Spontaneous Autoinflation of Saline Mammary Implants: Further Studies

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Background: Previous studies have reported a hyperinflation of saline-filled breast implants. On removal, the implant fluid had changed from clear saline to a yellowish-brown color, with a viscous consistency similar to serum.

Objectives: Our objective was to identify further the components of saline from implants that had undergone spontaneous autoinflation. Our hypothesis was that if serum albumin is present in the fluid, then other proteins would likely be found.

Methods: To screen and identify proteins in implant fluid, we used a proteomics-based approach that included 1- and 2-dimensional gel electrophoresis and mass spectrometry of protein samples.

Results: Four known proteins and 1 unknown protein product were identified. Based on 2-dimensional gel electrophoresis and mass spectrometry, 2 general observations can be made about the saline from the autoinflated implants: serum albumin was the most prevalent protein, and there are a large number of proteins that remain to be identified.

Conclusions: There are multiple macromolecules that cross into the lumen of the prosthesis. We believe spontaneous autoinflation is occurring more often than is believed or reported. (Aesthetic Surg J 2005;25:582-586.)

The implantation of gel-filled or saline-filled breast implants is a widely accepted procedure that has been performed for more than 3 decades. Most recipients of this procedure undergo aesthetic breast augmentation, and a smaller number receive implants in the course of breast reconstruction. A phenomenon associated with saline-filled implants known as “spontaneous autoinflation” has been investigated by only a few groups,1-4 each reporting on different aspects of this complication. Our first report in 19971 involved 5 patients. Since that time, 9 additional patients have presented with the same phenomenon in our practice, for a total of 14 cases in more than 3000 patients that have undergone the procedure. The chief complaint of these patients was a gradual increase in the size of one breast over a period of weeks or months (Figure 1). None of the 14 patients has experienced a recurrence of autoinflation.

In all cases, the original surgery was performed in the operating room under general anesthesia with a closed system. The pocket location for all 14 patients was subpector al. Irrigation was performed with bacitracin; steroids or Betadine were not used. All surgery was performed with blunt dissection. Two people measured the amount of saline placed in all implants, and all implants were filled according to the manufacturer’s specifications.

On gross examination of the implants, 3 common observations regarding this condition were observed. First, the implants’ liquid volume had increased in size since implantation; second, the consistency of the fluid removed was described as serum-like; and third, the removed fluid was yellow to brown in color (Figure 2), suggesting a high-protein concentration. Two previous studies1,3 have shown serum albumin to be present within explanted, hyperinflated implants. We used a proteomics-based approach to conduct a more thorough investigation to determine what other proteins might be present within this fluid. The results of this study show that, in addition to serum albumin, numerous other proteins of varying molecular mass pass through the lumen of the implant into the saline-filled interior.

Methods

One-dimensional sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE)

Protein concentrations of fluid from autoinflated and normal implants were determined using a modified
Bradford protein assay (BCA assay, Pierce Biotechnology Inc, Rockford, IL). A BSA standard curve and sample measurements were done in triplicate to determine total protein concentration of the implant fluid samples. Implant fluid was added to Laemmli sample buffer (62.5 mM Tris-HCl, pH 6.8, 2% SDS, 25% glycerol, 0.01% bromophenol blue, 5% β-mercaptoethanol), heated at 95°C for 5 minutes, and separated using SDS-PAGE on 4% to 20% gradient Tris/glycine gels (Bio-Rad, Hercules, CA) by applying a constant 150 V for a minimum of 1 hour at room temperature. After the samples were separated, the gel was washed 3 times in a minimum of 50 mL of diH2O for 5 minutes each at room temperature. The gel was Coomassie-stained using 30 mL of GelCode (Pierce Biotechnology Inc) for 1 hour at room temperature, followed by a 1-hour diH2O wash at room temperature. The gel was scanned using an HPScanJet flatbed scanner (Hewlett-Packard Co., Palo Alto, CA) and imported into PhotoShop 7.0 (Adobe Systems Inc., San Jose, CA) for image analysis.

Two-dimensional (2-D) gel electrophoresis

Implant samples were diluted with lysis buffer (7 M urea, 2 M thiourea, 4% CHAPS, 2% ampholytes pH 3-10, 5 mM tributylphosphine (Amersham All Plus One Reagents, Amersham Biosciences, Piscataway, NJ) to a final volume of 200 μL. Two gels were run in parallel, one with a final protein concentration of 100 μg and the second with a total of 200 μg of protein. Diluted samples were added to 4% ReadyStrip pH 3-10 (Bio-Rad) immobilized pH gradient (IPG) strips, and a passive rehydration overnight (~14 hours) was performed. IPG strips were run on a Multiphor II IEF apparatus (Amersham Biosciences) for 1.5 hours, 300 V at 18°C, and then for 4.5 hours, 3500 V at 18°C, to separate the proteins based on their isoelectric point. IPG strips were then frozen at ~86°C overnight and equilibrated the following day for 20 minutes at room temperature. IPG strips were loaded onto Criterion gradient gels 8% to 16% Tris/glycine (Bio-Rad) and run with constant 200 V for 1.5 hours at 4°C to separate proteins based on their molecular weight. Gels were fixed for 30 minutes in 40% methanol/10% acetic acid at room temperature with gentle agitation, and were then stained overnight with SYPRO Ruby (Invitrogen-Molecular Probes, Carlsbad, CA) at room temperature with gentle agitation. The following day, the gels were destained in 10% methanol/7% acetic acid for 2 hours at room temperature and then imaged using a Bio-Rad FX Pro Laser Scanner. Image analysis was performed in PDQuest (Bio-Rad) and ProGenesis (Discovery by NonLinear Dynamics, Durham, NC).

Mass spectrometry of protein samples

Protein spots were picked from the 2-D gels, in-gel digests were performed, and the tryptic digests were analyzed using matrix-assisted laser desorption ionization-time of flight (MALDI-TOF). Peptide masses were entered into Mascot (Matrix Science Inc, Boston, MA: http://www.matrixscience.com), and the NCBI database was searched to identify the protein. Briefly, the in-gel trypsin digest procedure was adapted from what was developed at the University of California–San Francisco Mass Spectrometry Facility (http://donatello.ucsf.edu/ingel.html).

Results

The implant fluid from the hyperinflated implant was assayed for total protein concentration and determined to have a protein concentration of 10 mg/mL. Our control consisted of using a normal implant that had been removed from a patient after 10 years of implantation.
The normal implant’s total protein concentration was determined to be 0 mg/mL. Figure 3 is an image of a SDS-PAGE gradient gel (4% to 20%) loaded with equal volume amounts of both the hyperinflated implant fluid and the normal implant fluid for comparison. Different amounts of protein from the implant fluid were loaded for comparison as well. Lanes 1 to 4 were loaded with 10 μg total protein (10 μL of sample), 5 μg total protein (5 μL of sample), 1 μg total protein (1 μL of sample), and 0.5 μg of total protein (0.5 μL of sample), respectively. Lanes 5 to 7 were loaded with fluid from a normal implant. Lane 5 was loaded with 10 μL of sample; lane 6, 5 μL of sample; and lane 7, 1 μL of sample. A prominent band below the 75-kDa marker (approximately 60 to 65 kDa) was observed in all lanes; this is the expected region of the gel where serum albumin would be found. Lanes 1 and 2 exhibited the same protein profile, with detectable protein bands ranging from 75 kDa to 25 kDa. In lanes 3 and 4, only the prominent protein band located slightly below the 75-kDa marker remained detectable. Figure 4 presents the result of 2-D gel electrophoresis. The numbers indicate protein spots that were picked and identified to be statistically significant ($P < 0.05$). This gel had a total of 100 μg of protein loaded onto it. A particularly large spot can be seen in the center of the gel (spot 1); this is human serum albumin. The numbers of the protein spots on the gel correspond with the listing of the proteins identified and their molecular masses as determined by MALDI-TOF analysis in Table 1.

Table 2 shows laboratory analysis of the fluid from 3 patients with unilateral autoexpansion. Material from 12 of 14 hyperinflated implants was cultured, with no resultant growth (fungus, bacteria). No common denominator for patients with this result was observed. Factors such as lifestyle, habits, and occupation were not significant (Table 3).

**Discussion**

Although it is clear from the literature that spontaneous autoinflation of saline-filled breast implants occurs, it remains an underreported and poorly understood phenomenon of this prosthesis. Two studies have examined the contents of the autoinflated implants. However, no previous study has sought to ascertain, on a larger scale, which proteins are present in the implant solution beyond the apparently abundant serum albumin.

It can be clearly seen from the data presented in this article that a proteomics-based approach is well suited for this type of study. This proteomics-based approach requires only a minimal amount of sample and can screen many proteins from a 2-D gel in a short time. Although not measured quantitatively in this study, serum albumin appears to be the most abundant protein in the implant fluid samples. The protein with the highest level of staining is shown in Figures 3 and 4. The mass spectrometry data reported in Table 1 confirm that this protein is indeed serum albumin. A second implant sample from a different patient (data not shown) was so rich in serum albumin that it interfered with the identification of less abundant proteins using MALDI-TOF mass spectrometry. We are currently trying different methods to remove the serum albumin in an attempt to identify these other proteins.
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Careful observation of Figure 4 shows a large number of protein spots. Many of these were subject to MALDI-TOF; some of the protein spots were the same proteins reported in Table 1 but with modifications, so they were not included in the table. Some proteins (e.g., Acyl-CoA synthetase 4, T-cell receptor beta chain, and KIAA1037 protein) had scores that were below 74, which were not statistically significant and so are not presented in this study.

The most recent study of this phenomenon used magnetic resonance imaging, a noninvasive technique, as a method for postoperative volume determination of saline breast implants. The authors compared volume measurements of patients with breast implants at 3 months and 18 months postoperatively. They reported an initial increase in prosthesis volume size 3 months after implantation. This group measured the breast implants at 18 months after implantation and observed the implants remained autoinflated. They measured no significant difference in postoperative volume change between the 3 and 18 months and suggested that the autoinflation reached equilibrium during the 2 time points.

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Table 1. Proteins identified by MALDI-TOF and their molecular mass

<table>
<thead>
<tr>
<th>Protein spot number on 2-D gel</th>
<th>Proteins identified by MALDI-TOF*</th>
<th>Molecular mass (kDa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Human serum albumin</td>
<td>65.9</td>
</tr>
<tr>
<td>2</td>
<td>Transferrin precursor</td>
<td>77.0</td>
</tr>
<tr>
<td>3</td>
<td>Keratin, type I cytoskeletal</td>
<td>59.4</td>
</tr>
<tr>
<td>4</td>
<td>Unnamed protein product</td>
<td>41.4</td>
</tr>
<tr>
<td>5</td>
<td>IgM Kappa IIIb</td>
<td>13.7</td>
</tr>
</tbody>
</table>

*All proteins identified were significant (P < 0.05) with a Mowse score of greater than 74.

Table 2. Laboratory analysis of implant fluid from 3 patients with unilateral autoexpansion

<table>
<thead>
<tr>
<th>Test</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein (μg/mL)</td>
<td>9.1</td>
<td>9.9</td>
<td></td>
</tr>
<tr>
<td>Na+ (mEq/L)</td>
<td>150</td>
<td>148</td>
<td>141</td>
</tr>
<tr>
<td>K+ (mEq/L)</td>
<td>1.6</td>
<td>1.6</td>
<td>1.9</td>
</tr>
<tr>
<td>Cl– (mEq/L)</td>
<td>130</td>
<td>134</td>
<td>123</td>
</tr>
<tr>
<td>CO2 (mEq/L)</td>
<td></td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Glucose (mEq/L)</td>
<td>6</td>
<td>25</td>
<td>26</td>
</tr>
<tr>
<td>Osmolality (mOsm/kg)</td>
<td>290</td>
<td>291</td>
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</tr>
<tr>
<td>Specific gravity</td>
<td>1.010</td>
<td>1.012</td>
<td>1.011</td>
</tr>
<tr>
<td>Viscosity</td>
<td>15.38</td>
<td>4.188</td>
<td></td>
</tr>
<tr>
<td>pH</td>
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<td></td>
<td>8.00</td>
</tr>
</tbody>
</table>

Table 3. Summary of clinical data (14 patients)

- Mean patient age (range): 43 y (37-61 y)
- Mean time interval after implantation: 8+ y (3 y, 10 mo to 17 y, 1 mo)
- Procedure:
  - Aesthetic augmentation: 12 patients
  - Reconstruction: 2 patients
- Mean increase in volume: 159.9 cc
- Autoinflation location:
  - Left implant: 10 patients
  - Right implant: 3 patients
  - Both implants: 1 patient
Conclusion

The exact mechanism by which spontaneous autoinflation occurs is not completely understood, nor is it known why the autoinflation is often unilateral. However, it is clear that a variety of large macromolecules are penetrating the breast implants. It is likely that this imbalance of macromolecules across the water-permeable implant wall leads to osmotic swelling, akin to what occurs in capillary beds throughout the body. We conclude this phenomenon is occurring more often than is believed or reported.

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References