Journal of Biomaterials and Nanobiotechnology, 2021, 12, 21-33
https://www.scirp.org/journal/jbnb
ISSN Online: 2158-7043
ISSN Print: 2158-7027

An Impact of Different Silicone Breast Implants on the Bacterial Attachment and Growth

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How to cite this paper: Nam, S.Y., Zhang, X.R., Faruq, O., Chien, P.N., Dönmez, N. and Heo, C.Y. (2021) An Impact of Different Silicone Breast Implants on the Bacterial Attachment and Growth. *Journal of Biomaterials and Nanobiotechnology*, **12**, 21-33.
https://doi.org/10.4236/jbnb.2021.123003

Received: May 14, 2021
Accepted: May 25, 2021
Published: May 28, 2021

Abstract

Bacterial biofilms have been implicated with breast implant complications including capsular contracture, double-capsule formation, and breast implant-associated anaplastic large cell lymphoma. However, the relationship between implant surface texture and microbial biofilm formation is insufficiently evaluated. In the present study, we examined the antimicrobial activities of different types of silicone breast implant. The growth of bacterial including *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Pseudomonas aeruginosa* was compared using implants with various surface textures, including Hans Smooth, Hans SmoothFine, Allergan Smooth, Eurosilicone Smooth, Eurosilicone Texture, Sebbin Smooth, Sebbin Micro, Sebbin Texture, and Motiva Smooth. Microbial investigation revealed the increased growth of *S. aureus* on breast implants after 48 h, except Eurosilicone Smooth, Eurosilicone Texture, Hans SmoothFine and Sebbin Smooth material. At 48 hours, there was no major difference between the *S. aureus* attachment on smooth and textured implants. The results of *S. epidermis* attachment on the implant after 48 h showed that their growth decreased on surfaces of Motiva Smooth, Sebbin Smooth, and Eurosilicone Smooth. These results indicated that *S. epidermis* was unable to survive on these breast implants. Eventually, *P. aeruginosa* count had showed decrease of bacterial count after 48 hours compared to 24 hours in most of the implants except for Eurosilicone Texture, Sebbin Smooth and Sebbin Micro, where the count of *P. aeruginosa* slightly increased. This indicated that *P. aeruginosa* was unable to exist on the smooth surfaces. Our results show that the *in vitro* assay revealed no significant difference between smooth and textured surfaces and showed variable

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interactions and needed further molecular analysis to assess their adherence nature.

Keywords

Silicone Implants, Surface Texture, Biofilm, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, Bacterial Attachment

1. Introduction

Silicone is the most familiar and popular pharmaceutical agent used in the human body because of its molecular and physical properties [1]. It gains more popularity as breast implant over the years for the reconstructive as well as aesthetic attribution. In addition, their softness and natural appearance enhance patient satisfaction. Majority of the patients were pleased using silicone breast implant. However, silicone implant for long-term transplantation causes capsule contracture (CC) that is undesirable after surgery [2]. It entails contracting the collagen capsule formed around the breast, which may be uncomfortable and can distort the breast structure frequently [3]. The forming of capsules itself is understood to be a natural reaction to foreign bodies, but contracture has obscured. CC's etiology is not entirely known. Bacterial biofilms were associated with complications in breast implants, including CC and large-cell anaplastic lymphoma [4].

Biofilms are microbial communities, including skin tissues, implants, and medical equipment, which are connected to the surface and constitute a large amount of human microbial infections [3]. Many methods have been seeking to avoid the biofilm developments that subsequently reduce the risk of CC. These techniques include the elimination of implant and/or breast pocket microbial seed capacity as well as prevention of hematomas [5]. Another approach is using antimicrobial agents that reduce bacterial infection after surgery. However, their non-selective activity and surging resistance may lead to serious complications. Bacterial adhesion and biofilm formation to various abiotic surfaces are proposed and are supposed to get regulated and controlled by bacterial secretions, topography, and the surface of materials used [6]. These literatures indicated that textured implants provided the suitable link between the host response and microbial accumulation, which subsequently increased the rates of bacterial growth [7] [8]. In these cases, implant surfaces are more desirable to be less cellular adhesive, host responses and inflammation. Thus, surface improvements are a promising strategic approach for the prevention of cell adhesion and biofilm formation on healthcare devices is required [4] [9].

In this study, the bacterial attachment to different textured implants was studied by *Staphylococcus aureus*, *Staphylococcus epidermidis*, *and Pseudomonas aeruginosa*. The implants included were Hansbiomed Smooth, Hans-

biomed Smooth Fine, Eurosilicone Smooth, Eurosilicone Texture, Sebbin Smooth, Sebbin Micro, Sebbin Texture, Allergen Smooth, and Motiva Smooth. Previously, several studies had been carried out regarding bacterial adhesion and their growth pattern in breast implants. However, no such study has been conducted with these specific bacteria and implants. In the current work, we investigated the bacterial attachment and prevalence on the breast implant. Besides, the attachment and growth, the topography of the implants surface and their gross difference were revealed by Scanning electron microscopic (SEM).

2. Materials and Methods

2.1. Test Materials

Nine different silicone surfaces and a single untextured control substance were used in the current study. The equator side of silicone implants was closed off and the gel was removed, 2 cm 2 parts of the shells have been cut with clean scalpels for surface metrology. The implants included Hans Smooth, Hans SmoothFine, Eurosilicone (Smooth and Texture), Sebbin (Smooth, medium, and texture), Allergen, and Motiva Smooth surfaces for the analysis.

2.2. Scanning Electron Microscopy of Implant Surfaces

The samples (up to 1 cm^2) were dehydrated in ethanol after fixing in 3% glutaraldehyde. For 3 minutes, samples were submerged in hexamethyldisilazane (Sigma Aldrich, Germany). In Emitech K550 gold coater (Emitech Ltd., England), the samples had been placed on the aluminum stubs (M.E. Taylor Engineering, Inc, MD, USA) and sputtered with a 20-nm gold film. A scanning electron microscope (Hitachi, Tokyo, Japan) was used to visualize the gold-coated breast implant samples. SEM is used to compare the surface textures of the breast implant using a single implant-type shell [10] [11].

2.3. Growth Parameters for the Selected Bacteria Used for Analysis

In the current study, three bacterial organisms were used to study their adherence behavior on different textured silicone implants. The microbes like *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 14990, and *Pseudomonas aeruginosa* ATCC 10145, were employed for nine different implants. Before inoculation, the strains were sub-cultured twice in Tryptic Soy Broth (TSB) (Merck Millipore, Germany) and later maintained at −70˚C as frozen stock cultures.

2.4. Bacterial Growth Assay

By removing an implant shell, the blunt edges from the inner surface of the implants were primed and scrapped. The implant shell was protected using a punching biopsy unit of 5 mm. In a glass Petri plate, the implant parts were placed and sterilized at 115˚C for 39 hours under dry heat (source). After sterili-

zation, the implants were immersed and pressed in sterile water. The air was released from each petri-dish, and further analysis was performed.

According to JIS Z 2801, *S. aureus*, *S. epidermidis*, and *P. aeruginosa* were pre-incubated for 24 and 48 hours and then diluted to 1.0 - 10.0 × 10^5 colony formation unit (CFU)/ml. Further, 225 μL of the diluted bacterial solution was dropped onto the 40 × 40 standard PP film and covered with the top of the sample facing down. After 24 hours, CFU was measured according to the method of JIS Z 2801 and the result was calculated.

2.5. Statistical Analysis

Statistical analyses were carried out using Prism software (San Diego, CA, USA). The implants surfaces and bacterial attachment sores were analyzed by t-tests. The values represented are the mean ± standard error (SE) for n = 5 replicates. The $p < 0.05$ is set as a significant difference.

3. Results

3.1. SEM Images of Silicone Breast Implant Surfaces

SEM imaging shows that visually the implant texture varies in surface uniformity, pores appearance, pores size and the texture depth. General similarities among the groups of textures have been noticed in Hans Smooth, Eurosilicone Smooth, Sebbin Smooth and Allergen Smooth (Figure 1). These implant surfaces were almost same and appeared smooth, flat with no depth in texture. Eurosilicone Texture and Sebbin Texture had presented pores and showed complex

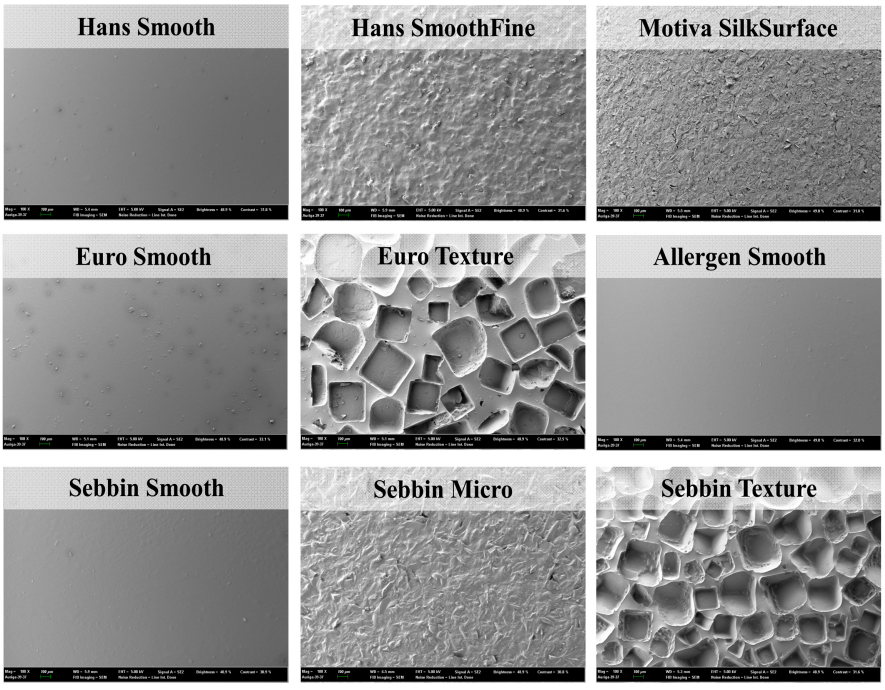

Figure 1. Scanning electron microscopy (SEM) images of different silicone biomaterials used for the study.

texture while other materials like HansBiomedBellaGelSmoothFine, Sebbin Micro, and Motiva Smooth Surface had unevenness and depths in their texture surfaces.

3.2. Bacterial Growth of *S. aureus* ATCC 6538 on Different Silicone Material

In Figure 2(a), the number of *S. aureus* ATCC 6538 attachment to different silicone implant materials was evaluated at 24 h and 48 h. The Hans Smooth material had the least *S. aureus* count at 24 h, highly resistance to bacterial attachment with 26.48% of control (p = 0.0027) and also compared to other smooth and textured silicone implants that had significant attachment of bacteria on surface implant materials from 76.05% to 99.81% of control (Figure 2(a)). However, the *S. aureus* count on Sebbin Smooth, Motiva Silk Surface and Sebbin Texture slightly increased at 1.76%, 2.34% and 9.72% respectively, while the bacterial attachment count on Allergan Smooth and HansSmooth had significantly increased at 23.57%, 71.29% respectively (p = 0.0464) at 48 hours. In contrast, Eurosilicone Smooth, Eurosilicone Text, Hans SmoothFine and Sebbin Smooth materials had decreased at 1.16%, 1.56%, 8.44% and 15.03%, respectively (Figure 2(a)). These results indicate that *S. aureus* was unable to survive on these silicone materials. The plate count images of the *S. aureus* on different silicone implants materials are presented in Figure 2(b) (at 24 hours) and Figure 2(c) (at 48 hours), where the bacterial attachment seems to vary depending on the material used.

3.3. Bacterial Growth of *S. epidermidis* ATCC 14990 on Different Silicone Material

In Figure 3(a), the number of *S. epidermidis* ATCC 14990 attachment to different silicone implant materials was evaluated at 24 and 48 hours. The Eurosilicone Texture and Hans Smooth had the least *S. epidermis* count at 24 h, highly resistance to bacterial attachment with 18.04% and 26.42% of control respectively (p = 0.013) and compared to other smooth and textured silicone implants. In contrast, the bacterial attachment of Motiva Silk Surface and Eurosilicone Smooth significantly attached and grew on silicone implant materials at 98.20%, 92.78% of control respectively.

At 48 hours. The *S. epidermis* attached count significantly increased from 29.53% to 70.87% in almost silicone implant materials. Moreover, the bacterial attachment on surfaces of Motiva Silk Surface, Sebbin Smooth, and Eurosilicone Smooth had decreased, from 6.08%, 13.01% and 21.96% respectively. These results indicate that *S. epidermis* was unable to survive on these silicone materials (Figure 3(a)). The maximum growth of bacteria was observed in Hans Smooth and Sebbin Micro with 70.87% and 51.02% of *S. epidermis* count from 24 to 48 hours respectively (p = 0.0162), and the least increase was observed in Hans SmoothFine with 34% (p = 0.0490). The *S. epidermidis* attached to smooth and textured implants had shown significant difference, wherein Hans SmoothFine,

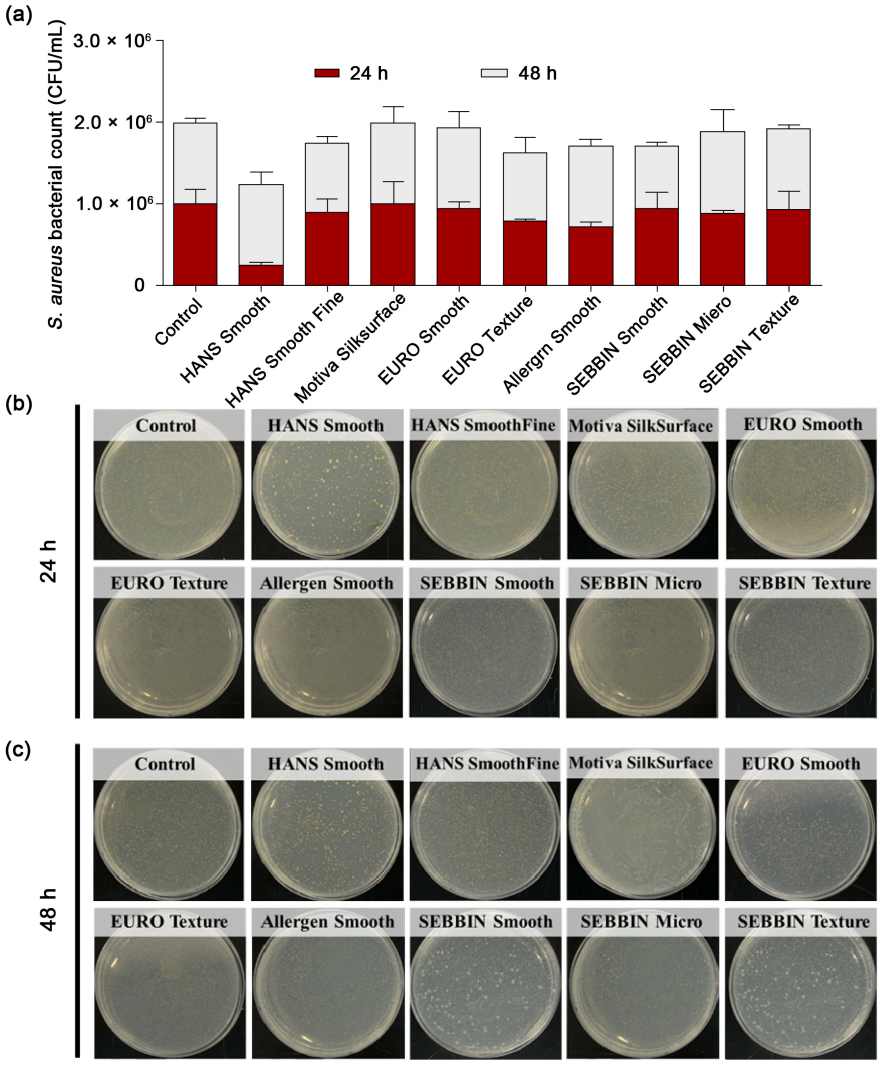

Figure 2. Growth (a) and bacterial attachment of *S. aureus* ATCC 6538 on different silicone implants measured at 24 hours (b) and 48 hours (c).

Eurosilicone Texture, Sebbin Texture, and Allergan Smooth displayed an increase of bacterial count of about 29%, 48.70%, 36.97%, and 36.29% respectively ($p < 0.05$). This indicates that these silicone implants had significantly higher bacterial count and attachment to them when compared to other implants of Eurosilicone Smooth, Sebbin Smooth, and Motiva Silk Surface. The plate count images of the *S. epidermidis* on different silicone implanted materials are presented in **Figure 3(b)** (at 24 hours) and **Figure 3(c)** (at 48 hours), where the different bacterial attachments were observed based on the materials used.

3.4. Bacterial Growth of *P. aeruginosa* ATCC 10145 on Different Silicone Material

In **Figure 4(a)**, the number of *P. aeruginosa* ATCC 10145 attachment to different silicone implant materials was evaluated at 24 and 48 hours. The Hansbiomed

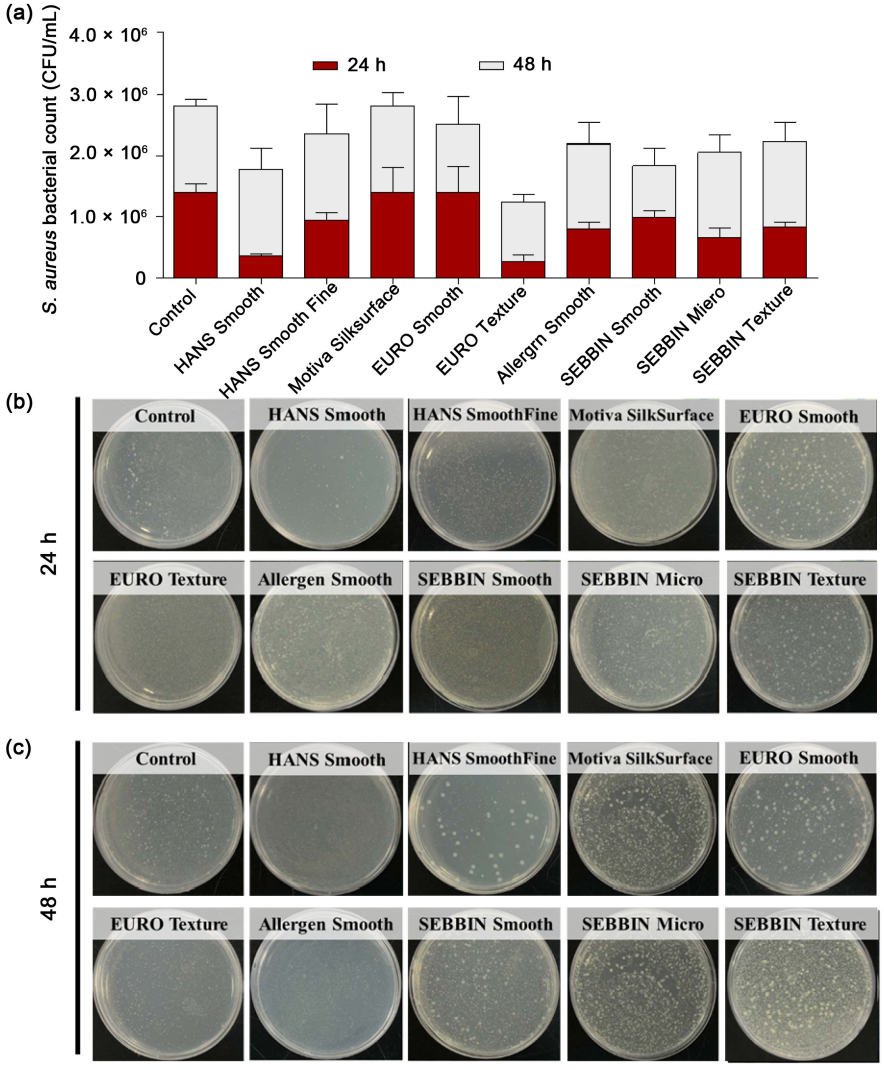

Figure 3. Growth (a) and bacterial attachment of *S. epidermidis* ATCC 14990 on different silicone implants measured at 24 hours (b) and 48 hours (c).

BellaGel Smooth Fine had the least *P. aeruginosa* count with 26.92% of control at 24 h ($p = 0.0318$), compared to all smooth and textured silicone implant materials from 68.83% to 100% of control. However, it was perceived that the *P. aeruginosa* bacterial count decline after 48 hours when compared to 24 hours in most of the materials used, except for Eurosilicone Texture, Sebbin Smooth and SebbinMicro materials, where the count of *P. aeruginosa* slightly increased by 22.09%, 7.91% and 10.57% respectively ($p = 0.0255$) by 48 hours. Vast variation was observed in the count of *P. aeruginosa* in HansBiomedBellaGel type of materials, where Hans Smooth had a decline in the bacterial count by 37.58% ($p = 0.0243$). Similarly, in Hans SmoothFine, the biofilm formation was reduced by 48 hours and the count of the bacteria was 9.10%. The smooth surface of silicone materials like Eurosilicone, Allergen, and Motiva Smooth had a decline of 53.40%, 43.15%, and 50.99%, respectively ($p = 0.0086$); this indicates that the *P. aeruginosa* was unable to survive on the smooth surfaces. At 48 hours, the

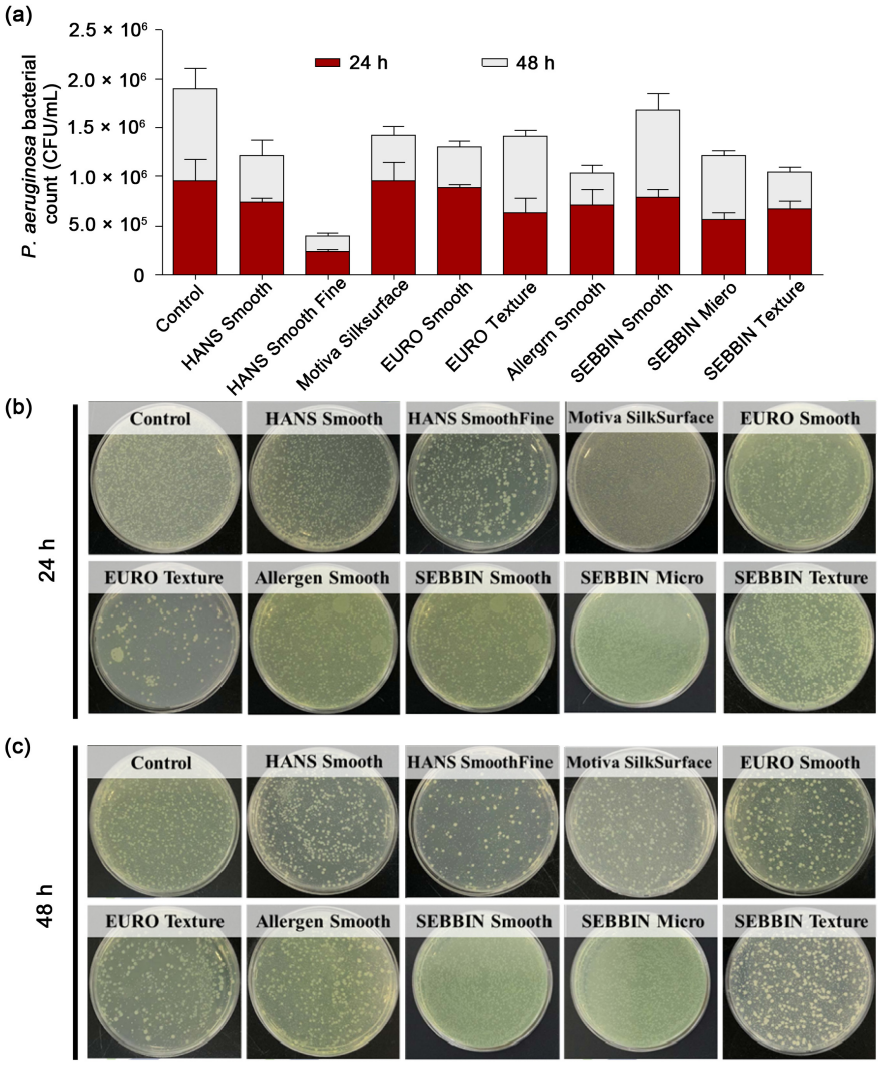

Figure 4. Growth (a) and bacterial attachment *of P. aeruginosa* ATCC 10145 on different silicone implants measured at 24 hours (b) and 48 hours (c).

P. aeruginosa attached to smooth and textured implants showing the significant difference, wherein Hans Smooth, Hans SmoothFine, and Sebbin Texture fall into a group with a decline of 9.10% - 37.58%, others like Eurosilicone Smooth, Allergan Smooth and Motiva Smooth showed a significant reduction of 43.15% - 53.40% when compared to 24 hours bacterial count ($p < 0.05$). Hence, indicating that the biofilm formation was less in *P. aeruginosa* when compared to *S. aureus* and *S. epidermidis*. The plate count images of the *P. aeruginosa* on different silicone implants materials are presented in **Figure 4(b)** (at 24 hours) and **Figure 4(c)** (at 48 hours), where the images were explicitly elucidated the variation of attachment of the bacteria on different materials according to their surface structure.

4. Discussion

Bacterial adherence on implant is a critical process that involves the implant

surfaces, physiological signal, bacterial species as well as their interaction in biological environment. Despite the bacterial response depending on several issues, implant surface topography significantly elucidates the consequences of bacterial attachment. It is postulated that higher surface area and roughness provide more auspicious environment for colonization [12]. Further bacterial colonization surges biofilm formation and infection on the implant site. Moreover, the biofilm may cause the capsular contracture and breast implant-associated anaplastic large-cell lymphoma (BI-ALCL), which eventually leads to failure of breast surgery. Therefore, successful breast reconstructive surgery, the surface topography of an implant is crucial in terms of bacterial attachment, infection, biofilm formation, and capsule contracture. However, the relationship between the surface texture and bacterial adhesion isn't always linear. Some author suggested slightly increase the surface roughness can drastically rise the bacterial adhesion, while other showed a large increase have no significant effect on bacterial attachment. [13] [14]. In this study, silicone implants with different surface morphologies were studied against three bacterial organisms and evaluated their adherence behavior on the implants. Our findings develop a rational approach for using these implants in breast reconstruction to overcome the biofilm formation as well as capsule contracture.

The implant surfaces have been classified into Macro-textured and Micro-textures, which are usually effect on cellular activities [15]. A study conducted by Barr *et al*. [11] accurately classified roughness features in macro, micro, meso and nano-textured implant surfaces. In our current study, the porous and nodular nature of EURO texture and Sebbin textured would promote the tissue in growth, (**Figure 1**) suggesting them to be categorized in Macro-textured class [15]. Such macro-texture implants are rarely found associated with Anaplastic Large Cell Lymphomas (ALCL) [16]. High surface textured implants with elevated ALCL rates showed bacterial contamination and biofilm [17]. Planar arrangements of fibroblasts were noticed in few breast tissues with smooth surface [11]. There's really no conclusion about which texture is best desirable for the surface of a breast implant in biological terms. Capsular formation in different patterns had seen in a series of macro and micro textures surfaces. Some breast implants with small tissue projection had showed little disorganization of collagen, in contrast other breast implants had larger tissue projection exhibited more irregular collagen orientation [10].

S. aureus biofilms and CC in a model of guinea pig were studied in another study that underwent bilateral implantation process. *S. aureus* culture was inoculated with experimental community implants overnight before placement and found an increase in the biofilm formation [18]. Interestingly, the initial bacterial attachment for textured implants was 20 times greater, which is not unexpected as several *in vitro* experiments showed increased bacterial adhesion and the formation of biofilms on rough surfaces [19] [20]. Under certain in vivo experiments, the *S. epidermidis* pockets when inoculated at different concentrations suggested a biofilm formation that affected the breast CC [3]. In certain

cases, *S. epidermidis* and *Propionibacterium* acnes were identified as presumed culprits. These organisms were recognized as normal flora on breast skin and surrounding tissue components [21]. The presence of bacteria was confirmed in patients with CC by bacteria isolation methods after surgery. In comparison, a statistically important improvement in the patient's serum hyaluronan level revealed a high Baker degree in CC patients with a microbial load like *S. epidermidis* [22] [23]. The incidence of CC was found to decline in antibiotic-treated or impregnated mesh in patients with *S. epidermidis* infection, resulting in the inhibition of bacterial growth and biofilm formation [23]. Translation study has now promoted the use of antibacterial mitigation to minimize CC formation and thus directly connect surface/microbial growth with a practical clinical effect [24]. It is also a known factor that implant texture can also affect CC, along with antibiotic pocket irrigation, biofilms reduction, sterile dissection, and contamination prevention [8] [25].

In the current studies, *S. aureus* growth on texture surfaces showed less variation after 48 hrs. The results demonstrated *S. aureus* was more susceptible to breast implant, regardless the surface morphology. This chronic contamination could be the result of the toxicity of silicone or silicone breakdown products. In other studies, showed *S. aureus* was involved in most of the implant associated infection where it utilizing fibrinogen to facilitate the colonization of bacteria. After coating the fibrinogen around the breast implant, the host on the breast implant led to formation of collagen rich capsule and enhanced the bacterial adherence [26]. On the other hand, our result had showed the variance in adhesion of *S. epidermidis* on breast implants according to the surface morphology. In contrast to previous studies, the *S. epidermidis* exhibited different adherence pattern based on the different implants after 24 h. In addition, the *in vitro* study revealed the bacterial adhesion and biofilm formation on different breast implants had varied on different time points. Moreover, the previous study suggested the biofilm formation on texture surface was thicker compared to smooth surface [27]. Similarly, our current results suggested the *S. epidermidis* adherence on textured surface was greater compared to smooth surfaces that subsequently increased the biofilm thickness. Eventually, our studies revealed the adhesion behavior of *P. aeruginosa* on the commercial breast implant where the bacterial count was compared at 24 h and 48 h. In contrast to other species, *P. aeruginosa* showed lower attachment on the implant surface. Therefore, it had less chanced to colonize on the breast implant. The data of our studies revealed the adhesion pattern of three microbial species on different breast implants at 24 h and 48 h. Hence, it can be speculated from the study that the presence of bacterial attachment would increase the microbial infection and each bacteria has its independent way to adherence mechanism irrespective of the silicone material, which needs to be investigated at the molecular level.

5. Conclusion

In conclusion, the surface area and topography of the textures of the breast im-

plant contribute greatly to the growth and adhesion of the bacterial pathogens. The findings of the study revealed differences between bacterial attachment to different textured and smooth surfaces of silicone implants. However, *P. aeruginosa* showed a decline in most of the materials used by 48 hs, suggesting no further biofilm on the studied textures. Whereas, *S. aureus* and *S. epidermidis* showed more differences between biofilm formation on these implants. There is no clear evidence of shell texture type with respect to implant characteristics and biofilm-related CC development.

Acknowledgements

This work was supported by grant No. 12-2013-028 from SNUBH Research Fund and by HansBiomed.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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