The Use of Antibiotic Impregnated Mesh Reduces the Formation of Biofilm-induced Capsular Contracture in the Porcine Model

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Introduction

Four decades after the introduction of silicone breast implants, capsular contracture remains the most common and most significant complication, with a reported prevalence from 0.6 to 100% (Iwuagwu 1997). Furthermore, it accounts for the majority of remedial surgery. There is growing evidence that capsular contracture is caused by sub-clinical infection of mammary implants by bacterial biofilm. In the absence of frank sepsis, *Staphylococcus epidermidis* was the most commonly isolated organism from bacteriologic cultures of contracted capsules. We have previously reported a prospective blinded study of capsules and implants following augmentation mammoplasty that showed isolation of *S. epidermidis* in 83% of samples obtained from women with symptomatic contracture (Pajkos 2003). It is increasingly clear that *S. epidermidis* biofilm is a major cause of prosthetic failure. Recent work has established its role in contamination of urinary catheters, cardiac valves, orthopedic prosthesis, vascular grafts as well as contact lenses (Donlan 2001).

We hypothesized that bacteria such as *Staphylococcus epidermidis* gain access to the mammary implant at the time of placement and once in contact with the prosthetic surface, forms a biofilm. This film forms the focus of ongoing irritation and stimulus for fibrous tissue formation and subsequent contracture (figure 1). Furthermore, that by preventing biofilm formation contracture around prostheses will be prevented.

**Figure 1: The role of biofilm in the genesis of capsular contracture**

Our most recent investigations using a pig model have now confirmed this hypothesis (Tamboto et al 2010). Miniature silicone gel textured mammary implants (McGhan, Dublin, Ireland) were placed in a submammary pocket using standard sterile operative techniques as employed in human. Implant pockets were inoculated with serially diluted clinical sample of *S. epidermidis* taken from a contracted breast. A total of 51 mammary augmentations were performed on 6 pigs. Pocket inoculation was strongly associated with biofilm formation (p = 0.0095). 80.6% of biofilm-positive implants developed contracted capsules. Biofilms were significantly associated with capsular contracture (p = 0.0213). Biofilm formation was
associated with a four-fold increased risk of developing contracture (OR = 4.1667, 95% CI = 1.1939 – 14.5413).

We aimed to investigate whether the use of an antibiotic mesh placed at the time if implantation would reduce biofilm formation and subsequent capsular contracture following breast augmentation in the porcine model.

**Methods**

Four cm diameter smooth, gel filled implants (TyRx Pharma, New Jersey USA) were placed into submammary pockets, dissected under the cranial, middle or caudal sets of teats, of adult female non-lactating pigs. Surgical methods were as described by Tamboto et al. All implants were inoculated with $10^5$ CFU of human clinical strain of *S. epidermidis*, originally isolated from a contracted breast in a patient. In the antibiotic treated group, a circular disc of antibiotic impregnated mesh (AIGISRx: TyRx Pharma New Jersey USA) was placed immediately beneath the implant. The mesh consists of a resorbable polymer carrying minocycline and rifampicin, which have been shown to be effective against *S. epidermidis* for a period of 10 days (TyRx Pharma, New Jersey USA).

The implants were left *in situ* for 16 weeks when they were subsequently assessed for contracture and harvested for biofilm analysis.

**Assessment of contracture**

Contracture of the implants was assessed by two methods:-

1. Blinded Baker manual scaling method, where:-
   - Baker grade I- the breast feels as soft as an un-implanted one and has no palpable capsule;
   - Baker grade II- there is minimal breast firmness and the implant can be palpated but is not visible.
   - Baker Grade III - with capsular contracture the breast becomes harder and distorted and the implant can be easily palpated or seen.
   - Baker Grade IV - with continued disease the breast is hard and distortion is often marked.

2. Applanation tonometry as adapted from Minami et al 2006 method. The implant and surrounding capsule was aseptically dissected and transported on ice to the laboratory. Further manipulations were conducted in a class II biohazard cabinet. The capsule and implant where overlaid with sterile foil and a Vaseline coated 300g weight was applied and the area of touch outlined. The area of touch was calculated using Adobe Professional software.

**Assessment of biofilm infection**

Biofilm infection was assessed by bacterial viability counts, total bacterial counts and SEM.
1. **Bacterial viability counts.**

Samples of capsule and implant were aseptically macerated with a scalpel blade, subjected to vigorous shaking (3 minutes before and after sonication) and ultrasonication (Soniclean bath, JMR, Sydney Australia) at a sweeping frequency of 42-47 kHz for 20 minutes in 15ml of Tryptone Soy Broth (Oxoid, Adelaide, Australia). The broths were incubated for at 37°C and inspected for signs of bacterial growth daily for 2 days and re-plated onto blood agar if positive. Classical microbiological techniques were used to identify positive cultures.

Quantitative viability counts (colony forming units CFU/gram of tissue) were obtained by plating 100µL of neat sample and dilutions (10⁻¹ to 10⁻²) onto blood agar (Oxoid, Adelaide, Australia). These cultures were incubated aerobically at 37°C.

2. **Total bacterial counts**

Total numbers of bacteria per gram of tissue were estimated using quantitative PCR which can detect all bacteria including viable culturable, viable non-culturable and dead bacteria.

**Bacterial DNA extraction:**

50mg-100mg frozen samples of each pig capsule were added to 275µL digestion buffer (50nMTris/HCl pH7.5, 150nM NaCl, 2mM EDTA, 1% SDS) containing 400µg proteinase kinase (Sigma Aldrich) and incubation at 50°C overnight. After pig capsule tissue was fully digested, proteinase kinase was inactivated by boiling for 5 min. Then lysozyme (Sigma Aldrich) was added to final concentration at 0.5mg/ml and incubated at 56°C for 2 hours prior to addition of 200µg proteinase kinase and incubation at 50°C for another 2 hours. Genomic DNA was extracted using a phenol/chloroform and then ethanol precipitated. Extracted DNA was resuspended in 100µl TE (10mM Tris-HCl pH 8.0 and 1mM EDTA) buffer.

**Quantitative real-time PCR:**

The quantification of total bacterial DNA in each pig capsule tissue was achieved by real-time quantitative PCR (qPCR) using universal eubacterial primer 16s rRNA_341F 5’-CCTACGGGAGGCAGCAG-3’ and 16s rRNA_534R 5’-ATTACCGCGGTGCTGCTG-3’ to amplify a 194bp amplicon of 16s rRNA gene of all bacteria. Pig 18s rRNA gene was used as a reference gene to normalise the amount of pig tissue used in DNA extraction. The primer pair used in 18s rRNA gene real-time PCR was 18s rRNA_756F 5’-GGTGGTGCCCTTCCGTCA-3’ and 18s rRNA_877R 5’-CGATGCGGCGCCGCTATT-3’ to amplify a 122bp amplicon.

Real-time PCR was carried out in 25 µl reaction mix containing 1X Brilliant II Sybr Green qPCR Master mix (Stratagene), 400nM forward and reverse primer and 100ng DNA template and was performed in Corbett Rotorgene 3000 with the following cycling conditions: activation of Taq polymerase at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 sec, annealing at 56°C for 30 sec and extension at 72°C for 20 sec.
Each qPCR was run with standard samples of known concentrations (copies/µl). The standards for the quantitative PCR were prepared from PCR fragments excised from a 1.5% agarose gel electrophoresis and purified by QIAquick Gel Extraction Kit (Qiagen). All steps were checked by running on an agarose gel to confirm a single clean band of DNA of the correct size.

Concentration of the purified nucleic acid was calculated by measuring the absorbance at 260 nm and its corresponding concentration was converted into copies/µl of PCR amplicon by using the Avogadro constant ($6.023 \times 10^{23}$) and its molecular weight (number of bases of the PCR product multiplied by the average molecular weight of a pair of nucleic acids (660 Da) (Sambrook et al. 1989).

Ten fold serial dilutions of the quantified 16s rRNA gene and 18s rRNA gene PCR amplicon solution were kept in aliquots at -20°C and used as external standards of known concentration (copies/µl) in real-time PCR reaction. The standard samples were ranged 10–10⁶ copies/µl which used to construct a standard curve for each qPCR run. The calibration curve was created by plotting the threshold cycle (Ct) corresponding to each standard vs the value of their corresponding gene concentration (copies/µl).

Copy number of total bacteria 16s rRNA gene was normalised against copy number of pig 18s rRNA gene in each pig capsule.

3. Scanning electron microscopy

The presence of biofilm in capsules was confirmed visually by scanning electron microscopy (SEM). Five sections (1cm²) of implant and capsule were fixed in glutaraldehyde for 1.5hrs, dehydrated through ethanol, immersed in HMDS (Polysciences Inc.) for 3 min before being aspirated dry and stored desiccated. The dried sample was coated with 20nm gold film in a Sputter Coater and examined in a scanning electron microscope.

Statistical analysis

The Chi-square test was used to compare the differences in contracture rates between treatment and control groups. A t-test was used to examine for differences between the treated and untreated, contracted implants. To examine for variation in bacterial numbers or implant tonometry between multiple tests an Analysis of Variance (ANOVA) with the Holm-Sidak method of multiple comparisons was performed. All statistical analysis was conducted using the SigmaPlot 11 Statistical Program.

Results

A total of 28 implants; 14 control and 14 antibiotic treated were implanted into 5 pigs. The implants were left in situ for 16 weeks prior to assessment and harvest. 1 control implant was found to have been extruded and was, therefore, excluded from analysis.
Assessment of contracture
Assessment of Baker grading showed all 13 control implants were contracted (graded III/IV) as compared with the 14 antibiotic treated which remained non-contracted (graded I/II). This difference was highly significant ($\chi^2=23.14$, df=1, p<0.001). Individual pig results are found in Table 1 and results are summarized in table 2.

Table 1. Baker grading of control and treated breasts related to anatomical insert positions. Two numbers in one cell represent both right and left breast implanted.

<table>
<thead>
<tr>
<th>Position</th>
<th>Control</th>
<th>Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cranial</td>
<td>Middle</td>
</tr>
<tr>
<td>Pig 1</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Pig 2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Pig 3</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Pig 4</td>
<td>3, Lost</td>
<td>1, 1</td>
</tr>
<tr>
<td>Pig 5</td>
<td>3, 3</td>
<td>3, 4</td>
</tr>
</tbody>
</table>

Table 2 Baker grading and percentage of contracted implants related to treatment.

<table>
<thead>
<tr>
<th>Status</th>
<th>Baker Grade</th>
<th>Contracture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Grade 3 = 6</td>
<td>Contracture 13/13</td>
</tr>
<tr>
<td>n = 14</td>
<td>Grade 4 = 7</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>* extruded = 1</td>
<td></td>
</tr>
<tr>
<td>Antibiotic mesh</td>
<td>Grade 1 = 8</td>
<td>Contracture 0/14</td>
</tr>
<tr>
<td>n = 14</td>
<td>Grade 2 = 6</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No contracture 14/14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100%</td>
</tr>
</tbody>
</table>

Tonometry confirmed our findings on Baker assessment. Implants with a higher Baker grading (control implants) having reduced surface area indicating greater thickness of the capsule as compared with antibiotic treated implants (See table 3). Despite the trend in reducing surface area as Baker grading increased these differences were not significant due to
the large range of surface areas obtained.

Table 3: Surface area measured by applanation tonometry for control (Baker grade 3 and 4) versus antibiotic treated implants (Baker grade 1 and 2). Mean mm² ± standard deviation (std).

<table>
<thead>
<tr>
<th>Baker Grade</th>
<th>Mean</th>
<th>Mean ± std</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>940</td>
<td>Treated</td>
<td>913 ± 217</td>
</tr>
<tr>
<td>2</td>
<td>870</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>836</td>
<td>Control</td>
<td>772 ±196</td>
</tr>
<tr>
<td>4</td>
<td>716</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Biofilm evaluation

On enrichment culture most of implants grew coagulase negative Staphylococcus of pig species origin, generally *S simulans*. Quantitative counts, were generally low with only 7 implants have more than 10 colonies. There was no relationship to implant treatment.

The number of total bacteria per PCR reaction was lowest in Baker grade 1 and gradually increased until Baker grade 3 before decreasing slightly (figure 2) however, this relationship was not significant.

Figure 2. Total number of bacteria related to Baker grading

Scanning electron microscopy confirmed the presence of bacterial biofilm on both implant surface and capsule (See figure 3a and b)
Figure 3a: biofilm on surface of porcine implant

Figure 3b: biofilm on inner surface of contracted capsule

References


