

**Chronic biofilm infection in breast implants is associated with an increased  
T cell lymphocytic infiltrate – implications for breast implant associated  
lymphoma**

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Our disclosures are listed below: A/Prof Deva and Vickery are consultants to Allergan, Mentor (J&J) and KCI. They have previously coordinated industry-sponsored research for these companies relating to both biofilms and breast prostheses.

None of the material has been published previously, is under consideration, or has been accepted for publication elsewhere.

## Abstract

**Introduction:** Biofilm infection around breast implants is a significant potentiator of capsular contracture. We aimed to investigate if chronic bacterial biofilm infection could promote T cell hyperplasia, a possible prelude to tumorigenesis.

**Methods:** Pig study: Twelve textured and 12 smooth implants were inserted into submammary pockets in three adult pigs. Implants were left in situ for a mean of 8.75 months.

Human study: Fifty-seven capsules from patients with Baker grade 4 capsules were collected prospectively over a 4-year period.

Biofilm and surrounding lymphocytes were analysed using a variety of culture, nucleic acid and visualisation techniques.

### Results:

Pig study: All samples were positive for bacterial biofilm. There was a significant correlation between the bacterial numbers and grade of capsular contracture ( $p=0.04$ ). Quantitative real-time PCR showed that all lymphocytes were significantly higher on textured implants as compared with smooth ( $p<0.001$ ). T cells accounted for the majority of the lymphocytic infiltrate. Imaging confirmed the presence of activated lymphocytes.

Human study: All capsules were positive for biofilm. Analysis of lymphocyte numbers showed a T-cell predominance ( $p<0.001$ ). There was a significant linear correlation between the number of T and B cells and the number of detected bacteria ( $p<0.001$ ). Subset analysis showed a significantly higher number of bacteria for polyurethane implants ( $p<0.005$ ).

**Discussion:** We have shown that chronic biofilm load produces an increased T cell response both in the pig and in humans. A possible link between bacterial biofilm and T cell hyperplasia is significant in light of breast implant associated anaplastic large cell lymphoma.

## Introduction

Capsular contracture continues to be the most common complication following the use of breast implants in both aesthetic and reconstructive surgery.<sup>1,2</sup> Infection of mammary implants with bacterial biofilm has been shown to be a significant potentiator of capsular contracture.<sup>3-5</sup> The subclinical infection theory, first proposed by Burkhardt<sup>6</sup>, has now been validated by both clinical and laboratory evidence.<sup>2,3,7-11</sup> Bacteria that live on the skin and within the breast ducts can contaminate the surface of the breast implant at the time of insertion. These bacteria subsequently form a biofilm, defined as a combination of bacterially derived sticky glycoprotein and nearly dormant bacteria, which binds irreversibly to the underlying silicone elastomer. Bacteria within the biofilm are resistant to antibiotics and antiseptics.<sup>12</sup> If the biofilm reaches a threshold that overwhelms the local host defenses, it will continue to proliferate and expand, eventually causing local inflammation and subsequent fibrosis leading to the establishment of capsular contracture (see Figure 1).<sup>5</sup>

We have established a valid experimental model for studying the progression of biofilm contamination to contracture in mammary implants using the pig.<sup>3</sup> Additionally, we have shown that the use of local antibiotics at the time of implant insertion can significantly reduce the incidence of capsular contracture.<sup>13</sup> This has been further supported in a recent clinical study showing a reduction of capsular contracture from 6% to 0.6% using intraoperative pocket irrigation.<sup>14</sup> Our most recent findings have shown that, once contaminated, textured outer surface of breast implants support a significantly higher load of bacteria than implants with a smooth outer shell.<sup>15</sup> The issue of lymphocyte response to breast implants has been recently raised by the reporting of breast implant associated acute large cell lymphoma (ALCL).<sup>16</sup>

In order to further analyze the interaction between biofilm load, capsular contracture and host response in the pig, we left biofilm infected implants *in situ* 3 times longer than in our previous experiments. We also prospectively examined human capsules around textured implants from patients with Baker grade 4 capsular contracture undergoing revision surgery to generate comparative findings.

## Methods

Approval for the all study protocols was obtained from the University of Sydney Animal Ethics Committee. All pigs were housed as a group in designated animal care facilities at the University of Sydney Farms, New South Wales, Australia. Human ethical approval was obtained from Macquarie University Human Ethics Committee.

## **Pig study**

A total of 24 implants, 12 textured and 12 smooth implants (Allergan, Irvine, Ca, USA) were inserted into submammary pockets into three adult, female, non-lactating, domestic large white pigs (*Sus domesticus*) weighing approximately 400 kg using methods as described by Tamboto et al.<sup>3</sup> This model has been previously reported in detail and has become accepted as a valid model to study the development of capsular contracture following biofilm infection of breast implants<sup>3,13,17</sup>. In summary, mini implants were inserted into a submammary pocket and inoculated with a dose of human *Staphylococcus epidermidis* previously titrated to generate consistent periprosthetic biofilm infection<sup>3</sup>. The animals were monitored daily in the postoperative period by a veterinary surgeon until their wounds were healed and during their period of housing by support staff at the veterinary school. Implants were left *in situ* for a mean of 8.75 months after which Baker grading<sup>18</sup> was performed (See Table 1). Baker grading was performed by qualified plastic surgeons, blinded to the implant type and has been supported as a valid outcome measure of contracture<sup>3,13,17</sup>. Baker grading has also been shown to be consistent with applanation tonometry when measuring degree of capsular contracture using the porcine model<sup>13</sup>.

## **Human study**

A prospective collection of capsules from patients undergoing total capsulectomy and removal of implants for Baker Grade 4 contracture was commenced in January 2009.

## **Sample Analysis**

### *Bacterial culture and identification*

The capsule was separated from the implant using aseptic techniques in a class II laminar flow cabinet. The capsule was macerated and transferred to a 10ml tryptone soya broth (Oxoid) and sonication at a mean sweeping frequency of 43kHz in an ultrasonic bath (Soniclean: JMR, Sydney, Australia) for 20 minutes followed by shaken vigorously for 2 minutes.<sup>3</sup> Colony forming units were determined by serial dilution and standard plate culture.

### *Polymerase Chain Reaction*

50mg-100mg of pig or human capsule or breast implant was digested using a combination of proteinase K and lysozyme and the genomic DNA extracted using phenol/chloroform extraction followed by ethanol precipitation as described previously.<sup>15</sup>

Total bacterial number in each sample was determined by real-time quantitative PCR (qPCR) of the eubacterial 16 rRNA gene- present in all bacteria<sup>15</sup>.

The number of T cells and B cells in each pig or human capsule tissue or attached to breast implants removed from pigs was quantified by real-time quantitative PCR of CD3 gene (Total T cell), CD4 gene (T helper) and CD8a (T cytotoxic) and CD79a gene (Total B cell). Primers specific to these genes in pig and human capsule are listed in Table 2.

The total number of bacteria and lymphocytes in human and pig capsule was expressed as per mg of capsule based on the average number of copies of the 18S gene in a mg of pig or human tissue.

Quantitative 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 with the following cycling conditions 95°C for 10 min, followed by 40 cycles of 95°C for 15 sec, 56°C for 30 sec and 72°C for 20 sec as described previously.<sup>13</sup> Each qPCR was run with standard samples of known concentrations ranging from 10<sup>2</sup>-10<sup>8</sup> copies/µl.

#### *Scanning Electron Microscopy*

The presence of biofilm was visually confirmed on all implants and capsules using scanning electron microscopy (SEM) as described previously.<sup>3</sup>

#### *Confocal laser scanning microscopy (CLSM)*

Monoclonal antibodies directed against CD3 (T cell) and CD79a (B cell) (Alaxa Fluor Antibody labeling kit, Life technologies, Carlsbad, CA, USA) were utilized to obtain confocal images of lymphocytic infiltrate on breast implant surface samples using methods previously described by Malisius et al.<sup>19</sup> Stained samples were fixed in 4% paraformaldehyde for one hour followed by three rinses in phosphate buffered saline (PBS) prior examination under Olympus Fluoview 300 inverted Confocal Laser Scanning Microscopy (CLSM) system at Macquarie University Microscopy Unit.

#### *Statistical Analysis*

One-way analysis of variance using the statistical package Sigma Plot 11 was utilized to test the relationship between cultured bacteria and Baker grade. A student T test was used to examine for differences in the number of bacteria and number of lymphocytes attached to different implants and in capsular tissue surrounding those implants. If the data was not normally distributed the Mann-Whitney rank sum test was utilized. Linear regression analysis, on log<sub>10</sub>-transformed data, was used to determine the relationship between T cells and number of bacteria in human capsules.

## Results

### Pig Study

Of the 24 implants, 4 were lost during the course of the experiment presumably due to exposure and subsequent extrusion. These lost implants were placed caudally in the pig and were therefore subjected to pressure from the pigs' haunches with resulting wound dehiscence and extrusion. There were 10 capsular specimens surrounding both smooth and textured implants available for analysis which were sufficient to allow comparative statistical analysis of outcomes.

#### *Bacterial culture*

20 capsular samples were subjected to culture analysis. Of the 20 implants, blinded assessment yielded 2 Baker 1, 6 Baker 2, 9 Baker 3 and 3 Baker 4 contracted implants. The overall contracture rate was 60%. Figure 2 summarizes the colony forming unit counts of cultured bacteria per mg of capsule for each Baker grade. They confirm a significant increase in the number of bacteria for increasing Baker grade ( $p=0.045$ ).

#### *Total bacterial number detected by quantitative real-time PCR*

Twenty capsule samples were also subjected to qPCR to detect the bacterial 16S RNA gene. There was no significant difference between the number of bacteria/mg in capsules surrounding different types of implants ( $2.7 \times 10^5$  versus  $3.5 \times 10^5$  bacteria/mg of capsular tissue for smooth and textured implants respectively). Consistent with our findings on culture, there was an increasing amount of detectable bacterial 16S RNA gene with increasing Baker grade (See figure 3). This did not reach significance.

Significantly more bacteria were attached to textured implants ( $4.2 \times 10^5$  bacteria/mg implant) compared with smooth implants ( $1.52 \times 10^3$  bacteria/mg implant)  $p < 0.001$ .

#### *Lymphocyte response*

##### Capsules

Quantitative PCR analysis of lymphocytes breast implant capsules showed a significant predominance of T cells (CD3, Mean  $3.7 \times 10^7$ ) as compared with B cells (CD79a, Mean  $4.7 \times 10^4$ ),  $p < 0.001$ . There was no significant difference in the number of CD3, CD4, CD8 or B cells/mg of capsular tissue surrounding smooth or textured implants ( $p > 0.7$ ).

## Implants

Analysis of the lymphocytic infiltrate on implants showed that textured implants had a significantly higher number of both B and T cells on their surface as compared with smooth implants (see Table 3). There were a mean of  $8.23 \times 10^5$  lymphocytes/mg of implant for textured as compared with a mean of  $1.3 \times 10^4$  lymphocytes/mg of implant for smooth ( $p < 0.001$ ), a 63 fold increase for textured implants. The majority of the lymphocytes associated with the surface of implants contaminated with bacterial biofilm were T cells.

### *Immunohistochemistry*

Six textured and 4 smooth implants were subjected to immunohistochemical staining with antibodies against CD3 (T cell) and CD79a (B cell) followed by CLSM imaging. All textured implants showed a high number of CD3 positive lymphocytes and scant CD79a positive lymphocytes (see Figure 4). Smooth implants had much less biological material to image but also showed a predominant CD3 (T cell) positive infiltrate (see Figure 4).

Confocal laser scanning microscopy images of lymphocytes attached to textured and smooth implants stained with CD3 stain (labeled with Alexa Fluor 488, green) and CD79a stain (labeled with Alexa Fluor 543, red).

### *Scanning electron microscopy*

Scanning electron microscopy confirmed the presence of numerous activated lymphocytes on the surface of textured implants (see Figure 5). Activation is indicated by increase in lymphocyte size, membrane ruffling and active replication.<sup>20,21</sup> By comparison, smooth implants had little or no attached infiltrate (see Figure 6).

## **Human Study**

### *Lymphocyte response*

A prospective collection of 57 periprosthetic capsules from 34 patients undergoing capsulectomy and removal of breast implants for Baker grade-4 contracture was undertaken over a 4-year period. All removed implants were textured reflecting the greater use of textured implants by surgeons who contributed to the study.

Capsules were subjected to quantitative PCR analysis to determine the number of lymphocytes, their CD status and the total number of bacteria as with the porcine samples. All capsules were positive for biofilm bacteria with a mean of  $2.52 \times 10^7$  bacteria/mg of capsule. In this cohort, there were a number of varying

texture types. There were 34 implants with biocell texture, 14 implants with siltex texture, 5 implants with PIP texture and 4 implants with polyurethane texture.

There were significantly more T cells (CD4+CD8a) as compared with B cells ( $p < 0.001$ ). The number of lymphocytes correlated with the number of bacteria/mg of capsular tissue (see Figure 7) ( $p < 0.001$ ,  $R = 0.71$  CD3,  $R = 0.83$  CD4,  $R = 0.71$  CD8a,  $R = 0.74$  CD79a). Analysis of bacterial number versus texture type showed that polyurethane implants had significantly more bacteria as compared with other textured implants ( $p < 0.005$ ) see Figure 8.

## Discussion

It has been over 30 years since Burkhardt proposed the subclinical infection hypothesis to explain capsular contracture and recommended the use of betadine irrigation of the pocket.<sup>6</sup> Since that time, with advances in our understanding of bacteria and their propensity to form biofilm on medical devices, the underlying science to support this theory has been elucidated.<sup>5</sup> Biofilm contamination of other medical devices is being recognized as an increasingly important cause of device associated infection and revision surgery with associated cost and patient morbidity.<sup>5</sup>

Our study sought to further investigate the important interactions between established biofilm and the surrounding host tissue around infected mammary implants. A number of important findings have arisen from this study.

From our pig model, we have shown that there are increasing numbers of bacteria for increasing Baker grade (See figure 2). These data suggest that there is a threshold of bacterial biofilm above which, host responses are triggered that ultimately lead to contracture. In Baker 1 implants, the host is able to clear or contain the biofilm to a level that does not produce further inflammation. Whilst it is unlikely that all bacteria are removed, the symbiosis between bacterial load and host immunity is able to restrict the inflammatory response. Once a critical load is reached, however, bacteria overwhelm the host response, continue to proliferate and trigger an inflammatory response leading to subsequent fibrosis and contracture. It is likely that this threshold will vary depending on host immunity, bacterial pathogenicity and the type of implant surface.

The use of PCR identification of total bacterial 16S RNA gene provides an alternative means of detecting biofilm to traditional culture techniques. PCR is a more sensitive tool for diagnosis and will potentially detect contaminating bacteria as well.<sup>22</sup> It is important to corroborate findings of PCR with either imaging of bacterial biofilm and/or culture techniques. The PCR data did show a similar increasing bacterial load with Baker grade for capsules surrounding both smooth and textured implants. Unlike the culture data, however, this did not reach significance.



The analysis of lymphocytes in both capsules and on the surface of implants contaminated with biofilm has shown that there is an overwhelming T cell response to the presence of bacteria. Furthermore, in the pigs, biofilm infected textured implants elicited a 63 fold increase in the number of T cells as compared with smooth implants. In our previous study<sup>15</sup>, we have shown both *in vitro* and *in vivo*, that textured implants, once contaminated, support up to 72x more biofilm bacteria as compared with smooth implants. In this study we have confirmed that if textured implants become contaminated they support 30x more biofilm bacteria than contaminated smooth implants. The use of texturization, whilst conferring biological benefits for tissue incorporation, also increases the surface area producing a more ideal surface for biofilm to form. The higher bacterial load on textured implants may explain the observed higher lymphocyte numbers and predominantly T cell hyperplasia.

The analysis of capsules from patients with grade 4 contracture and textured implants confirmed the predominance of T cells in the infiltrate and a linear relationship between the numbers of lymphocytes with increasing bacterial load. These findings are consistent with our prior findings in the pig. Analysis of capsules from the varying textured implants showed that the polyurethane coated implants had significantly higher numbers of bacteria as compared with other textured implant types ( $p < 0.005$ ). These findings, however, have to be tempered by the variation in time to explantation in this cohort and small comparative numbers of both PIP and polyurethane implants as compared with biocell and siltex textures. It has been suggested that textured implants cause less capsular contracture but comparative data to support this are conflicting<sup>17</sup>. Meta analyses are limited by significant variation in surgical technique, sample size, follow up period, variations in texture pore size<sup>23</sup>, implant placement, the use of antibacterial pocket irrigation, antibiotic utilization and the use of postoperative drainage<sup>23,24</sup> when pooling clinical studies. This variability in clinical practice does bring into question the “dictum” that it is surface texture alone that is responsible for consistently lower capsular contracture. Adams has postulated a cumulative effect of potentiators and suppressors that interplay to determine if a patient will progress to capsular contracture<sup>2</sup>. Avoidance of contamination, careful atraumatic dissection of surgical pockets and the use of pocket irrigation, for example, may be more significant suppressors of capsular contracture as compared with the suppressive contribution of surface texture alone. A recent study by Giordano et al<sup>14</sup>, for example, has shown a 10 fold reduction in capsular contraction with the use of povidone iodine pocket irrigation alone.

Our human data suggests that textured implants present a larger surface area to bacteria and support a higher bacterial load in the setting of established biofilm infection. In the setting of a higher biofilm load, any advantage conferred by surface texture could be potentially negated by more rapid biofilm growth, subsequent contracture and associated T cell hyperplasia. The infectious hypothesis, does not necessarily predict that textured implants will progress to higher rates of contracture as this is determined by the threshold levels of infection, above which, local inflammation is both initiated and perpetuated. We have shown, for example, that smooth implants with significant biofilm contamination will also equally progress to contracture. It is interesting to note the finding of a decreasing trend of bacterial counts with decreasing

aggressiveness of implant texture<sup>25</sup> (see figure 8). This supports our previous study showing that textured implants present an increased surface area for bacterial biofilm to form<sup>15</sup>. We plan to subject varying implant textures to our previously reported *in vitro* model<sup>15</sup> to further study this.

A possible link between bacterial biofilm and T cell hyperplasia is significant in the context of recent reports of breast implant associated anaplastic large cell lymphoma (BiALCL)<sup>16,26-28</sup>, a rare T cell lymphoma. Interestingly, the majority of cases have been associated with textured implants and more particularly, the more aggressive (biocell) texture.<sup>28</sup> The use of biocell textured implants has also been implicated in late seroma and double capsule, which is a common presentation of BiALCL.<sup>29</sup> Allan et al have also previously reported the finding of biofilm in double capsules in both human and pigs pointing to possible role for biofilm in the pathogenesis of both double capsule and ALCL.<sup>30</sup> Interestingly, the CD4 positive (T helper) cells showed the most significant correlation ( $r=0.83$ ) with increasing numbers of bacteria. It is these cells that undergo malignant transformation in ALCL.

The finding of a higher T cell numbers in periprosthetic capsules taken from both humans and pigs with chronic biofilm infection raises the question of a biologic link between the presence of biofilm, inflammation, T cell stimulation and the development of lymphoma. Chronic bacterial infection has been shown to be a causal agent in the development lymphoma in humans (*Helicobacter pylori*: gastric lymphoma<sup>31</sup>). In *H. pylori* infection, chronic inflammation of the gastric mucosa has been cited as the foundational mechanism underlying the occurrence and development of gastric lymphoma.<sup>32</sup> Deregulation of T cell stimulation has been identified as a potential pathway to the development of malignancy.<sup>33</sup> More recently, virulence factors from *H. pylori* such as the cytotoxin-associated gene A (CagA) protein have been shown to deregulate intracellular signaling pathways and promote lymphomagenesis.<sup>34</sup> It is biologically plausible that chronically infected breast implants may mediate similar inflammatory and neoplastic processes that lead to development of a T cell lymphoma. This is in contrast with mucosal associated and other implant-associated lymphomas, which are primarily of B cell origin and could reflect the unique microenvironment in which breast implants reside. The pathway to malignancy, however, is likely to be a multistep process with possible variation in bacterial phenotypes, patient genotypes and other immune mediated factors contributing to the eventual development of BiALCL. This would explain why some patients with biofilm infection around breast implants proceed to contracture and why other patients (less commonly) proceed to lymphocytic hyperplasia and BiALCL. It would also account for the variation in BiALCL aggressiveness seen in patients who have other phenotypic or immune mediated risk factors for the development of malignancy. Further study of the relationship between bacterial biofilm, lymphocytes and the local breast environment is needed to more clearly elucidate the pathway from biofilm infection to the development of malignancy.

A recent study published by Kellog et al<sup>35</sup> have described lymphoma (both T and B cell) arising in association with a variety of other prosthetics including stainless steel, pacemakers, venous access devices, cardiac prostheses and orthopedic hip prostheses. It is possible that device associated infection with biofilm and subsequent inflammation combined with a maladaptive immune

response is the common causal link to the development of these rare malignancies. These findings further reinforce the importance of applying known and effective intra-operative strategies<sup>5</sup> to reduce the risk of biofilm contamination at time of insertion, especially when choosing textured implants.

## **Conclusion**

Our study has confirmed that there is a progressive increase in the number of bacteria as capsular contracture becomes more apparent. This points to a likely threshold for the establishment of a proliferating biofilm and development of capsular contracture on mammary implants. Chronic biofilm infection of mammary prostheses in both pigs and humans are associated with a predominantly T cell lymphocytic infiltrate, which is directly linked to the bacterial load attached to the implant. In the pig, the T cell numbers were highest for infected textured implants consistent with our previous finding of higher bacterial numbers on these implants. The finding of activated lymphocytes on electron microscopy and proliferation of T cells on the surface of textured implants is significant in light of recent reports of breast implant associated ALCL. Further analysis of the immune response to biofilm around mammary implants and other medical devices should be undertaken as a matter of priority to investigate the link between biofilm mediated inflammation and malignancy.

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## Legend to Figures and Tables

Table 1 : Baker grading of capsular contracture in pig cohort

Table 2: Primers used for quantitative PCR of biofilm and lymphocytic response obtained from pig capsules/implants and human capsules. Bp=base pair

Table 3: Mean number of lymphocytes as detected by qPCR /mg of implant for textured vs. smooth implants in pig cohort ( $p < 0.001$ )

Figure 1: Subclinical theory of capsular contracture

Figure 2: Colony forming unit counts of cultured bacteria per mg of capsule for each Baker grade in pig cohort. They confirm a significant increase in the number of bacteria for increasing Baker grade ( $p = 0.045$ ) (Standard error shown).

Figure 3: Total number of culturable and non-culturable bacteria, as determined by quantitative PCR per mg of capsule for each Baker grade in pig cohort (Standard error shown).

Figures 4: T/B cell staining for textured versus smooth implants in pig cohort  
4a: Textured implant at low power (200x) showing predominant CD3 (green) staining of material attached to surface of implant.  
4b: Textured implant at high power (400x) showing dense CD3 (green) positive lymphocytes  
4c: Smooth implant at low power (200X) showing scant CD3 (green) and some CD79a (red) material attached to surface of implant  
4d: Smooth implant at high power (400x) showing predominance of CD3 (green) positive lymphocytes

Figures 5: Scanning electron microscopy of activated lymphocytes on the surface of textured implants in association with biofilm.

5a: Biofilm and lymphocytes attached to a textured implant (magnification 1000x)

5b: Higher magnification showing many activated lymphocytes in close association with large quantities of biofilm attached to a textured implant (magnification 2500x)

5c: Close up of two activated lymphocytes in close association to biofilm attached to a textured implant (magnification 4500x)

Figure 6: Surface of Smooth implant showing scant biological material

Figures 7: CD3, CD4, CD8 and CD79a from human capsules versus total bacteria/mg of capsular sample

Figure 8: Bacterial numbers versus type of textured implants from human cohort (standard error shown)

Table 1: Baker grading for capsular contracture<sup>18</sup>

Baker grade	Clinical findings
1	The breast appears natural on examination. There is no evidence of thickening around the implant and minimal palpability of the implant on examination.
2	There is palpable thickening around the implant but no visible change in shape.
3	There is a palpable capsule and hardening of the breasts on examination. There is a visible change in the shape of the breast.
4	There is palpable thickening of the breast tissue and tissue capsule. Grossly visible changes with severe distortion are apparent on examination.

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Table 2: Primers used for quantitative PCR of biofilm and lymphocytic response obtained from pig capsules/implants and human capsules. Bp=base pair

Gene	Genbank accession number	Amplicon size	Primer Pair Sequence 5'-3'
16S rRNA (Eubacteria)		194bp	CCTACGGGAGGCAGCAG
			ATTACCGCGGCTGCTGG
18S rRNA	AY265350.1 NR_003286.2	122bp	GGTGGTGCCCTTCCGTCA CGATGCGGCGGCGTTAT
Sus scrofa CD3	AY323829.1	125bp	TCCCTGGGCAAATCTTGAC AATATCCTTGGGCTGGGTG
Sus scrofa CD4	NM_001001908.1	61bp	CGCGTGGGACTGGACCTG
			ACCATGACTGCCCTGTGCTT
Sus scrofa CD8a	AY590798.1	114bp	AACGCAGACCCGAGGAAG
			GCGGTGGCAGATGATGGTGA
Sus scrofa CD79a	NM_001135962.1	181bp	TGCTGATCTGTGCCGTGGTG
			TCCCTGGTAGGTGCCCTGGAG
Homo sapiens CD3e	NM_000733.3	66bp	TGCTGCTGGTTTACTACTGG
			CCGCTCCTCGTGTCAC
Homo sapiens CD4	BT019811.1	74bp	TTCATTGGGCTAGGCATC
			ATCTGAGACATCCGCTCTG
Homo sapiens CD8a	NM_001768.6	71bp	CAGCGTTCTCGGGCAAGA
	NM_171827.3		TCGTTCTCTCGGCGGAAGTC
Homo sapiens CD79a	NM_001783.3	54bp	ACTTCCAATGCCGCACAAT
	NM_021601.3		CGCGCCACCAGGTGACGTT

Table 3: Mean number of lymphocytes as detected by qPCR /mg of implant for textured vs. smooth implants in pig cohort (p<0.001)

	CD3 Total T Cells	CD4 Helper T cells	CD8a Cytotoxic T cells	CD79a B cells
Textured	$8.23 \times 10^5$	$6.44 \times 10^4$	$1.71 \times 10^3$	$3.94 \times 10^2$
Smooth	$1.30 \times 10^4$	$1.10 \times 10^3$	8.27	2.37

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