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ARTICLE

The effects of human amniotic membrane on silicone related capsule formation in rats

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ABSTRACT

Silicone breast implants are commonly used materials in plastic surgery for breast augmentation and reconstruction and the most severe complication of silicone implants are capsule contraction which occurs in 40% of patients. The aim of our study is to evaluate how the amniotic membrane alters the capsule formation effects of silicone 24 wistar rats were used in the study. We placed a bare silicone block into the left side (Subgroup A) and single layer amniotic membrane coated silicone block into the right side (Subgroup B) of the rats back. The rats were then separated into three groups and in group 1 rats were euthanized after 3 weeks, in group 2 after 12 weeks and in group 3 after 24 weeks. Then capsule thickness, fibroblast and lymphocyte cell counts were evaluated for each sample. In Group 2 and group 3, the capsule thickness in Subgroup B was detected to be statistically significantly lower than that in Subgroup A. In Group 1, 2, and 3, the lymphocyte count in the capsule tissue taken from Subgroup B was lower than Subgroup A but the difference was not statistically significant. In Group 2 and 3, the fibrocyte count detected in the capsule tissue in Subgroup B was found to be statistically significantly lower than Subgroup A. the amniotic membrane was demonstrated to reduce capsule thickness by the antifibrinolytic effect in our study.

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Introduction

Silicone breast implants are commonly used materials in plastic surgery for breast augmentation and reconstruction [1]. Although complications such as hemorrhage, pain, hematoma, infection, numbness, implant escape, and implant rupture can be observed after implantation of the breast implant, the most severe complication is capsule contraction which occurs in 40% of patients [2,3]. In capsule contraction, an extremely hardened, fibrotic, contracted capsule is formed around the implant, and this capsule causes complaints such as pain, stiffness, and breast distortion in the patient [4,5]. The exact cause of capsule contraction is not known and is thought to be caused by multifactorial effects [3,4,6].

Although treatment methods such as surgical capsulotomy, capsulectomy, or injection of steroids into the capsule can be implemented in the treatment of capsule contraction, the capsule frequently repeats once it is formed and therefore, it is necessary to focus on treatment methods that prevent it from forming. To prevent the formation of capsule contraction in the clinic, it is recommended to use textured surface implants, to create a submuscular pouch, to pay attention to contamination during surgery, and to wash the pouch with antibiotic/antiseptic drugs [3,4,6]. However, all these methods can not preclude the formation of capsule contraction completely.

In the literature, in order to prevent capsule contraction, injection and topical application of vitamin E, vitamin A, amniotic fluid, mitomycin C, various antibiotics, antineoplastic agents, steroid, and leukotriene inhibitors were tried in the pouch where the implant was placed [3,7]. Although some success has been achieved experimentally, a treatment method used in routine practice has not been developed yet [8,9]. Another method that can prevent capsule contraction other than material infiltration into the pouch is to prevent contact with body tissues by coating the surface of the implant with another substance [4,10].

The amniotic membrane is the lowest layer of the fetal membrane. It contains many cytokines related to cell proliferation and differentiation, growth factors, and a high amount of hyaluronan. The anti-inflammatory, bacteriostatic, reepithelization enhancing, and scar formation inhibiting properties of the AM have been demonstrated, and it is used in the clinic for ocular surface damage, reconstruction of pleura and pericardial defects, treatment of burns and chronic ulcers [11]. AM does not induce immune rejection so it can be used for allogeneic transplantation [12].

This study aims to demonstrate the effect of coating silicone implant surfaces with the AM, which has an anti-inflammatory and bacteriostatic effect, on capsule formation

Material and methods

Our study was carried out in the animal testing laboratories of Bezmialem Foundation University after obtaining the necessary approvals from the Local Ethics Committee of the Zeynep Kamil Women's and Children's Diseases Training and Research Hospital and the Animal Testing Local Ethics Committee of Bezmialem

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Foundation University. In our study, we used 32 Wistar rats weighing 250–350 g. All rats were obtained from the Experimental Animals Center of Bezmialem Foundation University, and throughout the experiment, the rats were housed in the animal testing laboratories of Bezmialem Foundation University. The animals were kept at a temperature of 22 degrees with a cycle of 12 h light and 12 h dark, without any food and water restrictions. All surgical procedures were performed under 10% ketamine HCl (50 mg/kg - Alfamine ® - IM) and xylazine HCl (2.5 mg/kg - Rompun ® - IM) anesthesia and under sterile conditions by the same surgical team. After surgery, the rats were kept in separate cages in order to prevent them from damaging each other.

Obtaining the amniotic membrane

In Zeynep Kamil Women's and Children's Diseases and Obstetrics and Gynecology Clinic, the placentas of term pregnant women, whose consent forms prepared following the Helsinki criteria had been obtained previously, were taken in a sterile environment after cesarean section and transferred to sterile containers. The amniotic membranes were separated from these placentas by blunt dissection in a laminar airflow incubator sterilized with UV light in advance. The obtained amniotic membranes were washed with 0.9% isotonic solution, and the blood clots on them were removed. Afterward, in accordance with the International Federation of Eye and Tissue Bank (IFTEB) procedure, 500 cc Medium 199 solution (Sigma-Aldrich, Steinhauser, Germany) was prepared in which 50 mcg/ml streptomycin (İ.E. Ulagay, Istanbul), 50 mcg/ml penicillin (İeciline®, İ.E. Ulagay, Istanbul), and 2.5 mcg/ml amphotericin b (Ambisome ®, Gilead Sciences Ltd, County Cork, Ireland) were put. The amniotic membranes were washed four times with this solution.

Experimental protocol

A total of 64 silicone gel sheets (Bioderm™, NY, USA) with the dimensions of 1 × 1 cm and a thickness of 0.18 cm were prepared. Thirty-two of the silicone sheets were wrapped with one layer of the amniotic membrane, and the remaining 32 silicone gel sheets were left without being wrapped with the amniotic membrane.

After the appropriate anesthesia was provided with ketamine and xylazine, the rats' back skins were shaved, and under sterile conditions, a 1 cm incision was made on the cranial side of the rats' backs to the right and left areas, and 2 pockets with the dimensions of 1.5 × 1.5 cm were created under the skin. A 2 cm undamaged space was left between the pockets. Bare silicone was placed in the right pocket, silicone wrapped with one layer of the amniotic membrane was placed in the left pocket, and incisions, made to form the pockets, were sutured with 4/0 Vicryl (Doğsan, Turkey).

The rats were then divided into three groups with eight rats in each group. The rats in the first group were euthanized with a high dose of anesthesia after three weeks, the rats in the second group were euthanized after 12 weeks, and the rats in the third group were euthanized after 24 weeks. The silicone-implanted regions were excised with the skin on them, and tissue samples were taken in capsule formation and separated for histopathological measurements.

Histopathological measurements

In each group, the tissue samples taken from the area, where bare silicone was placed in the dorsal region, were named as Subgroup A, and the tissue samples taken from the silicone-placed area wrapped with one layer of the amniotic membrane were named as Subgroup B.

Tissue samples were embedded in paraffin. Sections 6 μm in thickness were taken and placed on glass slides. By applying standard procedures, Hematoxylin - Eosin and Mason - Trichrome stainings were performed. Fibroblast and lymphocyte cells on the capsule structure in the cross-section were calculated by counting under ×200 magnification using a fluorescence microscope (Nikon eclipse NI). Under ×200 magnification, capsule thicknesses were measured in the thickest region using the NIS Elements D.4.00.00 program.

Statistical analysis

The Shapiro-Wilk test was conducted to investigate whether the distribution of continuous numerical variables was close to normal. Levene's test was used to determine whether the assumption of homogeneity of variance was provided or not. Descriptive statistics were expressed as median (1st quarter–3rd quarter) for continuous numerical variables.

When the localizations were kept constant, the significance of the difference in terms of capsule thickness, fibrocyte count, and lymphocyte count between the groups, respectively, was evaluated by the Kruskal-Wallis test. According to the Bonferroni correction, the results were considered statistically significant for $p < 0.025$. In case the results of the Kruskal-Wallis test statistics were found to be significant, the condition(s) causing the difference was(were) determined using the Dunn-Bonferroni multiple comparison test.

When the sacrification days were kept constant, the significance of the difference in terms of capsule thickness, fibrocyte count, and lymphocyte count between the localizations, respectively, was examined by the Wilcoxon sign test. According to the Bonferroni correction, the results were considered statistically significant for $p < 0.0167$.

Data were analyzed using IBM SPSS Statistics Version 17.0 (IBM Corporation, Armonk, NY, USA). The Bonferroni correction was applied in the present study to control Type I error in all possible multiple comparisons.

Results

Amniotic membrane integrity was detected in 80% of the rats in the group euthanized after three weeks (Group 1), but the amniotic membrane was not observed in the rats euthanized after 12 weeks (Group 2) and after 24 weeks (Group 3).

Capsule thickness

In Group 1, in which the silicone implant remained for 3 weeks, the mean capsule thickness in Subgroup B (the subgroup to which a silicon block wrapped with one layer of the amniotic band was placed) was lower than the mean capsule thickness in Subgroup A (the subgroup to which a bare silicone block was placed), but the difference between them was not statistically significant (*p*:0.674). (Figure 1(a,b)). In Group 2 in which the silicone implant remained for 12 weeks and in Group 3 in which the silicone implant remained for 24 weeks, the capsule thickness in Subgroup B was detected to be statistically significantly lower than that in Subgroup A (*p*:0.015, *p*:0.012). (Figure 2(a,b))

When compared between themselves, there was no statistically significant difference (*p*:0.87) in the capsule thicknesses detected

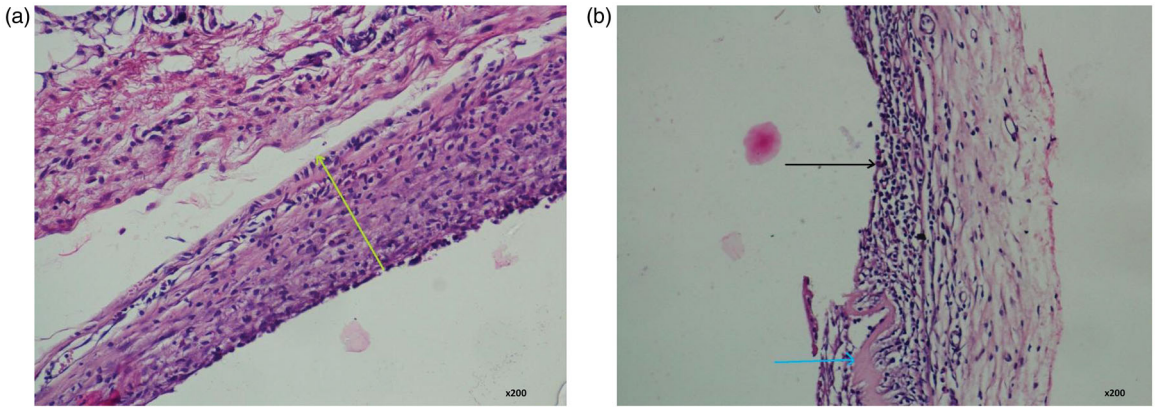

Figure 1. (a) Capsule thickness with bare silicone block at 3rd week (Hematoxylin and eosin; original magnification × 200). (b) Capsule thickness with single layer amniotic membrane folded silicone block at 3rd week (Hematoxylin and eosin; original magnification ×200).

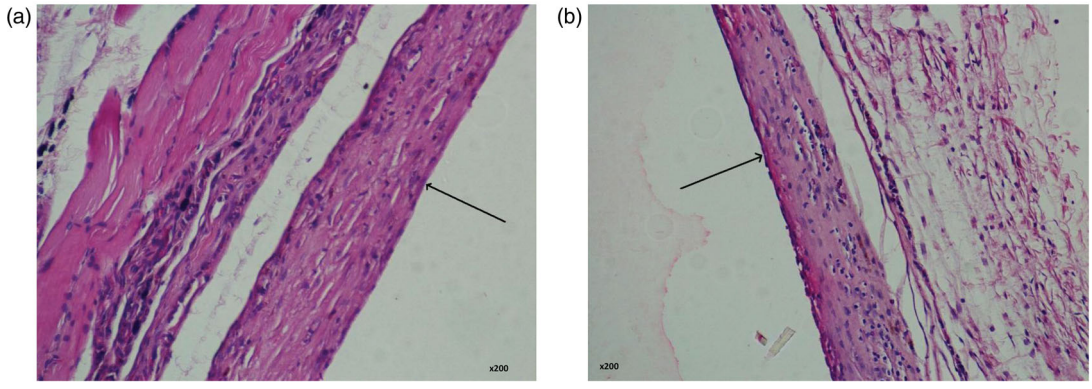

Figure 2. (a) Capsule thickness with bare silicone block at 12th week (Hematoxylin and eosin; original magnification × 200). (b) Capsule thickness with single layer amniotic membrane folded silicone block at 12th week (Hematoxylin and eosin; original magnification ×200).

at the 3rd, 12th, and 24th weeks in subgroup A, while in subgroup B, when the capsule thicknesses detected at the 3rd, 12th, and 24th weeks were compared between themselves, it was determined that the capsule thickness at the 3rd week was statistically significantly higher than the thickness at the 12th and 24th weeks ($p < 0.001$), and there was no difference between the values at the 12th and 24th weeks. (Figures 3(a,b) and 4), (Table 1).

Lymphocyte count

In Group 1, Group 2, and Group 3, in which silicone implants remained for 3, 12, and 24 weeks, respectively, although the lymphocyte count in the capsule tissue taken from Subgroup B (the subgroup to which a silicon block wrapped with one layer of the amniotic band was placed) was lower than the lymphocyte count in the capsule tissue taken from Subgroup A (the subgroup to which a bare silicon block was placed), it was determined that the difference was not statistically significant (p:0.106, p:0.061, p:0.040).

When the capsule thicknesses detected at the 3rd, 12th, and 24th weeks in Subgroup A and Subgroup B were compared between themselves, in both groups, the lymphocyte count in the capsule tissue at the 3rd week was determined to be statistically significantly higher than the lymphocyte count in the capsule tissue at the 12th and 24th weeks. No difference was detected between the values detected at the 12th and 24th weeks (p:0.003, p:<0.001). (Figure 5, Table 1).

Fibrocyte count

In Group 1, in which the silicone implant remained for 3 weeks, there was no statistically significant difference between the fibrocyte count detected in the capsule tissue in Subgroup A (the subgroup to which a bare silicone block was placed) and the fibrocyte count in the capsule tissue in Subgroup B (the subgroup to which a silicone block wrapped with one layer of the amniotic band was placed) (p:0.484). In Group 2, in which the silicone implant remained for 12 weeks and in Group 3 in which the silicon implant remained for 24 weeks, the fibrocyte count detected in the capsule tissue in Subgroup B was found to be statistically significantly lower than the fibrocyte count detected in the capsule tissue in Subgroup A (p:0.012, p:0.010).

In the capsule tissue in Subgroup A, when the fibrocyte counts detected at the 3rd, 12th, and 24th weeks were compared between themselves, no difference was detected between the 3rd week and the 12th week and the 12th week and the 24th week in terms of the fibrocyte count, but there was a statistically significant difference between the 3rd week and the 24th week (p:0.005). When the fibrocyte counts detected at the 3rd, 12th, and 24th weeks in the capsule tissue in Subgroup B were compared between themselves, it was detected that there was a statistically significant difference between the 3rd week and the 12th week, and between the 3rd week and the 24th week in terms of the fibrocyte count, but there was no difference between the 12th and the 24th weeks ($p < 0.001$). (Figure 6),(Table 1).

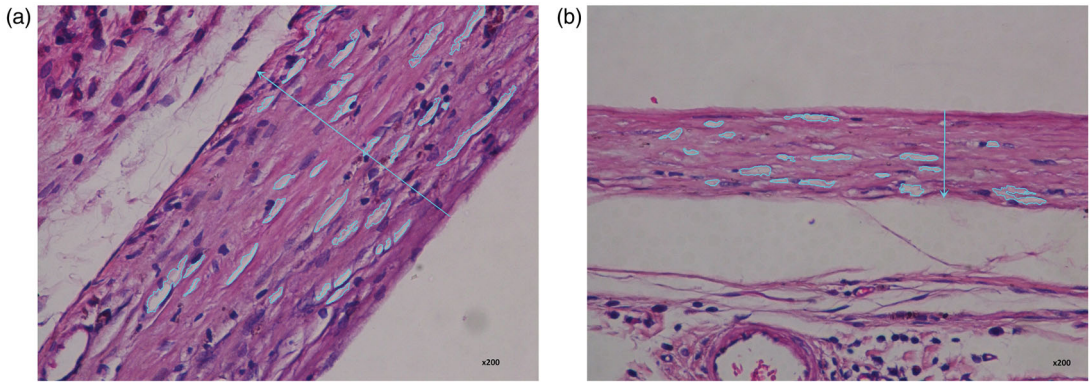

Figure 3. (a) Capsule thickness with bare silicone block at 24th week (Hematoxylin and eosin; original magnification ×200). (b) Capsule thickness with single layer amniotic membrane folded silicone block at 24th week (Hematoxylin and eosin; original magnification ×200).

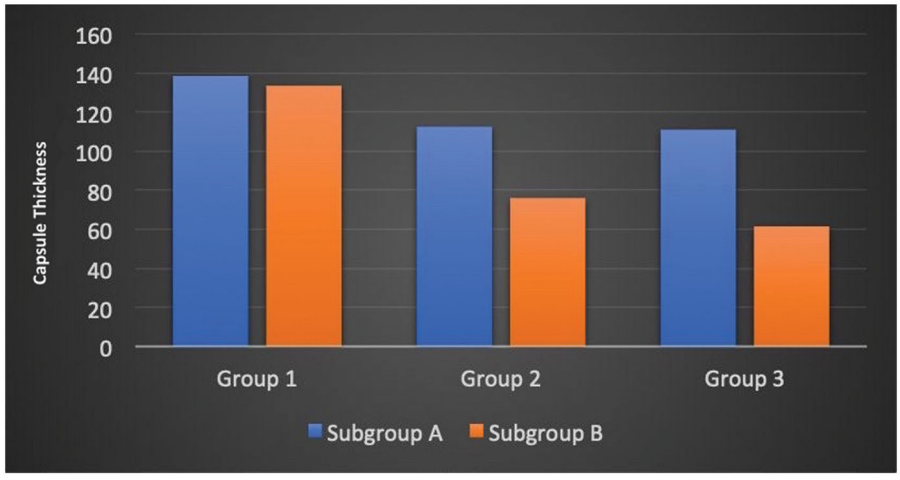

Figure 4. The difference in terms of capsule thickness between the groups.

Table 1. The difference in terms of capsule thickness, fibrocyte count, and lymphocyte count between the groups.

	Subgroup A (Bare silicone)	Subgroup B (silicone wrapped with AM)	*p* Value[†]
Capsule thickness			
Group 1(3rd *week)*	138.50 (71.50–193.50)	133.50 (118.00–156.00)[a,b]	0.674
Group 2(12th *week)*	112.50 (69.00–137.25)	76.00 (61.00–88.25)[a]	**0.015**
Group 3(24th *week)*	111.00 (65.75–135.25)	61.50 (56.00–73.75)[b]	**0.012**
p Value[‡]	0.870	**<0.001**	
Fibrocyte Count			
Group 1(3rd *week)*	98.50 (86.75–104.75)[b]	105.00 (88.75–121.75)[a,b]	0.484
Group 2(12th *week)*	85.00 (56.00–95.00)	31.00 (27.25–38.75)[a]	**0.012**
Group 3(24th *week)*	62.50 (60.25–70.25)[b]	28.50 (23.00–31.50)[b]	**0.012**
p-value[‡]	**0.005**	**<0.001**	
Lymphocyte count			
Group 1(3rd *week)*	28.00 (16.25–36.25)[a,b]	21.50 (11.75–27.00)[a,b]	0.106
Group 2(12th *week)*	7.00 (3.00–13.75)[a]	4.00 (0.50–8.00)[a]	0.061
Group 3(24th *week)*	9.00 (6.00–11.50)[b]	4.00 (2.00–8.00)[b]	0.040
p Value[‡]	**0.003**	**<0.001**	

Descriptive statistics were expressed as median (1st quarter–3rd quarter), [†]comparison of the difference between Subgroup A and B, Wilcoxon sign test, According to the Bonferroni correction, the results were considered statistically significant for $p < 0.0167$. [‡]comparison of the difference between Group1,2 and 3., the Kruskal-Wallis test, According to the Bonferroni correction, the results were considered statistically significant for $p < 0.025$ a; group 1 vs group 2, b; group 1 vs group 3 ($p < 0.025$).

Discussion

Although why capsule contraction occurs in patients using silicone breast prosthesis is not known precisely, the emphasis is put on two theories as the reason for this. The first one of these theories is the theory of subclinical infection, and this theory claims that inflammation formed secondarily to a subclinical infection, which is caused by various organisms, especially Staphylococcus epidermidis, which is planted in the medium during implant placement, causes capsule contraction [13]. The other theory is the hypertrophic scar theory, and this theory suggests that fibroblasts and myofibroblasts are overactivated as a result of the foreign body reaction caused by the implant similar to the scar in wound healing, and capsule contraction occurs due to the collagen overproduced by these cells [1]. These two pathways are thought to have a collective effect on capsule formation.

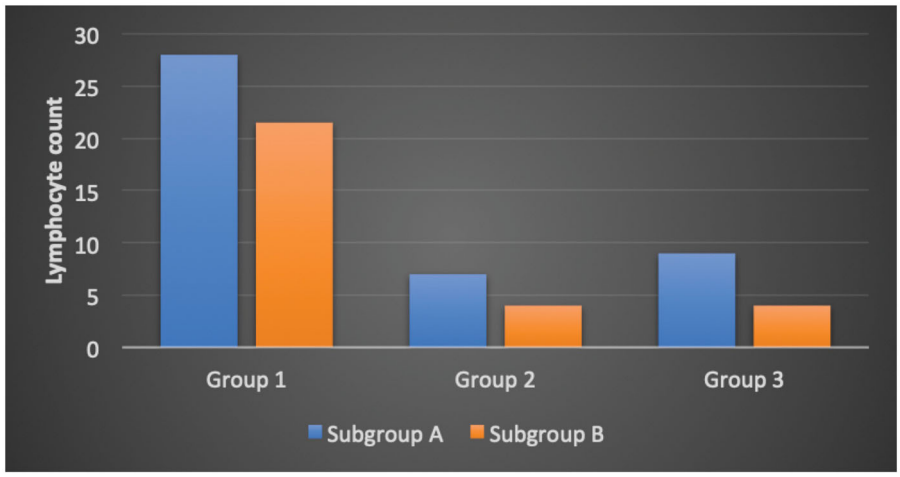

Figure 5. The difference in terms of fibrocyte count between the groups.

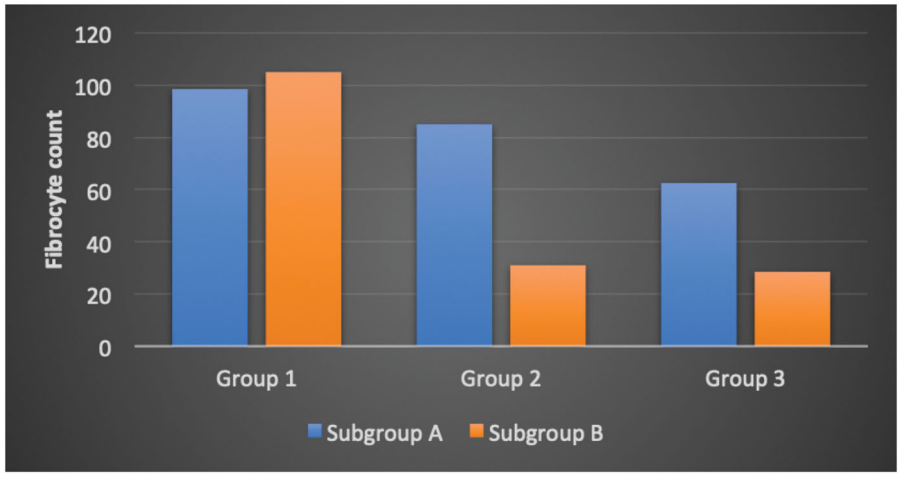

Figure 6. The difference in terms of lymphocyte count between the groups.

As it is suggested in the hypertrophic scar theory, theoretically, wrapping a silicone implant with organic material and preventing its contact with surrounding tissues may reduce the foreign body reaction caused by the implant, and consequently, capsule formation. With this thought, for the first time, Friedman et al. [14] wrapped the antiadhesive barrier membrane (AABM) in film form around the silicone disc in rats, and as a result, they reported that the capsule formation decreased. Afterward, it was published that wrapping an implant with the AABM in solution form, the mixture of alginate and poloxamer, the mixture of hyaluronate and carboxymethyl cellulose, AlloDerm reduces capsule formation [8,15,16].

In this study, the implant was wrapped with the amniotic membrane (AM) to prevent capsule formation. The amniotic membrane is the lowest layer of the fetal membrane and consists of a monolayer cuboid epithelium, a thick basal layer, and an avascular matrix. Amniotic epithelial cells contain many cytokines and growth factors, glycoprotein, proteoglycan, type 1–3 and 4 collagen fibers, and a high amount of polymeric hyaluronan, which are related to cell proliferation and differentiation [11,13]. The amniotic membrane, known to be used for the first time in traditional Chinese medicine, is used in tissue engineering as a scalfold for the migration and growth of cells [11].

In our study, wrapping the AM over the silicon layer was demonstrated to reduce capsule formation at the 12th and 24th weeks compared to the control group. Hyaluronan is the leading substance found in the amniotic membrane. Hyaluronan is a polymer, 4 kDa in weight, which is one of the structural components of the extracellular matrix [13]. The hyaluronan in the AM increases at the time of delivery and supports the separation of fetal membranes, especially by being present between the amnion and chorio decidua [17]. It reduces the secretion of proinflammatory cytokines such as TNF alpha and IL-6 and reduces inflammation by increasing the secretion of anti-inflammatory cytokines such as IL-10. Furthermore, hyaluronan inhibits fibrosis by reducing the amount of TGF-1 and TGF-2 and it was demonstrated to reduce tendon adhesion formation in tendon healing with this antifibrinolytic effect [13,18]. In our study, it was found out that besides capsule formation, the AM also decreased the fibrocyte count.

In our study, although it was not statistically significant, it was found that the lymphocyte count in the samples taken at the 12th and 24th weeks in the AM silicone-wrapped groups was lower compared to the control group. Amniotic membrane is an immune-privileged tissue because Amniotic epithelial cells are not secrete major histocompatibility antigens HLA-A, B, or DR but Amniotic epithelial and mesenchymal cells produced soluble HLA-G molecules [19]. It was shown that HLA-G inhibit lymphocyte proliferation and inactivated lymphocyte and dendritic cell activation [19–20]. Also Fas ligand–positive cells were detected in Amniotic membrane and these Fas ligands suppress the invasion of lymphocytes [18,20–22].

In our study, when the control and AM experimental groups were compared within themselves, it was determined that the capsule thickness was the highest in the control and AM groups at the 3rd week. In the control group, it was observed that although the thickness decreased at the 12th and 24th weeks compared to the 3rd week, no statistically significant difference was formed, and in the AM group, it was observed that it decreased statistically significantly at the 12th week and that there was no difference between the values of the 12th and 24th weeks. Similarly, the capsule thickness was shown to be the highest on the 30th day in the study conducted by Mendes et al. [23] on rats and on the 35th day in the study conducted by Moreira et al. [3] on rats, and in the samples taken after 2 months, this thickness was shown to decrease in the control and experimental groups.

Studies on silicone implants are mostly performed on rats due to histological similarity [23]. The average life of rats is three years and of humans is 85 years. Accordingly, the period of 1–3 months in rats corresponds to 2.5–7.5 years of human life [3]. Our study and the studies of Mendes et al. [23] and Moreira et al. [3] showed that when implants are placed in rats, capsule formation occurs in any case in the first 1-month period and that the capsule thickness decreases over time, and no change in thickness occurs after 12 weeks (3 months). Therefore, the examination of the three-month period in rats is considered sufficient for capsule formation.

In conclusion, the AM, which was wrapped in a single layer around the silicone prosthesis, was demonstrated to reduce capsule thickness by the antifibrinolytic effect in our study. As a result of our study, the AM can be recommended as a candidate tissue that can be tried in clinical studies for the treatment of capsule contraction.

Disclosure statement

The authors report no conflicts of interest and no funding.

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