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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Silicon breast implants' texture affecting bacterial biofilm formation

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SUMMARY

Introduction/Objective The most important etiologic factors for both, capsular contracture (CC) and breast implant-associated anaplastic large cell lymphoma (BIA-ALCL) is subclinical infection, defined as a response of an organism on presence of biofilm on the implant surface.
The aim of this research was to examine the possibility of biofilm formation of four different bacteria (*Staphylococcus epidermidis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Ralstonia picketti*) on three differently textured silicone breast implants (Siltex, Mentor, pore size 70–150 μm; MESMO®sensitive, Polytech, pore size 50–900 μm; and SilkSurface, Motiva pores 13 μm) *in vitro.*

Methods Samples of silicone breast implant capsules (sized 1 × 1 cm) were divided into three groups according to texture. After sterilization, 30 samples in every group were contaminated with 100 μl of examined bacterial broth, followed by incubation which led to biofilm formation. For testing the capability of biofilm formation, modified technique with microtitar plates described by Stepanović was used.

Results All four examined bacteria *(Staphylococcus epidermidis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Ralstonia picketti)* form more biofilm on implants with pore sizes 50–900 μm compared to implants with pore size 70–150 μm and those with 13 μm. Statistical significance was found in biofilm formation on implants with pores 70–150 μm compared to implants with pores 13 μm. The only exception was *P. aeuruginosa* which did not show significant difference in biofilm formation on implants 70–150 μm and 13 μm.

Conclusion Silicone breast implants with micro and nanotexture should be chosen in order to prevent biofilm formation and possible consequent complications.

Keywords: biofilm; bacterial adhesion; prosthesis-related infections; breast implants; silicon elastomers

INTRODUCTION

Breast implant surgery is followed with high level of satisfaction; however, occasional complications prolong treatment, increase costs in general, and reflect on quality of life of a patient [1]. The most common complication after breast implant surgery is capsular contracture (CC) which is also the most common cause for the reoperation [2]. The incidence of CC is up to 50% [2, 3]. It is usually left untreated, when CC is first or second degree of Regnault classification, while third and fourth stage cause breast disfigurement sometimes followed with mastodynia. This kind of CC requires reoperation including capsulotomy, capsulectomy and implant removal or exchange [3].

The most severe complication after breast implant surgery is breast implant-associated anaplastic large cell lymphoma (BIA-ALCL). Firstly, it was published by Keech and Creech in 1997, but it was not until 2011 when it was distinguished as a separate disease by defining specific immunophenotype CD30, found only in patients who developed ALCL and had silicone breast implants [4]. There is a growing number of reported patients with BIA-ALCL every day [5]. BIA-ALCL can have two forms: localized, presented as a solid mass on the capsule or late seroma or both; and systemic form. Localized disease is surgically treated by removing the implant and complete capsulectomy, while systemic disease needs multimodal therapy [4, 5].

Precise cause for both CC and BIA-ALCL remains unknown. However, many etiologic factors have been associated with its pathogenesis [6, 7, 8]. Common and most important risk factors for both CC and BIA-ALCL are presence of bacterial biofilm and silicone implant surface texture, where silicone implant surface is a distinguishing factor by itself for bacterial adherence [9]. According to Hu et al. [8], presence of bacterial biofilm promotes immunological response leading to BIA-ALCL. The most common bacteria isolated from biofilms found on silicone breast implants in patients with CC are *Staphylococcus epidermidis*, *Propionibacterium specieae*, *Staphylococcus aureus*, while *Ralstonia pickettii* is most common bacteria found in patients with BIA-ALCL [9–12].

Retrospective analysis showed increased incidence in formation of CC in patients with

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smooth surface implants in contrast to textured implants, while BIA-ALCL is found almost exclusively in patients with textured silicone implants [5, 7]. For these reasons, nowadays there are plenty of different breast implant textures on the market. According to pore sizes and implant surface roughness, many classifications have been suggested, such as: smooth, micro and macro textured [13]. In the literature there is a few papers published comparing possibility of biofilm formation on different textures [3, 7, 11, 14].

The aim of the study was to determine possibilities of biofilm formation *in vitro* of four bacteria (*Staphylococcus epidermidis, Staphylococcus aureus, Pseudomonas aeruginosa*, and *Ralstonia picketii*) on three different silicone breast implant textures.

METHODS

This research was conducted at the Clinic for Plastic and Reconstructive Surgery, Clinical Center of Vojvodina and at the Laboratory for Microbiology at the Institute for Public Health of Vojvodina in Novi Sad, Serbia. For the experiment, three differently textured breast implants, divided into three groups, have been used:

- Group 1 – texture with pore size 70–150 μm (SILTEX, MemoryGel®, Mentor, CA, USA);
- Group 2 – texture with pore size 50–900 μm (MESMO®sensitive, Polytech Health & Aesthetics GmbH, Dieburg, Germany);
- Group 3 – texture with pore size 13 μm (SilkSurface, TrueMonobloc®, Motiva, Establishment Labs S.A., Coyol de Alajuela, Costa Rica).

Capsules of implants were cut into pieces 1 × 1 cm, which were sterilized with hydrogen peroxide plasma (STERRAD 100S, Johnson & Johnson, New Brunswick, NJ, USA). In total, 30 sterile samples, from each different texture, were contaminated with four different bacteria: *Staphylococcus epidermidis* (n = 30), *Staphylococcus aureus* (n = 30), *Pseudomonas aeruginosa* (n = 30), and *Ralstonia pickettii* (n = 30), which consisted of 360 samples in general. For detecting capability of biofilm formation, modified technique with microtiter plates according to Stepanovic et al. [15] was used. This method considers growth of bacteria in liquid trypticase soy broth in polyvinyl microtiter plates in previously determined conditions, which enable growth of biofilm (previously refreshed 24-hour bacterial culture, incubated on 37 degrees C in aerobic conditions and resuspended in trypticase soy broth).

According to absorbance, all bacteria were divided in four categories, where cut-off absorbance (OD_c) was defined as three standard deviations of mean absorbance negative control, as shown in Table 1.

Table 1. Intensity of bacterial strain adherence

$OD \leq OD_c$	non adherent bacterial strain
$OD_c < OD \leq 2 \times OD_c$	weakly adherent bacterial strain
$2 \times OD_c < OD \leq 4 \times OD_c$	moderately adherent bacterial strain
$4 \times OD_c < OD$	very adherent bacterial strain

OD – absorbance; ODc – cut-off absorbance

Statistical analysis was done in program SPSS v.20 (IBM Corp., SPSS Statistics for Windows, Armonk, NY, USA).

RESULTS

χ^2 test of independence detected statistically significant influence of breast implant texture on *Staphylococcus epidermidis* biofilm production (χ^2 (4) = 44.628, p = 0.000). The results are shown in Figure 1. Results of χ^2 test were confirmed with Kruskal Wallis test, which detected statistically significant difference in biofilm production of *S. epidermidis* on all three types of breast implants (χ^2 (2) = 42.365, p = 0.000). According to Cohen criteria, breast implant texture has an intermediate influence on biofilm formation of *S. epidermidis* (0.2479). In order to detect differences among different textures, Mann–Whitney U test was used with Bonferroni correction alpha (0.05/3 = 0.017). It was confirmed that bacteria *S. epidermidis* produce statistically more biofilm on silicone breast implants in Group 1 compared to Group 3 (U = 297, p = 0.005) and in Group 2 compared to Group 3 (U = 57.5, p = 0.000). Finally, biofilm of *S. epidermidis* is produced more on implants in Group 2 compared to Group 1 (U = 185, p = 0.000).

χ^2 test of independence detected statistically significant influence of breast implant texture on *Staphylococcus aureus* biofilm production (χ^2 (4) = 71.036, p = 0.000). The results are shown in Figure 2. Results of χ^2 test, confirmed with Kruskal–Wallis test, detected statistically significant difference in biofilm production of *S. aureus* on all three types of breast implants (χ^2 (2) = 55.504, p = 0.000). According to Cohen criteria, breast implant texture has a high influence on biofilm formation of *S. aureus* (0.3946). Mann–Whitney U test with Bonferroni correction alpha confirmed that bacteria *S. aureus* produce statistically more biofilm on silicone breast implants in Group 1 compared to Group 3 (U = 195, p = 0.000) and in Group 2 compared to Group 3 (U = 30, p = 0.000). Finally, biofilm of *S. aureus* is produced more on implants in Group 2 compared to Group 1.

χ^2 test of independence detected statistically significant influence of breast implant texture on *Pseudomonas aeruginosa* biofilm production (χ^2 (4) = 17.872, p = 0.001). The results are shown on Figure 3. Results of χ^2 test were confirmed with Kruskal–Wallis test, which detected statistical significant difference in biofilm production of *Pseudomonas aeruginosa* on all three types of breast implants (χ^2 (2) = 16.856, p = 0.000). According to Cohen criteria, breast implant texture has a low influence on biofilm formation of *Pseudomonas aeruginosa* (0.099). Mann–Whitney U test with Bonferroni correction alpha confirmed that bacteria *S. aureus* produce statistically more biofilm on silicone breast implants in Group 1 compared to Group 3 (U = 426, p = 0.694) and in Group 2 compared to Group 3 (U = 255, p = 0.000). Finally, biofilm of *P. aeruginosa* is produced more on implants in Group 2 compared to Group 1 (U = 258, p = 0.000).

χ^2 test of independence detected statistically significant influence of breast implant texture on *Ralstonia picketii*

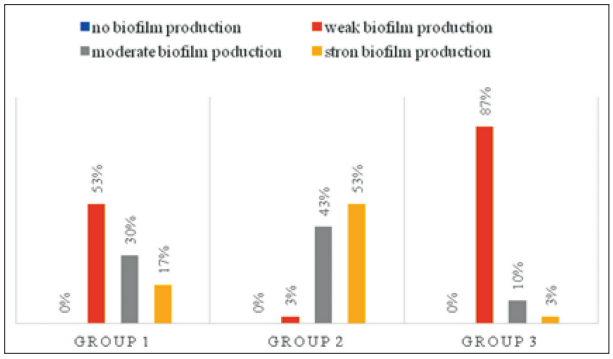


Figure 1. Frequency of *Staphylococcus epidermidis* biofilm production in all three differently textured silicone breast implants

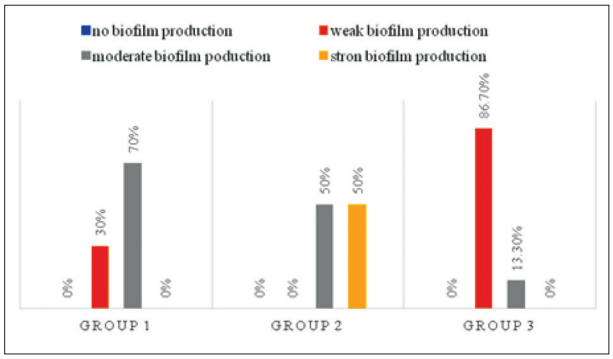


Figure 2. Frequency of *Staphylococcus aureus* biofilm production in all three differently textured silicone breast implants

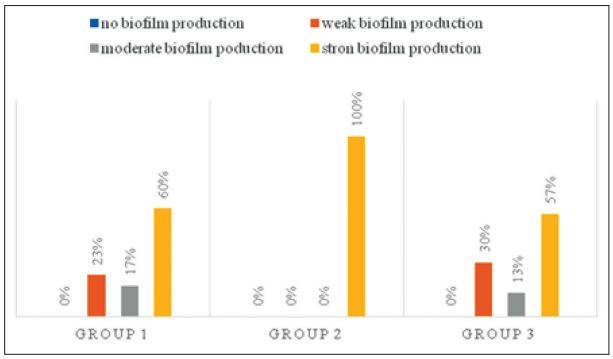


Figure 3. Frequency of *Pseudomonas aeruginosa* biofilm production on all three differently textured silicone breast implants

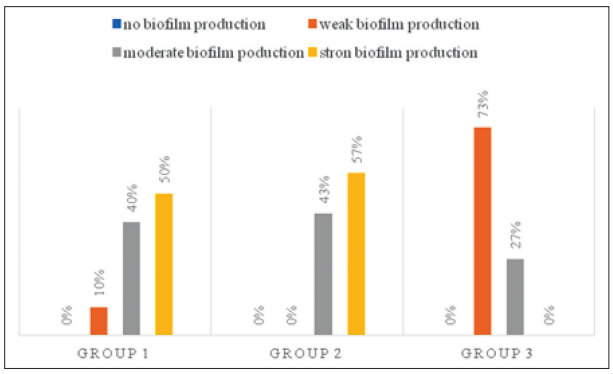


Figure 4. Frequency of *Ralstonia picketii* biofilm production in all three differently textured silicone breast implants

biofilm production (χ^2 (4) = 18.872, p = 0.001). The results are shown on Figure 4. Results of χ^2 test were confirmed with Kruskal–Wallis test, which detected statistical significant difference in biofilm production of *Ralstonia picketii* on all three types of breast implants (χ^2 (2) = 46.366, p = 0.000). According to Cohen criteria, breast implant texture has an intermediate influence on biofilm formation of *Ralstonia picketii* (0.2867). Mann–Whitney U test with Bonferroni correction alpha confirmed that bacteria *Ralstonia picketii* produce statistically more biofilm on silicone breast implants in Group 1 compared to Group 3 (U = 105, p = 0.694) and in Group 2 compared to Group 3 (U = 52, p = 0.000). Finally, biofilm of *Ralstonia picketii* is produced more on implants in Group 2 compared to Group 1 (U = 270, p = 0.000).

DISCUSSION

Breast implant surgery is one of the most common procedures in plastic and reconstructive surgery. Even though it has a high satisfaction rate, infrequent complications do happen. The most common complication is CC with the incidence of 8–50%, while the sincerest one is BIA-ALCL [4, 8].

The etiology of CC is still not known; however, many papers have been discussing it. [16–19]. Del Pozo et al. [17] found more bacteria on implants that were taken out due to the CC compared to those that were extracted for some other reason. Tamboto et al. [18] proved, in his *in vivo* experiment, that pocket inoculation with *Staphylococcus epidermidis* before positioning the implant increases the risk of CC four times [18]. Pocket inoculation with *Staphylococcus epidermidis* provokes CC in 80% of implants, while contracted capsules have 25% more bacteria compared to noncontracted, as published by Jacombs et al. [7]. Bergmann et al. [19] published an article detecting thicker capsule around those implants that were previously contaminated with *Staphylococcus epidermidis*.

Hu et al. [8] suggest that BIA-ALCL can arise as a consequence of chronic infection, such as chronic infection can be a cause for gastric lymphoma development in patients with *Helicobacter pylori* [20]. On experimental model *in vivo,* they detected more lymphocytes' infiltrate on textured implant surfaces compared to smooth surfaces. Furthermore, in that infiltrate T lymphocytes predominated in contrast to B lymphocytes, while polyurethane implants had significantly more bacteria than other implants with textured surfaces. Hu et al. [8] also detected bacteria *Ralstonia spp*. in capsules in 80% of patients diagnosed with BIA-ALCL.

In this study, possibility of biofilm formation of four different bacteria (*S. epidermidis, S. aureus, Pseudomonas aeruginosa* and *Ralstonia pickettii*) on three different breast implant surface textures was tested. *S. epidermidis* was tested as it the most common identified bacteria found, not only on the capsule but also on the implant itself, in patients with CC [10]. *S. aureus* is a common saprophyte on human mucosa and can be a virulent cause of sometimes

sincere infections. Gramm negative bacteria are rare cause of breast implant infections, but nevertheless *P. aeruginosa* was found to be the second most common cause of these infections and therefore was tested in this study [12]. Finally, *Ralstonia pickettii* was chosen as it was found in 80% of capsules in patients diagnosed with BIA-ALCL [8].

Cheesa et al. [10] tested the virulence and biofilm formation possibility of *S. epidermidis* and *S. aureus* taken from breast implants during the routine implant exchange or due to the complications. They found out that those bacteria were significantly stronger producers of biofilm compared to its controls. Prolonged incubation and biofilm formation allow them longer survival during time. *S. epidermidis* has an ability to produce slime which enhances attachment to different surfaces, and which act as a cement for other bacteria. Also, *S. epidermidis* is responsible for coagulase negative nosocomial infections, specifically infections on different implanted devices [3]. Presence of bacteria without signs of infection cause subtle inflammatory response and is called subclinical infection [21].

Since bacteria in biofilm are immersed in matrix, common swabs from infected surfaces are not sufficient for microbial detection. Those swabs are often negative. For biofilm detection on different surfaces, there are a few available methods [22]. Still, the most spread method in use today is sonification process. Even precise, this method besides providing information of biofilm presence and its intensity does not give any other information like number of bacteria [22]. Besides sonification, for biofilm detection electron microscopy, polymerase chain reaction and fluorescent *in situ* hybridization are being used. However, most of those methods are not easily reachable since they are found in specialized laboratories for biofilm research.

In his experiment, Rieger et al. [23] did sonification of whole protheses. Pajkos et al. [11] used only pieces of protheses (2 × 2 cm) as it was done in this study, but they also expected the samples with electron microscopy. According to their research, sonification process detected only one sample which was negative for biofilm, while electron microscope found biofilm on that sample [11]. In experiment conducted by Jacombs et al. [7], on four from six mini silicone breast implants installed into pigs, no biofilm was seen with electron microscopy, while sonification method showed its presence. Tamboto et al. [18] counted only biofilm identified with electron microscopy. In this study, for biofilm detection traditional method was used, concerning sonification process, bacterial growth and its identification with standardized microbiological procedures. Furthermore, the experiment was done on samples sized 1 × 1 cm, derived from capsules of three differently textured breast implants with pore sizes 70–150 μm, 50–900 μm and 13 μm. Using particles from breast implant capsules is not new. Pajkos et al. [11] used samples sized 2 × 2 cm, some authors used samples 1 × 1 cm and others even smaller 5 × 5 mm [3, 14].

Up till now, many studies have been published which examine the possibility of biofilm formation on different surfaces, but not so many on silicone breast implants [9]. Most studies compare biofilm formation on textured and smooth implants. In 1989, Sanger found with electron microscopy not only bacteria in pores of polyurethane implants but also in irregularities of smooth implants explanted for different reasons [24]. In study by Jacombs et al. [7], 20 times more bacteria were found in vivo and 72 times more in vitro, attached on textured breast implants compared to smooth. However, they did not specify which textured implant they used [8]. Del Pozo et al [17] examined bacterial cultures from contracted capsules and from implants itself explanted for different reasons. Majority were textured implants. In more than half of CC positive cultures were diagnosed on implants with sonification process.

Today, there are plenty of producers and production technologies of breast implants, so it is not enough to say only "textured" implants rather to precisely define texture. In 2016, Atlan et al. [25] compared characteristics of three differently textured implants with electron microscopy, X-ray microtomography and mechanical microscopy and found huge differences in surface textures which can reflect on clinical course. Abramo et al. [26] divided textured implant: on microtextured with open pores (Biocell®, McGhan) (600–800 μm) and depth (150–200 μm), and microtextured (Siltex®, Mentor) which have uniformly distributed wavy texture with open pores (70–150 μm) and depth (40–100 μm). A few years ago, implant with pore diameter 13 μm were produced, which according to Barr et al. [27] would be called mesotexture, but according to Maxwell intermediate texture [28].

Histologic studies show differently oriented collagen fibers in capsules around textured and smooth implants [24]. These fibers are intersected around textured implants, therefore lowering the incidence of CC as is shown in study by Stevens et al. [2] conducted among 5000 patients. The importance of textured surface is lowering the possibility of CC and anatomic implant rotation. However, the disadvantage is that they allow for more bacterial adhesion and biofilm formation [8, 29]. This is due to the fact that textured implants have more total surface area than smooth implants. Danino et al. [30] found quantitative increase in biofilm formation when bacterial count exceeds 2000 organisms per mm^2 and since macrotexture keeps more bacteria, it is no wonder that there is more biofilm formation. However, it is not only the texture that it is responsible for this phenomenon but the composition of capsule itself. Bacteria adhere easily to hydrophobic surfaces as it is case with elastomer. Hydrophilic surfaces lower bacterial count on the implant surface and contribute to apoptosis of inflammatory cells. Roughness of implant texture increase the contact angle with bacteria and lower the degree of wetness of hydrophobic surfaces, therefore allowing easier bacterial adhesion [25].

On the other hand, *in vivo* studies show different effects of bacterial contamination of macrotextured implants and effects of texture as a whole. Even though Jacombs et al. [7] found in their study more bacteria on textured implants compared to smooth ones, higher incidence of CC was not found around textured implants. She postulated hypothesis that there must be critical level of biofilm colonies, which, when exceeded, lead to CC. Furthermore,

this concentration is not dependent on surface texture. Bergamann et al. [19] made an experiment on the rats, which were implanted with textured implants and those covered with polyurethane foam. Half of the implants were contaminated with *S. epidermidis*. They expected the incidence of CC, histologic composition and possible infection. Even though they found significantly more inflammatory cells in capsules around polyurethane compared to smooth implants, this higher number did not correlate with bacterial contamination. Their results show thicker capsule around those implants (either textured or polyurethane) that were previously contaminated. Furthermore, they found significantly more T lymphocytes in both contaminated and non-contaminated polyurethane implants in contrast to textured one [19]. This would mean that texture itself would promote host inflammatory response, which, according to Hu et al. [8] can be one of the reasons for development of BIA-ALCL. In their multicentric study, Rieger et al. [23] observed capsules and implants extracted for whatever reason. They found out that bacterial contamination, confirmed with sonification method, contribute to intensity of CC but does not correlate with implant texture. Texture has higher influence on histologic capsule composition [19]. It is speculated that macrotextured implants, which usually have pores larger than cell size, influence the intensity of foreign body reaction therefore helping the tissue ingrowth on its surface and contribute to double capsule formation and late seroma accumulation. This is not the case with micro textured and meso textured implants [27].

In this research, three differently textured implants (70–150 μm (SILTEX, Mentor); 50–900 μm (MESMO®sensitive, Polytech Health & Aesthetics GmbH,); and 13 μm (SilkSurface, TrueMonobloc®, Motiva, Establishment Labs S.A.) were used. These implants are found at the moment on the market in our country and are used most often. Possibility of biofilm formation on these implants *in vitro* was examined. Results show that all examined bacteria (*S. epidermidis, S. aureus, P. aeruginosa* and *Ralstonia pickettii)* form statistically more biofilm on implants with pore sized 50–900 μm compared to pores 70–150 μm and compared to pores 13 μm, as well as on implant with pores 70–150 μm compared to 13 μm ($p = 0.000$). The only exception is *Pseudomonas aeruginosa* where no statistical difference was found in biofilm production on implants with pores 70–150 μm compared to those with pores 13 μm ($p = 0.694$).

CONCLUSION

In this experiment, it is shown that different implant surface texture influences different potential for biofilm formation of examined bacteria, which are most commonly found in contracted capsules and in capsules in patients who developed BIA-ALCL. Since bacterial contamination occurs most probably during the implant placement, further studies would be needed to identify which irrigation protocol would be recommended for each bacterium and texture.

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Утицај текстуре силиконских имплантата за дојку на формирање бактеријског биофилма

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САЖЕТАК

Увод/Циљ Најважнији фактор ризика за настанак капсуларне контрактуре и анапластичног лимфома великих ћелија удруженог са силиконским имплантатима за дојку је суклиничка инфекција, која се дефинише као одговор организма на присуство биофилма на површини имплантата.
Циљ рада је био да се испита могућност формирања биофилма четири различите бактерије (*Staphylococcus epidermidis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* и *Ralstonia picketti*) на три различито текстурисана силиконска имплантата за дојку (*Siltex*, *Mentor*, величина пора 70–150 *μm*; *MESMO®sensitive*, *Polytech*, величина пора 50–900 *μm* и *SilkSurface*, *Motiva*, величина пора 13 *μm*) у *in vitro* условима.
Методе Узорци (величине 1 × 1 *cm*) капсула силиконских имплантата за дојку су подељени у три групе према текстури. После стерилизације, 30 узорака из сваке групе контаминирано је са 100 *μl* испитиваног бактеријског бујона, после чега је уследила инкубација која је довела до формирања биофилма. За тестирање могућности формирања биофилма коришћена је модификована техника са микротитарским плочама по Степановићу.
Резултати Све четири испитиване бактерије (*S. epidermidis*, *S. aureus*, *P. aeruginosa* и *Ralstonia pickettii)* више су формирале биофилм на имплантатима са порама 50–900 *μm* у односу на имплантате са величином пора 70–150 *μm* и 13 *μm*. Статистичка значајност је утврђена у формирању биофилма на имплантатима са величином пора 70–150 *μm* у односу на оне са порама 13 *μm*. Једини изузетак је био *P. Aeuruginosa*, који није показао значајну разлику у формирању биофилма на имплантатима са порама величине 70–150 *μm* и 13 *μm*.
Закључак У циљу превенције формирања биофилма и следствених компликација требало би користити микротекстурисане и нанотекстурисане силиконске имплантате за дојку.
Кључне речи: биофилм; адхезија бактерија; инфекције узроковане уградњом протеза; имплантати за дојку; силиконски еластомер