted that tear specimens from the saline implants were also tested, which left less shell material for the Die C full-scale tensile specimens.) The tensile strength ratio varies from a minimum of 0.68 to a maximum of 1.50, with an average value of 1.10 (SD = 0.19). The elongation ratio varies from 0.75 to 1.30, with an average of 1.04 (SD = 0.13).

These three investigations comparing Die C and Die C half-scale tensile data demonstrate a relationship between their testing results. In general, the differences obtained with the two dies are most likely overshadowed by mechanical property variations associated with lot-to-lot variability, variability within a lot, and variations in the elastomer taken from different areas of a single implant shell. Our protocol assumes that tensile data obtained with the two dies are essentially identical.

TABLE 2. Ratios of Die C Half-Scale to Die C Average Values of Tensile Strength and Elongation

Implant	Tensile strength ratio	Elongation ratio	No. Die C specimens tested
Silastic I – #1 gel	0.838	0.817	4
Silastic I – #2 gel	1.077	1.032	6
Silastic I – #3 gel	1.128	1.029	6
Silastic I – #4 gel	1.286	1.072	6
Silastic I – #5 gel	0.948	0.972	4
Silastic I – #6 gel	1.098	1.007	6
Silastic I – #7 gel	1.067	1.060	6
Silastic II – #1 gel	0.838	0.859	4
Silastic II – #2 gel	0.681	0.751	4
Silastic II – #3 gel	1.280	1.176	4
Silastic II – #4 gel	0.922	1.052	6
Silastic II – #5 gel	1.140	1.018	4
Silastic II – #6 gel	1.497	1.291	4
Mfg X – #1 saline	1.213	1.061	2
Mfg X – #2 saline	1.400	1.297	2
Mfg X – #3 saline	1.348	1.062	2
Mfg Y – #1 saline	1.050	1.104	3
Mfg Y – #2 saline	1.024	1.053	3
Mfg Y – #3 saline	0.976	0.982	3
Mfg Z – #1 saline	1.068	1.117	3
Mfg Z – #2 saline	1.128	1.063	2
Mfg Z – #3 saline	1.084	1.072	3

V.D. Chemical Analysis

When studying silicone gel or saline breast implants, especially those that have failed, it is useful to look for any chemical changes in the elastomer by measuring its crosslink density, which can markedly affect the mechanical properties of an implant shell. A large increase in crosslink density leads to a higher modulus material with associated embrittlement and decreased elongation to failure. The crosslink density of an elastomeric polymer, such as polydimethysiloxane (PDMS), is directly calculated from swelling measurements using a custom protocol developed by CIRA.

The crosslink density of explant shells is measured after specimens are extracted to remove the non-crosslinked, low-molecular-weight silicones. The extraction process leaves the elastomer more like it was prior to implantation. Material for this testing usually comes from scraps of shell left over after the mechanical property specimens are cut. Typically, two small (~1" × 1" [2.54 × 2.54 cm]) shell specimens are cut for swelling measurements. The shell samples are first extracted with hexane, dried to constant weight, and swollen in toluene to constant weight. The samples are then patted dry and placed in a tared glass weighing jar. The crosslink density of the shells is calculated by applying Flory and Rehner's classic theory.[19]

Other chemical tests can be incorporated into this protocol for analysis of retrieved explants. For example, the extract may be analyzed with gas chromatography–mass spectrometry (GCMS) to identify low-molecular-weight silicone constituents in the elastomer. Fourier-transform infrared spectroscopic analysis of the shell is useful for detecting higher molecular weight materials and biological components such as lipids.

VI. APPLICATION OF PROTOCOL

VI.A. Silicone Gel Breast Implant Analysis

This protocol has been used extensively to investigate various types of silicone gel explants and never-implanted controls manufactured in the US over the past 30+ years.[7,11,12,14,15] The implants studied were drawn from the world's largest known inventory of explanted breast implants with lot-matched controls[7] and included the oldest known explants from first, second, and third generations that have been analyzed to date, including two Cronin seamed explants removed intact after 32 years of implantation.[15] Most of our analyses have focused on measurement of mechanical and chemical properties of intact and ruptured silicone gel implants to determine whether and how implant failure relates to possible elastomer degradation. With this protocol, we can characterize the primary failure mechanisms of breast implants and identify those shell failure sites caused by surgical instruments.[2]

VI.B. Saline Breast Implant Analysis

Most published implant properties data relate to silicone gel devices.[11-15,20] The mechanical properties of explanted saline-filled breast implants have not received as much scientific attention. The lack of information on inflatable shells can be addressed only by testing them with methodologies such as those outlined above. For both silicone gel and saline implants, we must have sufficient experimental data to determine whether implantation degrades elastomer, how in vivo aging affects the shell, and what processes might contribute to failure.

TABLE 3. Implantation Time and Status of Five Tested Saline Explants

Manufacturer	Years of implantation	Implant status at explantation	Shell type
Simaplast	23	intact	smooth
Heyer-Schulte	22	intact	smooth
Heyer-Schulte	21	failed	smooth
McGhan	5.5	failed	textured
McGhan	3	failed	textured

We have recently applied this protocol to investigating several saline explants that were removed after varying implantation times. A brief description of the five explants tested is presented in Table 3. To our knowledge, the Simaplast device, with 23 years in vivo, is the oldest saline implant analyzed to date. It was manufactured in France and explanted at the University of Toronto.[21] A photograph of the anterior surface of this intact explant, which is extensively calcified, is shown in Figure 1. We believe the intact Heyer–Schulte explant with 22 years of implantation (Fig. 2) is the oldest US-made saline device that has been tested. Data from a failed Heyer–Schulte explant with 21 years of implantation and failed McGhan textured explants with 5.5 and 3 years in vivo are presented to demonstrate the application of SEM in the analysis of failed shell regions found in saline implants.

A major reason for analyzing explants with the types of tools described in this protocol is to determine the effect(s) of implantation on shell properties. This is best accomplished by comparing explants to proper controls. Neither lot-matched controls nor controls that would yield shell property ranges were available for the saline explants listed in Table 3. Consequently, they were classified only by manufacturer and implantation duration and analyzed accordingly.

The ultimate strength properties of tensile strength, elongation, and tear resistance are presented in Table 4 for the five as-received (nonextracted) shells. Table 4 also gives the average shell thickness determined from all the tensile and tear specimens. With no controls available for these explants, any assessment of the effects of in vivo aging is limited because the values of the shell properties at implantation are unknown. We can, however, conclude that the shell thicknesses of the five explants are comparable. The tensile strength and tear resistance of the Simaplast explant are much higher than that of the other four explants, but its percent elongation is the lowest. Elongation values for the Simaplast explant and the McGhan explant with 5.5 years in vivo are below the ASTM standard of 350% for unimplanted shells. Even so, the Simaplast implant remained intact for 23 years.

The properties of tensile strength and elongation for shells extracted with hexane prior to analysis are shown in Table 5, along with the percent extracted. For the Simaplast and McGhan shells, the low percentages of material extracted (2.8, 2.7, and 3.1%) indicate a higher percentage of monomer was incorporated into the elastomer. The large amount of material extracted from the Heyer–Schulte shells is comparable to the percent extracted from many silicone gel implant shells, which contain silicone molecules from the gel filler. As with silicone gel shells,[15] properties measurements of the unextracted saline shells are lower

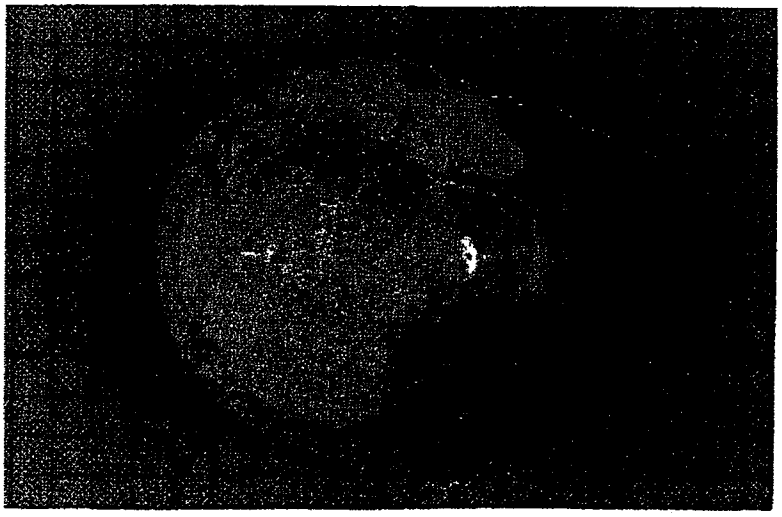

FIGURE 1. Anterior surface of intact Simaplast saline implant removed after 23 years. Extensive calcification is evident.

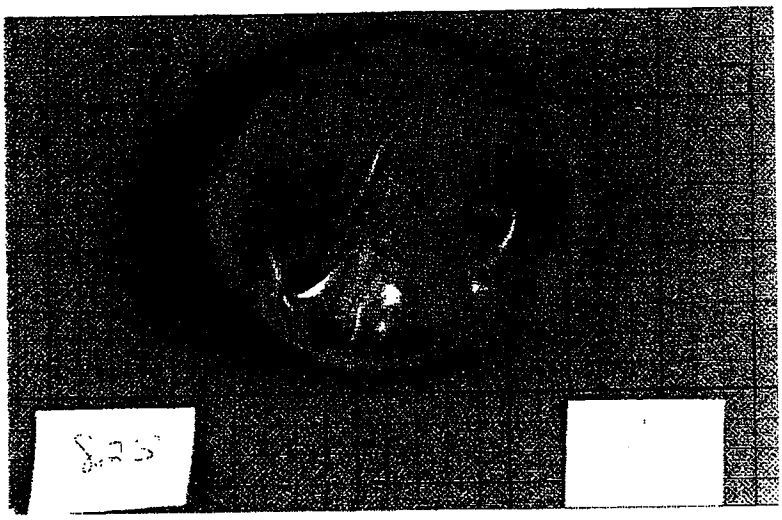

FIGURE 2. Intact Heyer-Schulte saline explant with 22 years of implantation. To our knowledge, oldest US-made saline implant tested.

than those of the extracted shells. The failed Heyer–Schulte explant with 21 years of implantation had the greatest decrease in ultimate properties, with the tensile strength and elongation of unextracted specimens being about 50% and 30% lower than extracted specimens.

The failure of the Heyer–Schulte explant with 21 years in vivo occurred in a fold near the outer perimeter of the smooth shell, shown in Figure 3. A 6.3-mm tear, which appears as a straight line in this 35-mm photograph, developed in the fold. Figure 4, which is an SEM micrograph

TABLE 4. Ultimate Strength Properties of Five Saline Explant Shells

Explant	Years in vivo	Tensile strength (PSI)	Percent elongation	Tear resistance (P/I)	Shell thickness (in)
Simaplast	23	866	299	74	0.023
Heyer-Schulte	22	371	387	33	0.018
Heyer-Schulte	21	284	421	25	0.018
McGhan	5.5	412	300	35	0.027
McGhan	3	642	420	52	0.023

PSI = pounds force per square inch (1 psi = 6.895 kPa)
P/I = pounds force per inch (1 P/I = 1.751 N/cm)

TABLE 5. Tensile Strength and Elongation of Extracted Saline Explant Shells

Explant	Years in vivo	Percent extracted	Tensile strength (PSI)	Elongation (%)
Simaplast	23	2.8	937	303
Heyer-Schulte	22	21.7	480	421
Heyer-Schulte	21	25.5	557	598
McGhan	5.5	2.7	449	373
McGhan	3	3.1	664	409

PSI = pounds force per square inch (1 psi = 6.895 kPa)

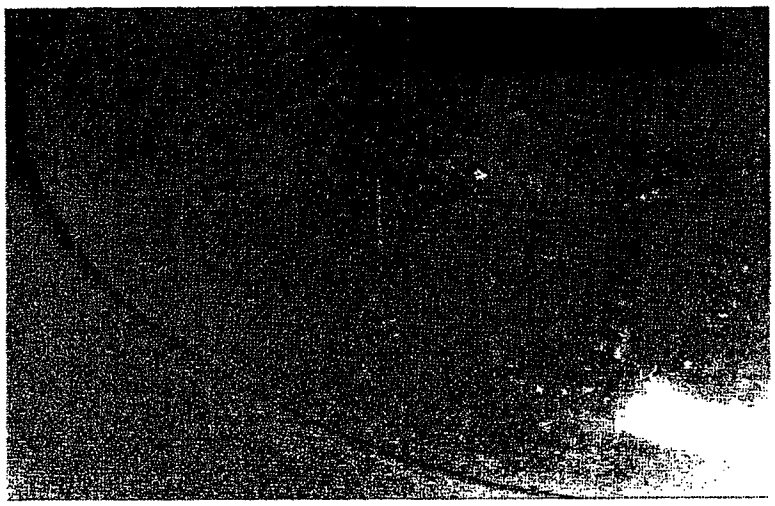

FIGURE 3. Heyer-Schulte saline explant with 21 years in vivo failed in fold near outer perimeter of smooth shell, where 6.3 mm tear developed. Tear appears as straight line in photograph.

of the outer surface of the failed region, reveals the morphology of the tear. There is no sign of significant surface abrasion on either the inside or outside surface of the shell at the fold region. A scale (in micrometers) is shown in the lower right of the micrograph.

The failed region of the McGhan implant that deflated after 5.5 years of implantation was not visible until the texturing on the surface was lightly spread apart on either side of the tear. The SEM micrograph in Figure 5 reveals that the tear near the outer perimeter is about 1.5 mm in length; no indication of surface abrasion is visible on either the inner smooth or outer textured side of the shell.

The failure of the McGhan explant with 3 years in vivo also occurred in a fold near the outer perimeter, where a small crack developed between two regions of wear. The SEM micrograph of the inner shell surface for this saline explant (Fig. 6) shows that the failure site consists of a 0.06-mm hole with a crack propagating outward from the hole and extending a length of about 0.20 mm. (For comparative purposes, the width of the hole is about the

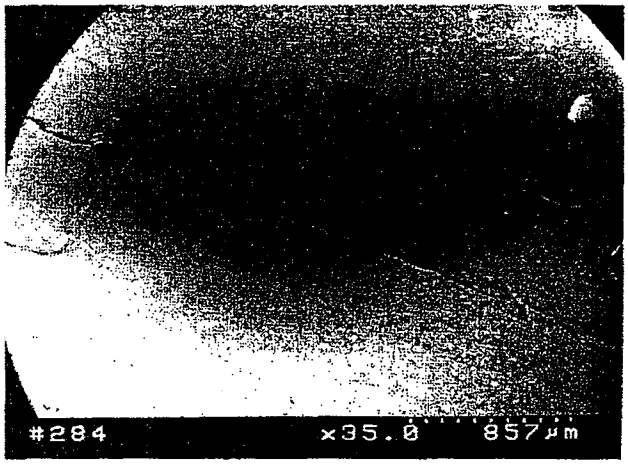

FIGURE 4. SEM micrograph of outer shell surface of failed region seen in Fig. 3. No evidence of significant surface abrasion on inside or outside surface of shell in fold area.

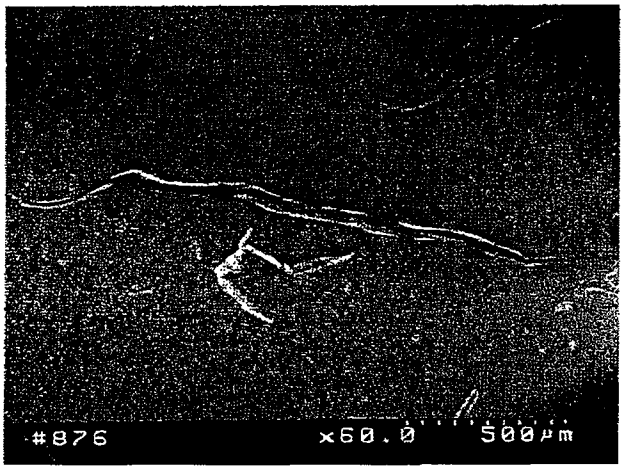

FIGURE 5. Textured McGhan saline implant deflated after 5.5 years in vivo. The SEM micrograph shows 1.5-mm-long tear near outer perimeter, but no evidence of surrounding surface abrasion. Micrograph taken of smooth inner surface of shell exposed to saline.

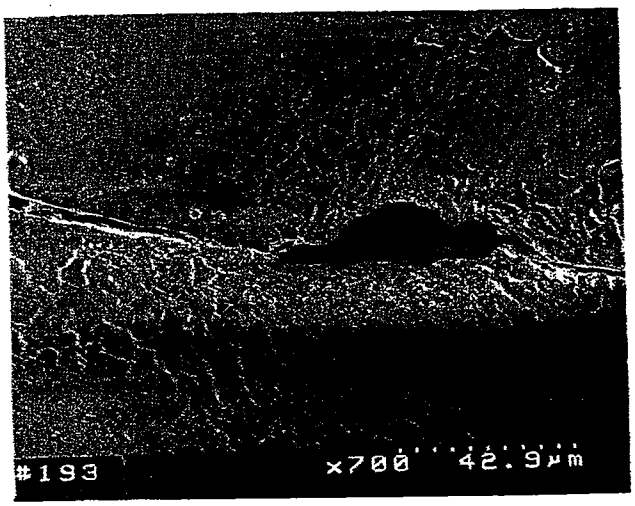

FIGURE 6. Textured McGhan saline explant with 3 years in vivo also failed in fold near outer perimeter, but abrasion evident around failure site. SEM micrograph of inner shell surface reveals 0.06-mm hole with crack propagating outward and extending about 0.20 mm.

size of a human hair.) An abrasion pattern around the small hole is also visible.

Although the three saline explants seen in Figures 4, 5, and 6 failed near the outer perimeter on the anterior surface, the morphology of the failed regions is quite different. These micrographs demonstrate that failure can exhibit drastically different morphology.

VII. CONCLUSION

The methods described herein are presented as a proposed standardized protocol for breast implant retrieval and analysis. The Center for Implant Retrieval and Analysis routinely tests explants to determine their ultimate strength properties (tensile strength, elongation, and tear resistance) and examines implant shells with SEM to document patterns and mechanisms of device failure. The plastic surgery community should be aware that the methodologies outlined above are available and are sometimes necessary to determine what happens to a breast implant while it is implanted in the body. To better appreciate material properties data reported in the literature, surgeons need to understand what is involved in the testing process and what the data mean. In addition, this protocol can be used by different research groups to acquire reliable data pertaining to silicone gel and saline breast implant analysis as part of the effort to explain the effects of long-term implantation on elastomer shells. Only then can the historical and clinical information related to retrieved implants be correlated with their material properties measurements and microscopic examination.

We have included examples from recent study of saline explants, one with an implantation time of 23 years. It is hoped that these examples will illustrate the testing process and the kinds of data attainable with different analytical tools. In addition, these data add to our understanding of saline implants, which have not been investigated as vigorously as have silicone gel devices.

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