Case report

Bacterial biofilm associated with a case of capsular contracture

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SUMMARY
Capsular contracture is one of the most common complications of implant-based breast augmentation. Despite its prevalence, the etiology of capsular contracture remains controversial although the surface texture of the breast implant, the anatomical position of the prosthesis and the presence of bacterial biofilm could be considered trigger factors. In fact, all medical implants are susceptible to bacterial colonization and biofilm formation. The present study demonstrated the presence of microbial biofilm constituted by cocci in a breast implant obtained from a patient with Baker grade II capsular contracture. This suggests that subclinical infection can be present and involved in low grade capsular contracture.

Key words: Microbial Biofilm, Capsular Contracture; Inflammation

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INTRODUCTION
Augmentation and reconstruction mammaplasty are among the most frequently performed cosmetic procedures and one of the relatively common complications is capsular contracture (CC) (Del Pozo et al., 2009). CC involves tightening of the collagen capsule that forms around the breast implant, which can be painful and very often distorts the breast (Galdiero et al., 2018). CC remains the most common cause of breast surgery revision. Various studies have indicated CC incidences ranging from 5% to 74% of breast reconstructive surgeries and approximately 45,000 patients with CC are diagnosed annually (Asplund et al., 1996; Wong et al., 2006; Handel et al., 2006; Cunningham and McCue, 2009). CC is classified according to the Baker classification system (Little and Baker, 1980) as follows: grade I, breast absolutely natural; grade II, minimum contracture; grade III, moderate contracture; and grade IV, severe contracture. Possible causes of contracture could be chemical composition and surface texture of the implant and the presence of bacterial biofilms (Rieger et al., 2013). Bacteria that live on the skin and within the breast ducts can contaminate the surface of the breast implant at the time of insertion forming biofilm (Chessa et al., 2016). Microbial biofilms could represent a trigger for chronic peri-implant inflammation and multiple animal and clinical studies have shown a correlation between biofilms and CC (Hu et al., 2015; Chessa et al., 2016). Biofilms are usually polymicrobial and display elevated resistance to antibiotics, disinfectants, and the immune response (Donlan and Costerton, 2002). Microbiological identification of the bacteria involved in biofilm formation is essential to develop preventive measures and to establish a correct treatment. In general, the microorganisms most commonly reported are Staphylococcus aureus, coagulase-negative staphylococci (CoNS), particularly Staphylococcus epidermidis, and Propionibacterium acnes (Pajkos et al., 2003; Netscher et al., 1995).

This report describes a case of a patient with Baker grade II capsular contracture in which microbial agents from capsula and prosthesis were identified and characterized by their ability to form biofilms. Furthermore, ultrastructural analysis revealed biofilm directly on the breast implant, confirming results obtained by microbiological assay.

CASE REPORT
Breast implants and capsules removed from a 35-year-old patient (Figure 1) with Baker grade II capsular contracture were analyzed by microbiological procedures. The patient had undergone her first surgery two years before with another surgeon. Clinical symptoms of capsular contracture had started six months after the first surgery with gradually increasing breast firmness and lateral implant dislocation. During explantation, two specimens were taken (Figure 2). These included capsule biopsies and breast implants. For microbiological studies, each specimen was cut into small fragments, approximately 10-20 mm, transferred to a 6-ml of Brain Heart Infusion Broth (Oxoid,
Rome, Italy) and incubated up to 72 hours at 37°C under vigorous shaking. Positive cultures were plated on blood agar, Mannitol Salt Agar and Mac Conkey 3 agar (Oxoid, Rome, Italy), and incubated for 24 to 72 hours at 37°C. Bacterial colony identification and antibiotic susceptibility were done using the Vitek 2 system (bioMérieux) and molecular identification method. 16S rDNA gene sequencing was used to identify the isolated strains. Genomic DNA extraction was achieved by suspending three to five bacterial colonies in TE buffer (10 mM Tris, 1 mM EDTA, pH 7.8) and heating at 100°C for 20 min. Universal 16S rDNA bacterial primers (V1-V9) 8F (5’-AGAGTTTGATCCTGGCTCAG-3’) and 1492R (5’-GGTTACCTTGTTACGACTT-3’) were used to amplify the 16S rDNA using 10 ng of genomic DNA isolated from each strain. PCR products were visualized on a 1% agarose gel. Amplicons were quantitated and bi-directional sequenced using primers 8F and 1492R (Bio-Fab Research labs). The 16S rDNA sequences were compared with those available in the GenBank using the BLAST program (http://blast.ncbi.nlm.nih.gov/blast). S. epidermidis, S. hominis, and S. warnerii were identified. The antibiotic susceptibility analysis showed that among antibiotic tested (Cefoxitin screen, Penicillin, Oxacillin, Gentamicin, Levofloxacin, Clindamycin, Erythromycin, Linezolid, Daptomycin, Teicoplanin, Vancomycin, Tetracycline, Tigecycline, Fosfomycin, Fusidic acid, Mupirocin, Rifampin, Trimethoprim-sulfamethoxazole), S. epidermidis was resistant to Erythromycin (MIC≥8 μg/ml) and Teicoplanin (MIC=16 μg/ml); S. warneri was resistant to Clindamycin (MIC≥4 μg/ml) and Daptomycin (MIC=4 μg/ml); S. hominis was resistant to Erythromycin (MIC≥8 μg/ml), Clindamycin (MIC=0.5 μg/ml) and Teicoplanin (MIC=8 μg/ml).

Biofilm formation was assayed as previously described (Stepanović et al., 2007). The S. epidermidis ATCC 35984 strain was used as a positive control. Biofilms were quantified by measuring the absorbance at λ 570 nm and the ratio 570/595 nm was calculated to normalize bacterial biofilm production to bacterial growth. The average of OD values was calculated and cutoff values (ODc) were established. ODc is defined as the average of OD of negative control + three standard deviations. According to their absorbance, isolates were defined as strong when (4xODc<OD), medium (2xODc<OD≤ 4xODc), weak (ODc<OD≤ 2xODc) or non-biofilm producers (OD≤ ODc). Results obtained showed that all strains were strong biofilm producers being OD>4xODc: S. epidermidis (4.5±0.7), S. warneri (4.12±1.2) and S. hominis (4.73±1.1) and S. epidermidis ATCC 35984 a strong biofilm producer used as positive control (4.72±0.85).

The presence of biofilm on implant was revealed by Ultra-high resolution Field Emission Gun Scanning Electron Microscopy (FEG-SEM, FEI Company). A fragment of the implant was fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) at 4°C for 24 h, and post-fixed in 1% OsO4 solution. Samples were then dehydrated through a graded series of ethanol solutions, dried with
hexamethyldisilazane and gold sputtered. Secondary electron images were performed with an acceleration voltage of 20 KV. The images were processed for display using Photoshop (Adobe Systems Inc., San Jose, CA, USA) software. FEG-SEM analysis showed two morphological types of biofilm images (Figure 3). Coccoid cells, found by ultrastructural analysis, confirmed results obtained by microbiological assay. Bacterial cells appeared aggregated to form biofilm: coccoid cells were either saturated with extracellular material that obscured individual cells or aggregated with many individual cells.

**DISCUSSION**

The etiology of CC is not completely understood. Capsule formation itself is known to be a normal response to all kind of foreign bodies, but contracture is not. CC formation is likely a multifactorial process and several putative factors have been proposed, such as a previous contracture, and oncologic patients treated with radiotherapy (Galdiero et al., 2018). Other factors are placement of an incision site, hypertrophic scarring, overactive inflammatory response, and foreign body reaction from powdered gloves, dust, or silicone gel leakage (Adams, 2009). Several lines of preliminary evidence suggest a role of microbial infection in CC pathogenesis (Virden et al., 1992; Dobke et al., 1995). Local skin flora, such as coagulase-negative staphylococci, *Propionibacterium acnes*, and *Corynebacterium species* (Byrd et al., 2018) is the most frequently involved in CC (Bartsich et al., 2011). The breast skin may harbor bacteria that remain present during surgery in sterile conditions and may gain access to breast implants during or following placement. Moreover, in specific breast regions like the nipples, contamination during surgery is more frequent for the highest concentration of local bacteria (Bartsich et al., 2011). A significant association between CC and the presence of skin bacteria on the explanted breast implants has been also demonstrated (Del Pozo et al., 2009). Indeed, bacteria of the normal skin flora, such as *S. epidermidis*, have been found involved in the formation of biofilm on mammary implants (Chessa et al., 2016). Some have suggested that bacteria form biofilms on the implant, stimulating fibrosis around the implant and, ultimately, capsular contracture (Dobke et al., 1994; Netscher, 2004). Biofilms are microbial communities that are attached to a surface, including living tissue, implants and medical devices. These infections are difficult to treat, and as a result, they become persistent and chronic. Different literature data show a correlation between the presence of microbial biofilms on various medical implants and persistent inflammation of the surrounding tissue (Costerton et al., 2005; Arciola et al., 2012). It appears that microbial biofilms also form on breast implants and might contribute to a chronic inflammatory response and thus formation of capsular fibrosis and subsequent contracture (Schreml et al., 2007). Investigations of biofilms on mammary implants began by studying CC. Virden et al. (1992) were among the first to demonstrate a correlation between biofilms on silicone shells and the risk of CC.
Several additional studies have attempted to determine the pathophysiology and prognosis of biofilm-related CC (Dobke et al., 1995; Pajkos et al., 2003) as well as potential prophylactic and therapeutic measures. Studies conducted in an animal model have observed a correlation between bacterial biofilm and CC. *S. epidermidis* was inoculated before implantation of a breast prosthesis and after two weeks biofilm was present both on the implant and on the capsule (Ajdic et al., 2016). Gualdiero et al. (2018) reported that patients with a clinical history of breast surgery or chronic inflammation are most susceptible to developing a CC caused by the formation of biofilm on the protheses. Based on the scientific evidence, we speculate that in our reported clinical case, biofilm formation could play a role in chronic inflammation and pathogenesis of CC in a patient with Baker grade II, although little evidence has been reported. Moreover, based on the types of isolates belonging to skin flora such as *S. epidermidis*, *S. warnerii* and *S. hominis*, all strong biofilm producers, we could assess that endogenous flora could be a source of contamination (Bartsich et al., 2011). Interestingly, Arslan et al. (2011) reported *S. warnerii* as a cause of endocarditis in a patient who had a silicone mammoplasty. This leads us to speculate that *S. warnerii* could be a potential pathogen causing subclinical infection during breast surgery in patients with the implant. Preventive strategies to minimize implant contamination from endogenous breast flora appear to be the only preventive measures to reduce the risk of CC (Ajdic et al., 2016). Therefore, prevention rather than treatment might be a better strategy.

**Conflict of interest statement**

The authors declared no potential conflicts of interest with respect to the research, authorship, and publication of this article.

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REFERENCES


**Figure 1**: Preoperative view of a 35-year-old patient with Baker grade II capsular contracture. The patient had undergone her first surgery two years before with another surgeon.
Figure 2: Implants and capsules removed.
Figure 3: Bacterial biofilm revealed by SEM. Thin biofilm matrix appeared due to bacterial cells saturated with amorphous material (Figure 3A and B), which obscured individual cells and voluminous bacterial aggregates with many individual cells consistent with cocci (Figure 3C and D). Bacteria (thin arrows) and possible bacterial micro-colonies (thick arrows).