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ORIGINAL ARTICLE   **BASIC SCIENCE/EXPERIMENTAL**

Is Salvage of Recently Infected Breast Implant After Breast Augmentation or Reconstruction Possible? An Experimental Study

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Abstract

Introduction The reinsertion of an infected implant when peri-prosthetic infection occurs early after breast augmentation or breast reconstruction remains controversial. In this experimental study, the authors tried to remove bacteria, and their biofilm, from the colonized surface of breast prostheses, without damaging their integrity.

Materials and Methods A total of 112 shell samples of silicone breast prostheses, smooth (SPSS) and textured (TPSS), were colonized by *S. epidermidis* (SE) or *S. aureus* (SA) strains, all able to produce biofilms. After 15 days, all

the samples were removed from the contaminated culture broth and constituted 4 groups of 20 contaminated samples: SPSS/SE (group I), SPSS/SA (group II), TPSS/SE (group III), TPSS/SE (group IV). In another group—group SEM−, 16 colonized samples were used for documentation with scanning electron microscopy (SEM). The remaining 16 samples were used to test the limits of detection of the sterility test. All samples of groups I–IV and 8 samples of group SEM were « washed » with a smooth brush in a povidone-iodine bath and rinsed with saline solution. A subset of the washed samples was sent for SEM and the others were immersed in sterile broth and were incubated at 35 °C for 3 weeks (groups I–IV).

Results Fifteen days after contamination, all the samples in groups I–IV were colonized. In the SEM group, SEM images attested to the presence of bacteria in biofilm attached to the shells. After cleaning, SEM did not reveal any bacteria and there was no visible alteration in the outer structure of the shell. Sterility tests performed after decontamination in groups I–IV remained negative for all the samples.

Conclusion Breast prostheses recently contaminated with *Staphylococci*, frequently involved in peri-prosthetic breast implant infection and capable of producing biofilms, can be efficiently decontaminated by the procedure used in this study. Our decontamination procedure did not alter the surface structure of the prostheses. This decontamination procedure could allow reinsertion of an infected implant when peri-prosthetic infection occurs early after breast augmentation or breast reconstruction and when a salvage procedure is indicated.

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Introduction

Infection is the leading cause of morbidity after breast implantation and complicates 2.0–2.5% of interventions in most case series. Two-thirds of infections develop in the acute post-operative period [1, 2].

Organisms most often involved in breast prosthesis infection are *Staphylococci* (68.2%), usually *S. aureus* or *S. epidermidis* [3]. These organisms belong to normal skin flora and can colonize mammary ducts [4–8]. After incision of the skin and transection of the mammary ducts, the breast pocket is rapidly colonised. A brief skin contact can be sufficient for prosthesis contamination. It has been demonstrated that organisms can adhere to breast implants after only 2 min of contact [9].

Management of early breast prosthesis infection after breast augmentation or breast reconstruction is contentious. Opinions remain divided regarding either a two-stage replacement procedure, with removal and delayed reinsertion after 6 months, which remains the gold standard [10, 11], or a more aggressive, one-stage management, which involves thorough cleaning of the pocket after prosthesis removal and insertion of a new implant [1, 12–17].

In the context of one-stage surgical management, the aim of this study was to determine if bacteria and their biofilms could be removed from a recently colonized breast prosthesis, without damaging the implant. In other words, when salvage is indicated, is reinsertion of the same prosthesis after decontamination feasible?

Materials and Methods

This experimental study was performed following the ethical guidelines of the University of Liège. A total of 112 shell samples of silicone breast prostheses, smooth (SPSS) and textured (TPSS), were colonized by *S. epidermidis* (SE) or *S. aureus* (SA) strains, all able to produce biofilms. The study protocol is summarized in Fig. 1.

Organisms

Two strains of *Staphylococcus* originally isolated from infected intra-vascular catheters cultured in the medical microbiology laboratory were used: *Staphylococcus epidermidis* F0038576 and *Staphylococcus aureus* F0041616. These strains were inoculated onto sheep's blood Columbia agar and incubated overnight at 35 °C. Colonies were harvested and suspended in sterile saline solution. The turbidity of each suspension was adjusted to 0.5 MacFarland barium sulfate standard, equivalent to 10^8 colony-forming units (CFU) per ml. To assess the number of CFU/ml, a series consisting of tenfold dilutions of the bacterial suspension was quantitatively inoculated onto sheep's blood Columbia agar. Numbers of colonies were assessed after overnight incubation at 35 °C.

Breast Implants

Two types of breast implants were studied: Smooth surface, cohesive gel filled prosthesis (Mentor® Corporation Santa Barbara, CA, USA) and Siltex® textured surface, cohesive gel filled prosthesis (Mentor® Corporation Santa Barbara, CA, USA). In sterile conditions, 56 rectangular samples of $2.5 \times 1\ cm^2$ were punched from the prosthesis shells. Any remaining silicone gel on the internal side of the samples was removed with sterile gauze. A total of 112 samples were tailored. Each one was placed in a single sterile Petri dish to constitute 4 groups of 20 samples: SPSS/SE (group I), SPSS/SA (group II), TPSS/SE (group III), TPSS/SE (group IV). In another group—group SEM−, 16 samples were used for documentation with scanning electron microscopy (SEM). The remaining 16 samples were used to test the limits of detection in the sterility test (group TEST).

Contamination and Colonization

Each sterile shell sample of groups I–IV and group SEM was immersed in 10 ml of Columbia broth and further contaminated with 100 μl of a 10^8 CFU/ml suspension of *Staphylococcus aureus* or *Staphylococcus epidermidis*. For each type of prosthesis, 24 segments were contaminated per species of *Staphylococcus*. These cultures were incubated for 15 days at 35 °C (Fig. 1). After 15 days, all the samples were extracted from the contaminated culture broth. Contamination of the sample was attested by the presence of bacteria fixed on the wall of the tube and turbidity of the culture medium.
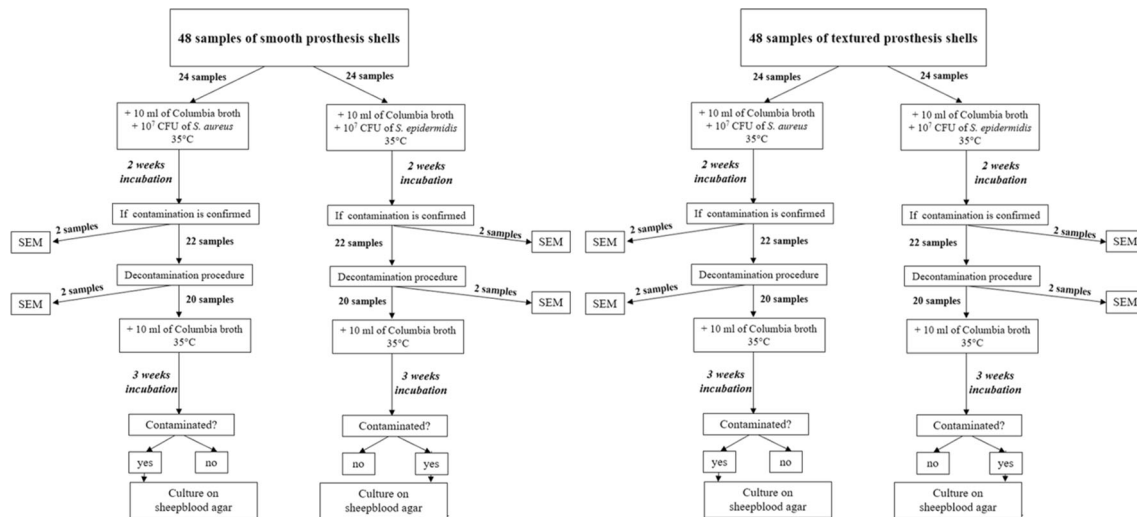
Fig. 1 Rectangular samples (48 2.5 × 1 cm²) were punched in the smooth and in the textured prosthesis shells. A total of 96 samples were tailored. For each type of prosthesis, 24 samples were contaminated per species of *Staphylococcus* and submitted to the sterility test. *CFU* colony-forming units, *SEM* scanning electron microscopy

Decontamination

After 2 weeks, the colonized segments of groups I–IV and 8 colonized segments of group SEM were taken from the culture tube and were submitted to the decontamination procedure (Fig. 1). They were immersed in a 100% povidone-iodine (Iso-Betadine® Dermique, PVP-I 10% solution aqueuse Purdue Frederick, Stamford, CT, USA) bath and both sides were cleaned with a sterile smooth brush. This procedure took < 1 min for each sample. Then, the samples were rinsed with a saline solution and dried with sterile gauze. Acute peri-prosthetic infection occurs, on average, 10–12 days (minimum 6 days and maximum 6 weeks) after surgery [2]. This is why our decontamination procedure was carried out on day 15, corresponding to the time of a potential salvage procedure in surgical practice.

Sterility Testing

To detect surviving microorganisms after decontamination, each sample was immersed in 10 ml of Columbia broth and incubated at 35 °C for 3 weeks. Cultures were examined for growth every day. Growth was assessed by the presence of turbidity. If a positive culture occurred, it was inoculated onto sheep's blood Columbia agar and incubated at 35 °C to check if the contaminant was the initial contaminating species, *S. aureus* or *S. epidermidis* (Fig. 1).

Limits of Detection in the Sterility Test (Group TEST)

To determine the lower limits of recovery and detection of surviving microorganisms in small numbers from contaminated samples of prosthetic materials (1 × 2.5 cm), 4 segments per type of prosthetic material and per species of *Staphylococcus* were contaminated on their outer surface with 100 μl of bacterial suspensions containing, respectively, 1, 10, 10^2 and 10^3 bacteria. These samples were left at room temperature for 1 h in an aseptic environment. Each segment was then examined for surviving microorganisms, as outlined above (Fig. 2).

Scanning Electron Microscopy (Group SEM)

Submission to the scanning electron microscopy (SEM) analysis was performed after 2 weeks contamination (subgroup SEM+) and after decontamination (subgroup SEM−). For each strain and for each type of prosthetic material, 2 colonized (SEM+) and 2 'washed' (SEM−) samples were submitted for SEM analysis (Fig. 1). All specimens were immersed in a 0.1 M cacodylate buffer solution. A fixing step was applied by soaking the samples for 45 min in a glutaraldehyde solution (2.5 vol% in 0.1 M cacodylate buffer) at room temperature. The samples were then rinsed with water and lyophilized. A Pt conductive coating was deposited by plasma sputtering (Balzers). The samples were mounted on aluminium holders and observed at 15 kV in a FEG-ESEM (XL30 from FEI company) under high vacuum. Each specimen was viewed at a magnification of 100–1000.
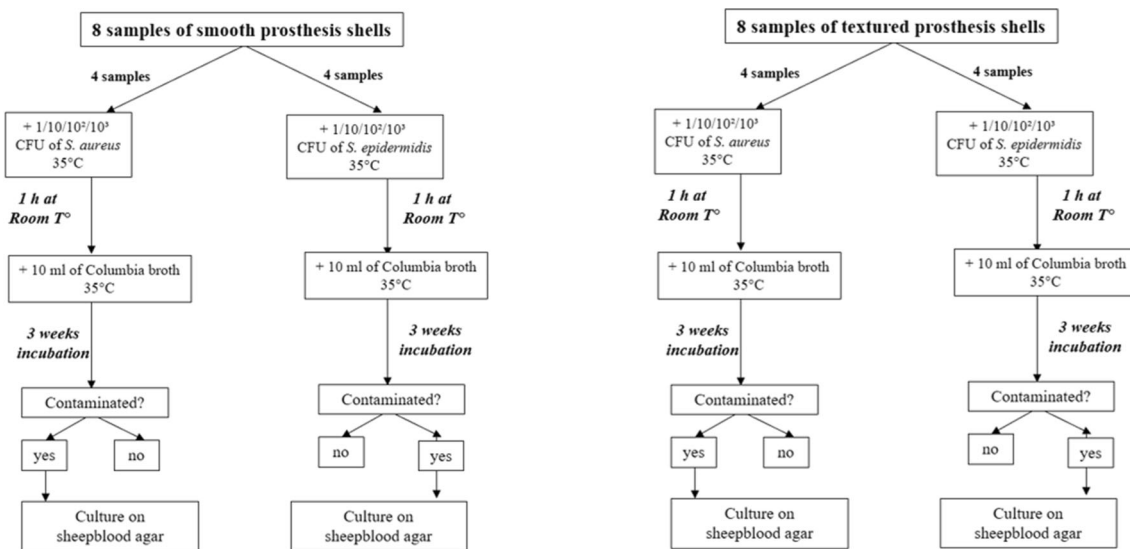
Fig. 2 Eight rectangular samples ($2.5 \times 1 \text{ cm}^2$) were punched in the smooth and in the textured prosthesis shells. A total of 16 samples were tailored. For each type of prosthesis and per species of

Staphylococcus, 4 samples were used to determine the limits of detection in the sterility tests. *CFU* colony-forming units, *SEM* scanning electron microscopy

Data Analysis

In this study, 112 shell samples of silicone breast prostheses, smooth and textured, were colonized by *S. epidermidis* or *S. aureus* strains, capable of producing biofilms. Data analysis was performed using the SPSS program (SPSS 22.0).

Results

Limits of Detection in the Sterility Test (Group TEST)

The lower limits of recovery of surviving microorganisms from quantitatively contaminated segments of prosthetic materials were 1 CFU for the smooth prosthetic material either for *S. aureus* or *S. epidermidis* and 10 CFU for the textured material. These results are not statistically different from microbiological cultures of serial 10th dilutions used for contamination.
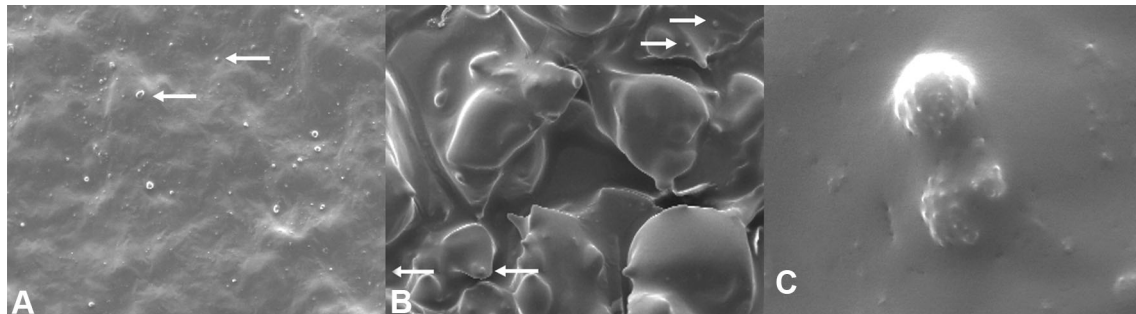
Groups I–IV

Two weeks after contamination, colonization of the silicone samples was confirmed for all 20 samples in group I (SPSS/SE), II (SPSS/SA), III (TPSS/SE), and IV (TPSS/SE) (Fig. 3). Three weeks after the decontamination procedure, the four sets of cultures corresponding to each contaminating organism per type of prosthetic material were negative. These results are summarized in Table 1.
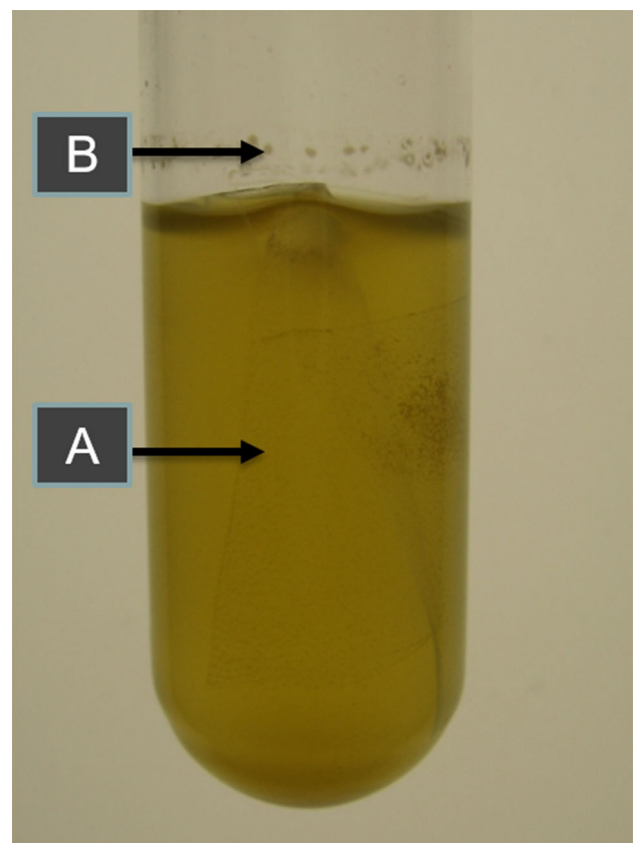
Fig. 3 Contamination of the sample was attested by turbidity of the culture medium (**a**) and the presence of bacteria fixed on the wall of the tube (**b**)

Table 1 Number of positive tests and limits of detection in the sterility tests in the four sets of cultures corresponding to each contaminating organism per type of prosthetic material 3 weeks after decontamination procedure

Type of prosthetic material	Contaminating bacteria	Number of positive tests after decontamination	Limits of detection in the sterility tests (CFU)
Smooth	*S. aureus*	0/20	1
	S. epidermidis	0/20	1
Textured	*S. aueus*	0/20	10
	S. epidermidis	0/20	10

Fig. 4 Scanning electron microscopy (SEM) shows bacteria colonies fixed on the outer surface of the prosthesis shells. **a** Smooth surface prosthesis covered by *Staphyloccus aureus* colonies, located with white arrows (Original magnification ×100), **b** textured surface prosthesis covered by *Staphylococcus epidermidis* colonies, located with white arrows (Original magnification ×100), **c** high magnification of two *Staphylococcus aureus* colonies (Original magnification ×2000)

Group SEM

After contamination, SEM analysis of the outer surface of the samples in group SEM+, textured or smooth, showed bacterial colonies gathered in groups of 10 bacteria, coated in bioslime (Fig. 4). Bacterial concentration on the outer surface of the implants appeared to be lower in the textured group. After decontamination, no remaining bacteria could be seen on the outer surface of the samples in group SEM−. The structure of the outer surface of the shell was not affected by the decontamination procedure.

Discussion

In this study, 112 shell samples of silicone breast prostheses, both smooth and textured, were colonized by *S. epidermidis* or *S. aureus* strains capable of producing biofilms. Fifteen days after contamination, all the samples were colonized. SEM images demonstrated the presence of bacteria and biofilm fixed on the shells. Sterility tests performed after decontamination in all groups of implants remained negative for all samples. After cleaning, SEM did not reveal any organisms and there was no alteration in the outer structure of the shell surface.

The organisms most commonly implicated in breast prosthesis infection are *Staphylococci* (68.2%), usually *S.*

aureus or *S. epidermidis* [3]. *Staphylococci* produce an exopolysaccharide extracellular material commonly referred to as slime. This slime production is enhanced in the presence of a foreign body [18]. Even in individuals with excellent cellular and humoral immunity, biofilm infections are rarely eradicated by the host defence mechanisms [19]. Antimicrobial therapy usually fails to kill bacteria within the biofilm [20]. After conservative treatment alone, biofilm and entrapped bacteria may continue to grow and cause subclinical infection, which may play a major role in capsular contracture [21, 22], and may act as a reservoir for acute exacerbations. Indeed, some of the "sessile" organisms may be released by the biofilm and become "planktonic", initiating a new acute peri-prosthetic infection. The only way definitively to eradicate infection is to surgically eliminate the organisms fixed on the surface of the foreign body.

In this context, the first step of our study was to create a model of an infected implant: Two strains of *Staphylococci* originally isolated from infected intra-vascular catheters and two frequently used types of prostheses (smooth and textured) were used to mimic the clinical scenario. After 2 weeks, all samples were colonized by groups of bacteria (*S. Epidermis* or *S. aureus*). SEM images showed colonies of about ten cells, embedded in their bioslime (Fig. 4c). It has been shown that the physical characteristics of a surface (rough or smooth) influence bacterial adhesion to only

a minor extent [23]. In our study, colonies seemed to be less dense in the textured group but this was not statistically significant and SEM was only used to obtain images of the colonization of the prosthesis samples.

The second step was to try to disunite the bioslime, and the embedded cells, from the implants. Once a biofilm has formed and the exopolysaccharide matrix has been secreted by the sessile cells, the resultant structure is highly viscoelastic and behaves rather like rubber. Donlan and Costerton have clearly demonstrated that biofilms formed in low-shear environments (as in our situation) have a low tensile strength and break easily, but biofilms formed at higher shear (i.e., rapidly flowing milieus) are remarkably strong and resistant to mechanical breakage [24]. To break the biofilm, the colonized shell samples were immersed in a povidone-iodine bath and gently cleaned with a smooth brush [2]. The mechanical action of the smooth brush is necessary to detach the fixed colonies. Moreover, the antiseptic action of Betadine against bacteria like *S. epidermidis* and their biofilm has been well documented in the literature [25]. The integrity of the samples after this combined procedure was evidenced in this study. The minor disruptions induced by a smooth brush on the prosthesis were insignificant compared to the tests performed at the factory (Mentor® Corporation Santa Barbara, Calif.). Moreover, the decision to use Betadine was also affirmed by the experimental study of Zambacos and Nguyen [26], who studied the effects of different concentrations of povidone-iodine (0.01–10%) on silicone shells and found no structural modification [26]. In other words, a brief contact of the implant with Betadine, during the decontamination procedure, and followed by copious saline solution irrigation, has no effect on the long-term integrity of the shell of the implant. This is also confirmed by the SEM analysis of the SEM− group, which demonstrates integrity of the samples. Finally, this combination of antiseptic and manual dissolution was effective because 3 weeks after the decontamination procedure, the four sets of cultures corresponding to each contaminating organism per type of prosthetic material were negative and analysis of the surface of the implants in SEM+ group did not reveal any residual bacteria. It should be emphasized that when a breast implant is inserted or reinserted, it is impossible to exclude bacterial contamination of the pocket and/or the implant, even if all necessary precautions are taken (decontamination of the implant, disinfection of the pocket, minimal operating time, no skin contact with the implant, and so on). The key is to reduce the bacterial load to such a point that the remaining bacteria can be eliminated or controlled by the host.

Despite the encouraging results obtained in this study, some limitations are to be noted: (1) Only two strains of *Staphylococcus* were used in this study. Samples contaminated with a higher number, or several strains of bacteria [27–30] may better mirror the numerous clinical situations with different time frames and hospital contaminants. Moreover, the bacterial content of the breast implant pocket could not be studied. However, Pittet et al. [2] clearly demonstrated that *S. aureus* or *S. epidermidis* are the most common causative organisms in breast prosthesis infections. (2) Additionally, the limits of detection in the sterility tests were slightly different in both groups, but as previously mentioned, this difference is not significant from the microbiological point of view. In the smooth prosthesis group, 1 CFU is the limit, versus 10 CFU in the textured prosthesis group. This means that even if the sterility tests are negative 3 weeks after the decontamination procedure, we cannot exclude that one bacterium might be left on the textured shell group and fewer than 10 bacteria on the smooth shell. Indeed, bacteria subject to harsh conditions may "hibernate" until conditions are more favorable for proliferation. (3) Finally, the low number of samples in the SEM groups meant they could only serve as photographic documentation. A higher number of samples may have better defined the link between the type of implant and its influence on bacterial adhesion.

Conclusion

Breast prostheses recently contaminated with strains of *Staphylococci*, frequently involved in peri-prosthetic breast implant infection and capable of producing biofilms, can be efficiently decontaminated by the procedure used in this study. Our decontamination procedure did not alter surface structure. This decontamination procedure could allow reinsertion of an infected implant when peri-prosthetic infection occurs early after breast augmentation or breast reconstruction and when a salvage procedure is indicated.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflicts of interest to disclose.

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