creative commons
COMMONS DEED

저작자표시-비영리-변경금지 2.0 대한민국

이용자는 아래의 조건을 따르는 경우에 한하여 자유롭게

- 이 저작물을 복제, 배포, 전송, 전시, 공연 및 방송할 수 있습니다.

다음과 같은 조건을 따라야 합니다:

저작자표시. 귀하는 원저작자를 표시하여야 합니다.

비영리. 귀하는 이 저작물을 영리 목적으로 이용할 수 없습니다.

변경금지. 귀하는 이 저작물을 개작, 변형 또는 가공할 수 없습니다.

- 귀하는, 이 저작물의 재이용이나 배포의 경우, 이 저작물에 적용된 이용허락조건을 명확하게 나타내어야 합니다.
- 저작권자로부터 별도의 허가를 받으면 이러한 조건들은 적용되지 않습니다.

저작권법에 따른 이용자의 권리는 위의 내용에 의하여 영향을 받지 않습니다.

이것은 이용허락규약(Legal Code)을 이해하기 쉽게 요약한 것입니다.

Disclaimer

dCollection

의학박사 학위논문

실리콘 유방 재건 모델에서 면역억제제 투여의 피막 형성 억제 효과 분석 연구

Study of analysis of the effect of Administration of Immunosuppressant in Silicone Breast reconstruction Model

울산대학교 대학원

의 학 과

김 형 배

실리콘 유방 재건 모델에서 면역억제제 투여의 피막 형성 억제 효과 분석 연구

지 도 교 수 엄 진 섭

이 논문을 의학 박사 학위 논문으로 제출함

2023 년 12 월

울 산 대 학 교 대 학 원

의 학 과

김 형 배

김형배의 의학박사 학위 논문을 인준함

심사위원 김 은 기 (인)

심사위원 엄 진 섭 (인)

심사위원 서 현 석 (인)

심사위원 한 현 호 (인)

심사위원 오 득 영 (인)

울 산 대 학 교 대 학 원

2023 년 12 월

차 례

그 림 차 례

영 문 요 약

Background: In this study, it was hypothesized that the capsule formation varies according to the systemic immunosuppressive drugs in contact with the silicone implant.

Materials and Methods: This study consisted of 18 SD rats that underwent premuscular plane implant reconstruction. They were divided into four groups: Group 1 as the untreated control (n = 6), Group 2 with tacrolimus (n = 6), and Group 3 with dexamethasone (n = 6), Three months after surgery, the histology and immunochemistry of the capsule tissues were analyzed.

Results: Systemic administration of Dexamethasone and Tacrolimus successfully reduces the capsule thickness and inflammation. Dexamethasone and Tacrolimus reduce the aggregation of the CD3 (T lymphocytes), and CD68 (histiocytes) positive cells around the capsule.

Conclusions: In this study, systemic use of Dexamethasone and Tacrolimus successfully reduces capsule formation and inflammation around the implant. Tacrolimus could be considered a potential preventive drug for capsular contracture in breast implant surgeries. Further studies for evaluating the effect of Tacrolimus are required through human study and local drug delivery.

Introduction

Silicone has been used widely in medicine for the last 70 years, the most frequent use of this material is in silicone breast implants. (1) Since 1961, when Cronin and Gerow developed the first silicone breast implant, silicone breast implants have been developed with various modifications. Meanwhile, silicone breast implants have been associated with several complications and risks. (2) Among the complications, the capsular contracture, which is the formation of a thick fibrotic capsule around the implant with a foreign body reaction, is known to have occurred up to 37.5% after breast augmentation and reconstructive surgeries. (3-6) Although capsular contracture has been the most common complication in breast implant surgery, surgeons continue to debate the etiology of capsular contracture.

The formation of the capsule is a natural response to all foreign bodies placed in the body. The capsule contains three layers: an inner cellular layer comprised primarily of fibroblasts, T-cells, and macrophages; a vascular middle layer of loose connective tissue; and an outer layer of dense connective tissues. (7) The foreign body reaction, particularly the inflammatory phase, is the main cause of the capsular contracture and is exacerbated by external factors, such as biofilm, chronic implant micromotion, or silicone particulate shedding. (8,9)

Authors have experience with several previous studies to see the various factors of capsule formation in a rat model. (10, 11) and authors have clinical impressions that transplant patients have a low rate of capsular contracture or capsule formation with breast silicone implants. Accordingly, the authors designed this study to find out the potential drugs for preventing capsular contracture. The pilot studies were conducted and found that tacrolimus and dexamethasone as potential effects on suppressing the capsule formation around the silicone implant in a rat model.

This study aims to study the relationship between capsule formation and systemic immunosuppression to determine whether various systemic immunosuppression can prevent and/or minimize capsular contracture. This experimental study could give us information on the etiology of capsular contracture and may help find new potential drugs for preventing capsular contracture.

Materials and Methods

Animal

All the animal experiments were performed by the protocol approved by the Asan Medical Center Institutional Animal Care and Use Committee

(Animal Welfare Assurance no. A20230597). Overall, 20 8-week-old adult Sprague Dawley (SD) male rats weighing 300 g to 350 g were included. All the rats underwent surgery, which mimics implant-based breast reconstruction. They were divided into three groups: Group 1 as controls that underwent implant-based reconstruction surgery (n = 6), Group 2 underwent implant-based reconstruction surgery with administration of tacrolimus (n = 6), and Group 3 underwent implant-based reconstruction surgery with administration of dexamethasone (n = 6).

Surgical techniques

Anesthesia was initiated and maintained by the inhalation of isoflurane at a concentration of 2%–3%. A 3 cm incision was made over the margin of the Latissimus dorsi muscle. A pre-muscular dissection above the Latissimus dorsi muscle was performed to make the implant pocket. The implant (1.5 × 1.5 cm smooth silicone implant, Hansbiomed, Seoul, Korea) (Figure 1B) was inserted and securely sutured with absorbable sutures.

Administration of immunosuppressive drugs

The intraperitoneal injection was utilized as the route of administration for immunosuppressive drugs in this study. The administered doses were as follows: tacrolimus at a dosage of 0.5 mg/kg, and dexamethasone at a dosage of 0.6 mg/kg Initiation of drug administration commenced on the day following the insertion of the implant. The drug was administrated for 15 days after surgery.

Histology

All the rats were sacrificed 3 months after surgery. (Figure 1A) The capsule and pericapsular tissues were harvested by En-bloc resection of the capsule and implant. (Figure 1C) The hematoxylin and eosin-stained sections were analyzed for capsule thickness. Immunohistochemistry evaluation was performed using the monoclonal antibody of α-smooth muscle actin (α-SMA, α-SMA monoclonal antibody, 1:100, 1A4 (asm-1), Thermoscientific, USA), Transforming growth factor beta (TGF-β, Anti-TGF beta 1 Antibody, 1:100, sc-130348, , Santa Cruz Biotechnology (SCBT), USA), CD 3 (Anti-CD3 Monoclonal Antibody, 1:100, ab16669, Abcam, Cambridge, UK), CD 68 (Anti-CD68 Monoclonal Antibody, 1:100, sc-20060, , Santa Cruz Biotechnology (SCBT), USA), and Toll-like Receptor 4 (TLR4, Anti-TLR4 Monoclonal Antibody, 1:100, ab20048, Abcam, Cambridge, UK). All tissues were randomly

selected at 3 points by our researcher. The analysis was performed by the first author. (H.B. Kim)

1) Capsule thickness

The hematoxylin and eosin-stained sections were analyzed for capsule thickness. Capsule thickness was measured by averaging the values at the center of three separate images (×100 magnification).

2) Immunohistochemistry

The immunohistochemistry for myofibroblasts was determined via alpha-smooth muscle actin staining (α-SMA) (×400 magnification). The immunohistochemistry for Inflammation was determined via TGF-β and TLR4 (x400 magnification). The analysis of the α-SMA, TGF-β, and TLR4 was the evaluation of the percentage of the stained area (% area). The segmentation and measurement of the % area were performed using ImageJ (ImageJ 1.53e, National Institute of Health, USA). The detailed evaluation for T-lymphocytes and histiocytes was determined via CD3 and CD68. The counts of hot spots of CD3 and CD68 positive cells were counted under x400 magnification.

Statistical Analysis

Statistical analysis was performed using the SPSS software (SPSS, Inc., Chicago, Version 21). Normality test was performed using the Shapiro-Wilk Test and Kolmogorov-Smirnov Test. The continuous data were compared with the Student t-test. Statistical significance was set at a p-value of <0.05.

Results

All the rats survived after irradiation, and no postoperative complications from the implant placement and administration of the drugs were observed.

1) Capsule thickness

Through hematoxylin and eosin staining, the capsule thickness was measured (x100 magnification) (Figure 2). The mean±SD capsule thickness was 496.4±191.3 μm in the group 1, 256.4±73.4 μm in the group 2, and 295.4±105.7 μm in the group 3. Significant differences were observed between group 1 and group 2 ($p<0.001$), and group 1 and group 3 ($p<0.001$). (Figure 3)

2) Immunohistochemistry of α-SMA

The mean±SD % area of the a-SMA stained area according to the tissues was 35.4% ±6.3 in the group 1, 19.5%±4.9 in the group 2, and 20.9%±7.3 in the group 3 tissue. Significant differences were observed between Group 1 and Group 2 ($p<0.001$), and Group 1 and Group 3 ($p<0.001$). (Figure 4)

3) Immunohistochemistry of TGF-β and TLR4

The mean±SD % area of the TGF-β stained area according to the tissues was 45.0% ±12.0 in the group 1, 24.8%±8.9 in the group 2, and 24.7%±7.4 in the group 3 tissue. Significant differences were observed between Group 1 and Group 2 ($p<0.001$), and Group 1 and Group 3 ($p<0.001$). (Figure 5A). The mean±SD % area of the TLR4 stained area according to the tissues was 24.3% ±11.7 in the group 1, 15.9%±5.9 in the group 2, and 14.8%±7.6 in the group 3 tissue. Significant differences were observed between Group 1 and Group 2 ($p=0.012$), and Group 1 and Group 3 ($p=0.007$). (Figure 5B)

4) Immunohistochemistry of CD3+ T lymphocyte and CD 68+ histiocyte

The hot spots of the CD3 and CD68 positive cells were measured. The CD3+ T lymphocytes were mainly observed around the loose connective tissue of the capsule and The CD68+ histiocytes were mainly observed around the

surface of the capsule. (Figure 6) The mean±SD count of CD 3 positive cells was 37.9±32.4 in the group 1, 10.5±6.1 in the group 2, and 16.2±11.1 in the group 3. Significant differences were observed between Group 1 and Group 2 (p=0.001), and Group 1 and Group 3 (p=0.014). (Figure 5C) The mean±SD count of CD68 positive cells was 23.0±17.2 in the group 1, 12.2±4.8 in the group 2, and 6.8±4.4 in the group 3. Significant differences were observed between group 1 and group 2 (p=0.02), group 1 and group 2 (p=0.001), and group 1 and group 3 (p<0.001). (Figure 5D) (Figure 7)

Discussion

This study described a rat model of the clinically relevant silicone implant-based breast reconstruction with various types of systemic immunosuppression with dexamethasone and tacrolimus. Considering the theory of capsule formation is based on inflammatory reaction, immunosuppression theoretically has a preventive effect on capsule formation, in addition, to potentially reducing capsular contracture. However, few structural studies evaluated and compared the effect of various types of systemic immunosuppression. This study was designed to evaluate the effect of various types of systemic immunosuppression. Therefore, we could get a better understanding of the etiology of capsule formation in silicone implants

and find another potential drug for preventing capsular contracture.

There have been several studies that reported the effect of systemic immunosuppression on capsular contracture. Ozlem et al examined the effect of systemic administration of Dexamethasone in a silicone implant rat model. They concluded that systemic administration of Dexamethasone resulted in decreased TLR4 expression and myofibroblast differentiation. (12) The angiotensin-converting-enzyme inhibitor Enalapril was reported to decrease the expression of fibrotic mediators, inflammatory markers, monoclonal antibodies (CD68), and the periprosthetic fibrosis process. (13) However, the use of Tacrolimus in breast implant surgery has not been introduced.

Tacrolimus is an immunosuppressive drug used for prophylaxis of organ rejection after transplantation. The main mechanism of action is that tacrolimus works by inhibiting the activity of an enzyme called calcineurin, which is responsible for activating certain immune cells, particularly T lymphocytes. (14) Tacrolimus has been used in various situations in foreign body insertion, including Tacrolimus releasing vascular stent and Tacrolimus coated sutures. (15-17) These uses aim to reduce the inflammatory reaction, and intimal proliferation, and finally increase the use of the foreign body. The results of our study could imply the potential effect of Tacrolimus to reduce the capsule formation of the breast implant and prevent capsular contracture. According to our results of CD3, Tacrolimus could reduce the

activity of T-lymphocytes in the capsule, finally reducing the capsule formation in a rat model.

Recently, Drug delivery using 3D printing is a rapidly growing approach in pharmaceutical manufacturing. (18, 19) 3D printing, a promising approach for pharmaceutical manufacturing, has gained considerable attention due to the advantages it provides over traditional pharmaceutical processes. 3D printing has been harnessed to develop local drug delivery systems for various clinical areas. (20) Although our study is conducted in the systemic administration of immunosuppressive drugs, this study could be fundamental to the future local drug release study for preventing capsular contracture. Local delivery of the immunosuppression may have a higher effect in local area, and minimize the side effects of systemic immunosuppression.

However, this study has some limitations. Considering the duration of our study is 3 months after surgery, which is a relatively short period, capsular contracture could occur any time after the insertion of the implants. This study was conducted with a single dose of the drugs, so further studies are needed to find out the optimal dose to suppress the capsule formation. The small sample size of 18 rats is also a limitation. The placement of the implant away from the breast tissue should also be considered. However, given the anatomical position of the rat, the placement of the implant under the chest area is difficult due to the mechanical stress and small

volume. Furthermore, clinical studies are needed to prove the preventive effect of the capsular contracture of these immunosuppressants. Finally, this study evaluated the capsule formation and immunohistochemistry of myofibroblasts and inflammatory factors, not directly the capsular contracture.

Conclusion

In this study, systemic use of dexamethasone and tacrolimus successfully reduces capsule formation and inflammation around the implant. T lymphocytes were successfully suppressed by both dexamethasone and tacrolimus. Tacrolimus could be considered a potential preventive drug for capsular contracture in breast implant surgeries. Further studies for evaluating the effect of Tacrolimus are required through human study and local drug delivery.

Figure Legends

Figure 1. The implant was inserted over the Latissimus dorsimusle of the dorsum of the rat (A). The 1.5 × 1.5 cm implant smooth silicone implant (B). The capsule and pericapsular tissues were harvested by En-bloc resection of the capsule and implant (C)

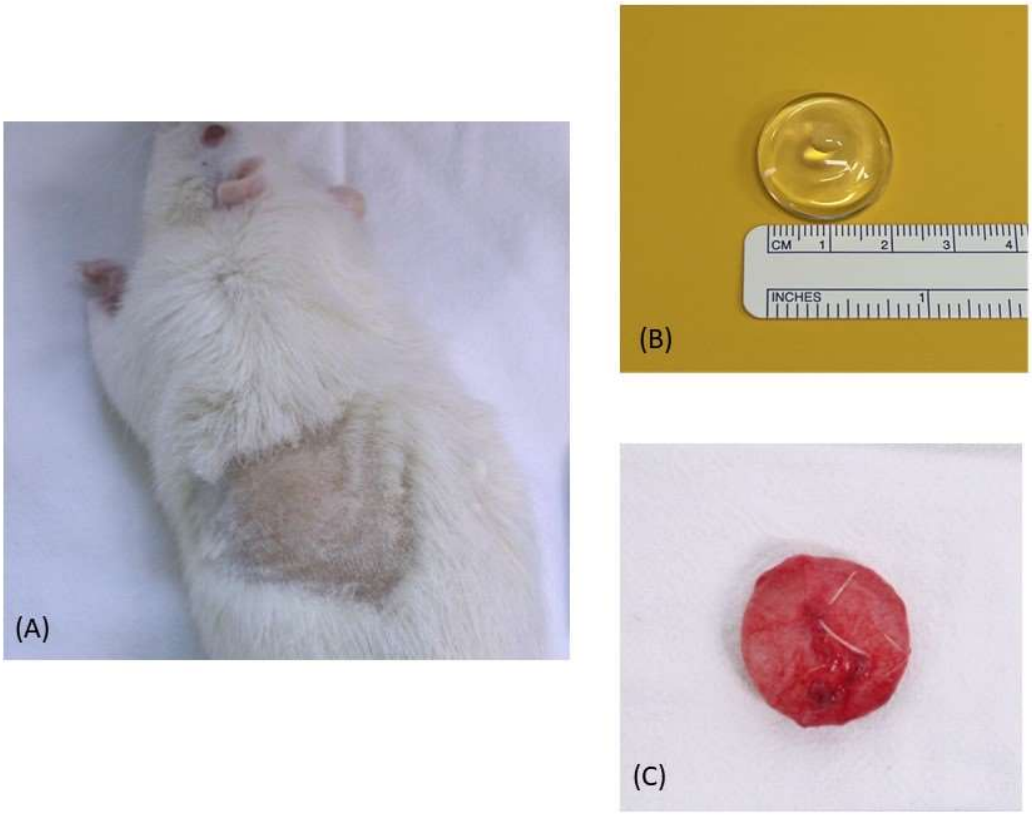


Figure 2. The examples of H&E staining of the capsule tissue.

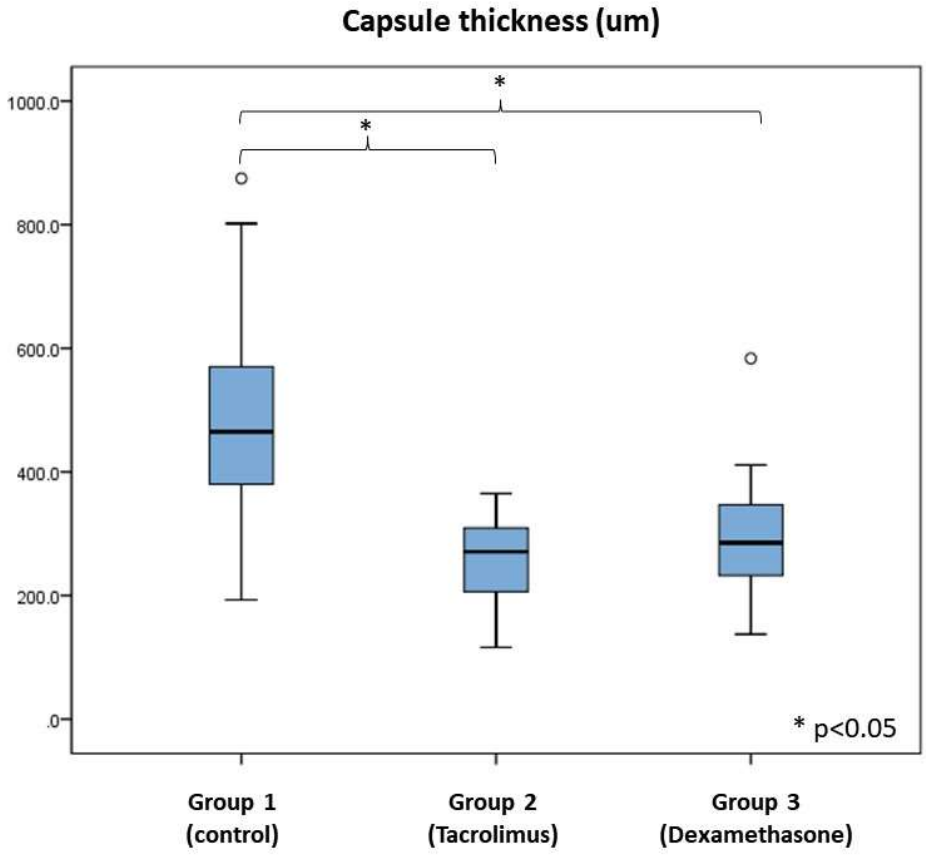


Figure 3. The results of the capsule thickness.

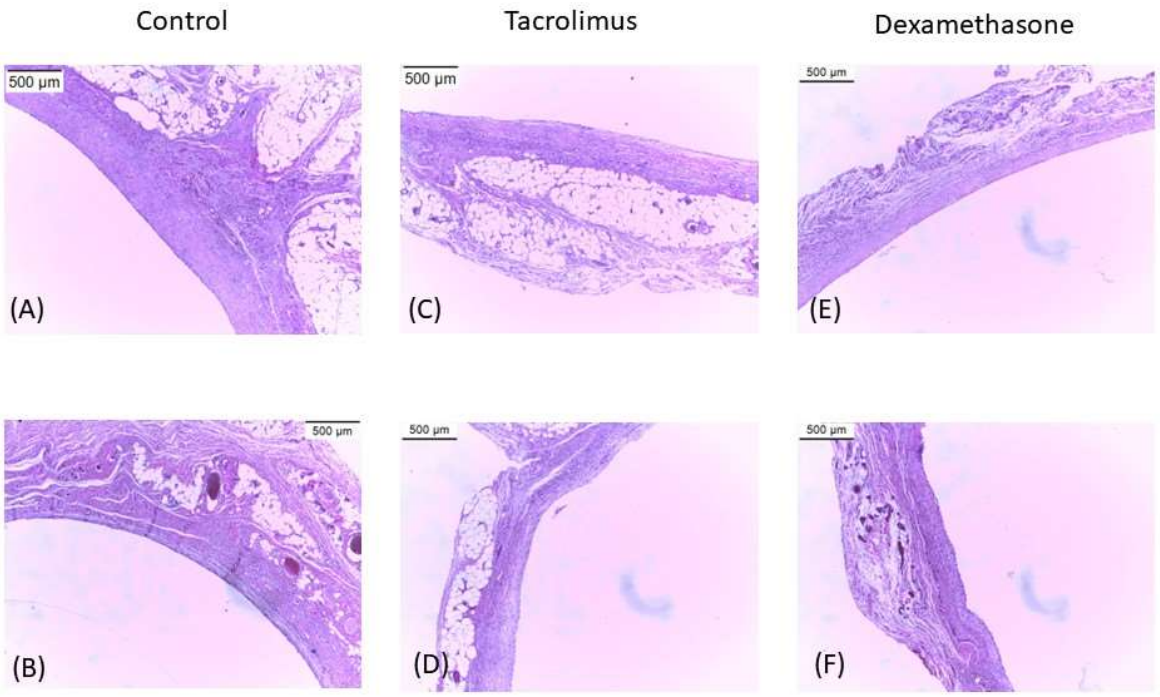


Figure 4. The immunohistochemistry results of the activity of the myofibroblasts (Alpha-SMA)

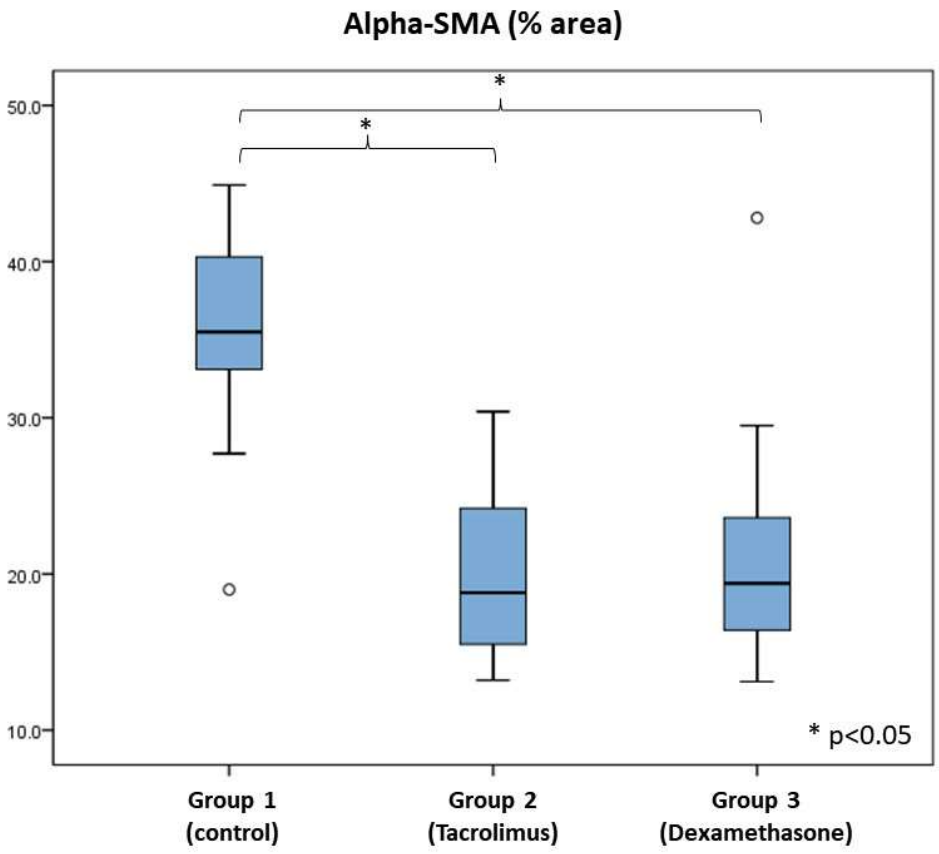


Figure 5. The immunohistochemistry results of the TGF-β, TLR4, CD3, and CD68

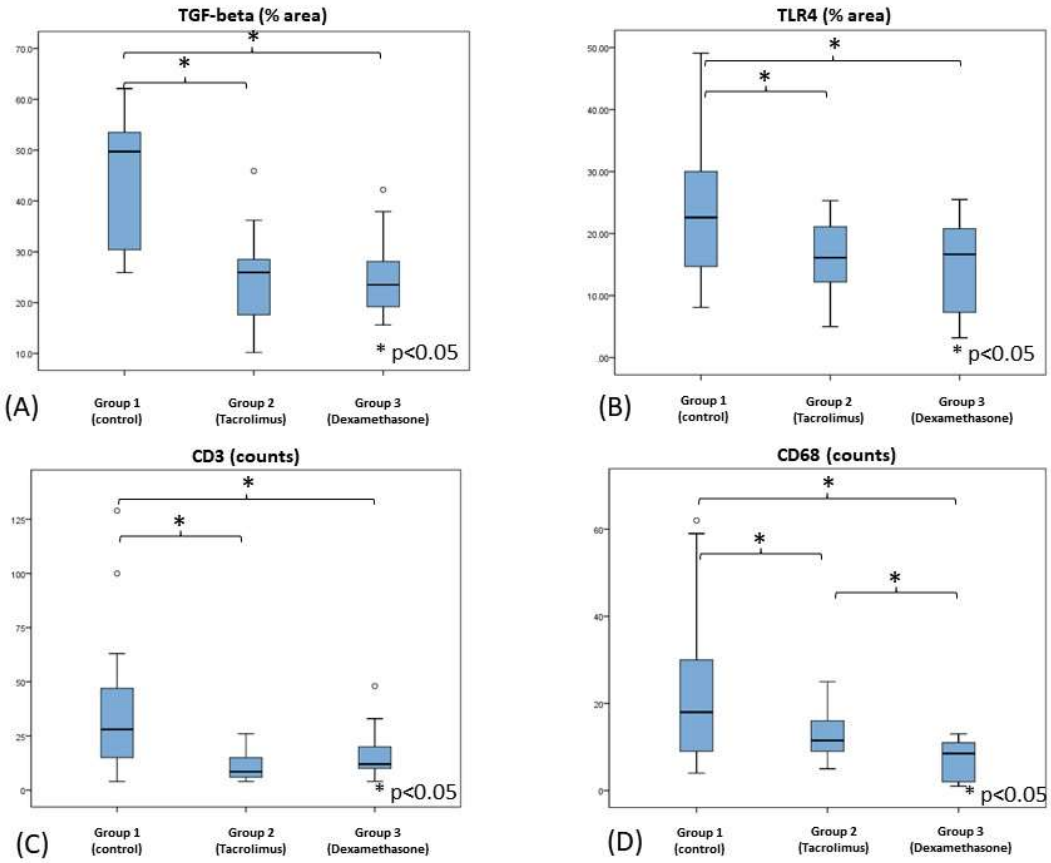


Figure 6. The counting of CD68 (A) and CD3 (B) positive cells.

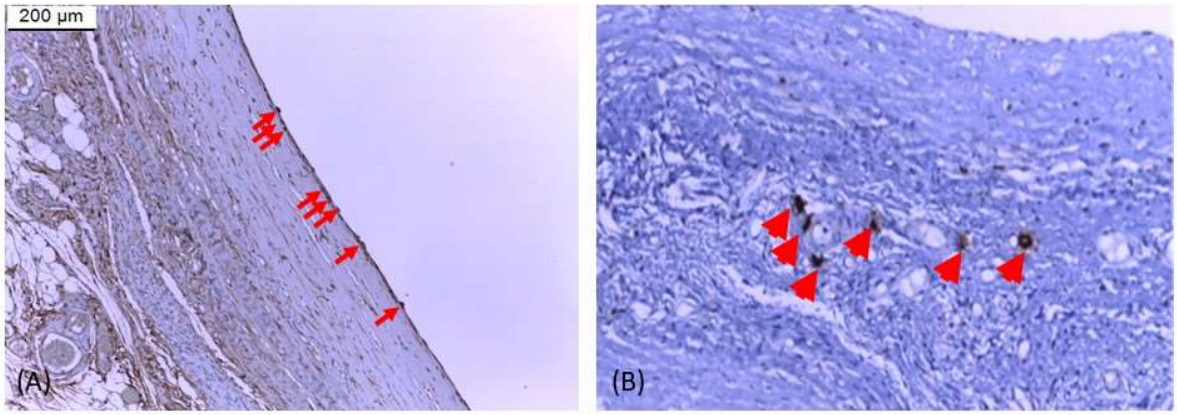
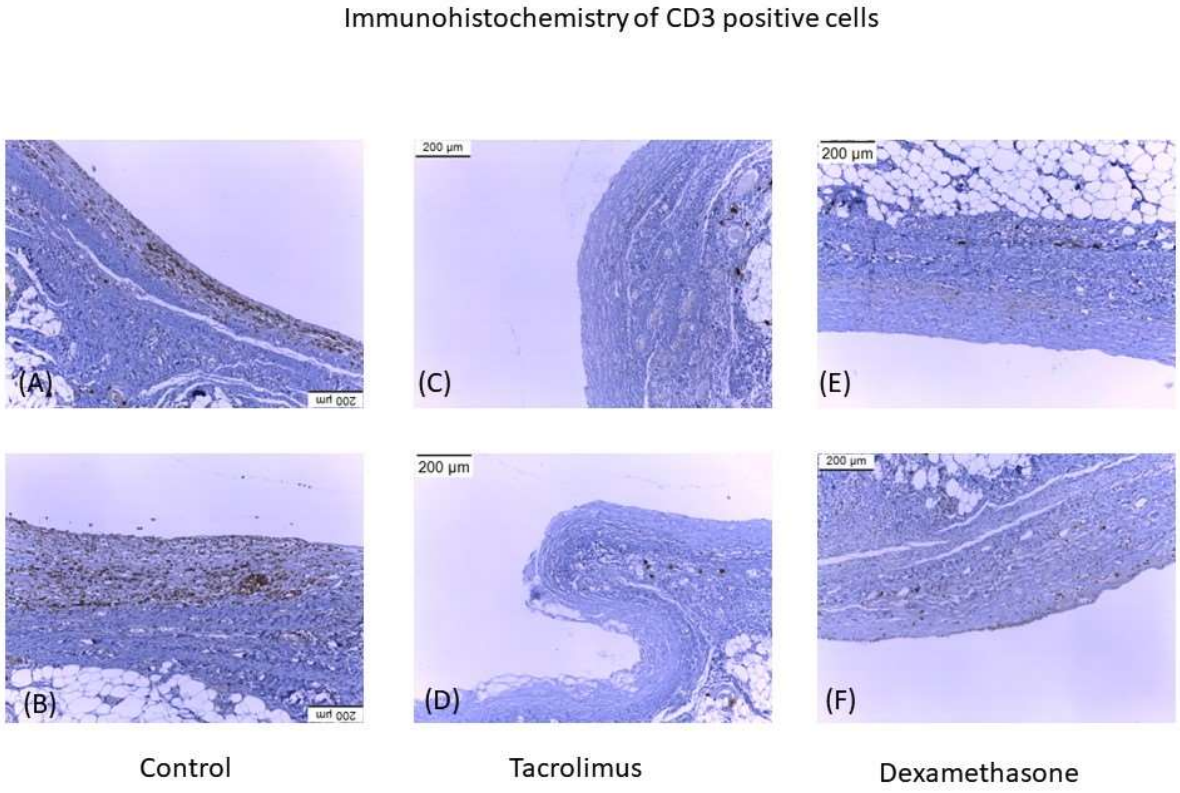


Figure 7. The examples of CD3 positive T lymphocytes in each group.



18

References

1. Pirjavec Mahić, A., Grebić, D., Čargonja, P., Kustić, D. Silicone Gel Breast Implants: Past, Present, and Future. *Acta medico-historica adriatica : AMHA* 2020;18:165-176.

2. Coroneos, C. J., Selber, J. C., Offodile, A. C., 2nd, Butler, C. E., Clemens, M. W. US FDA Breast Implant Postapproval Studies: Long-term Outcomes in 99,993 Patients. *Annals of surgery* 2019;269:30-36.

3. Bachour, Y. Capsular Contracture in Breast Implant Surgery: Where Are We Now and Where Are We Going? *Aesthetic plastic surgery* 2021;45:1328-1337.

4. Haran, O., Bracha, G., Tiosano, A., et al. Postirradiation Capsular Contracture in Implant-Based Breast Reconstruction: Management and Outcome. *Plastic and reconstructive surgery* 2021;147:11-19.

5. Araco, A., Gravante, G., Araco, F., Delogu, D., Cervelli, V. Capsular contracture: results of 3002 patients with aesthetic breast augmentation. *Plastic and reconstructive surgery* 2006;118:1499-1500.

6. Safran, T., Nepon, H., Chu, C. K., et al. Current Concepts in Capsular Contracture: Pathophysiology, Prevention, and Management. *Seminars in plastic surgery* 2021;35:189-197.

7. Wolfram, D., Rainer, C., Niederegger, H., Piza, H., Wick, G. Cellular and molecular composition of fibrous capsules formed around

silicone breast implants with special focus on local immune reactions. *Journal of autoimmunity* 2004;23:81-91.

8. Bachour, Y., Poort, L., Verweij, S. P., et al. PCR Characterization of Microbiota on Contracted and Non-Contracted Breast Capsules. *Aesthetic plastic surgery* 2019;43:918-926.

9. Walker, J. N., Pinkner, C. L., Pinkner, J. S., Hultgren, S. J., Myckatyn, T. M. The Detection of Bacteria and Matrix Proteins on Clinically Benign and Pathologic Implants. *Plastic and reconstructive surgery Global open* 2019;7:e2037.

10. Kim, H. B., Han, H. H., Eom, J. S. Difference in the Occurrence of Capsular Contracture According to Tissue Characteristics in an Irradiated Rat Model. *Plast Reconstr Surg* 2023;152:655e-661e.

11. Kim, H. B., Han, S. Y., Eom, J. S., Han, H. H. Human-Mimic Submuscular and Premuscular Irradiated Rat Model: Histologic Characteristics of the Capsule Tissue in Contact with the Breast Implant. *Breast J* 2023;2023:4363272.

12. Colak, O., Ozer, K., Dikmen, A., Ozakinci, H., Ozkaya, O. Evaluation of Safe Systemic Immunosuppression Created with Dexamethasone in Prevention of Capsular Contracture: A Glance to Distinct Perspectives with Toll-Like Receptors. *Aesthetic plastic surgery* 2018;42:1133-1143.

13. Zimman, O. A., Toblli, J., Stella, I., Ferder, M., Ferder, L.,

Inserra, F. The effects of angiotensin-converting-enzyme inhibitors on the fibrous envelope around mammary implants. *Plastic and reconstructive surgery* 2007;120:2025-2033.

14. Ho, S., Clipstone, N., Timmermann, L., et al. The mechanism of action of cyclosporin A and FK506. *Clin Immunol Immunopathol* 1996;80:S40-45.

15. Scheller, B., Grandt, A., Wnendt, S., Lorenz, G., Böhm, M., Nickenig, G. Comparative study of tacrolimus and paclitaxel stent coating in the porcine coronary model. *Z Kardiol* 2005;94:445-452.

16. Siller-Matula, J. M., Tentzeris, I., Vogel, B., et al. Tacrolimus-eluting carbon-coated stents versus sirolimus-eluting stents for prevention of symptom-driven clinical endpoints. *Clin Res Cardiol* 2010;99:645-650.

17. Ak, K., Ak, E., Dericioglu, O., et al. Tacrolimus-Eluting Suture Inhibits Neointimal Hyperplasia: An Experimental In Vivo Study in Rats. *Eur J Vasc Endovasc Surg* 2017;53:431-437.

18. Kotta, S., Nair, A., Alsabeelah, N. 3D Printing Technology in Drug Delivery: Recent Progress and Application. *Curr Pharm Des* 2018;24:5039-5048.

19. Hwang, S. H., Kim, J., Heo, C., et al. 3D printed multi-growth factor delivery patches fabricated using dual-crosslinked decellularized extracellular matrix-based hybrid inks to promote cerebral angiogenesis. *Acta Biomater* 2023;157:137-148.

20. Yi, H. G., Choi, Y. J., Kang, K. S., et al. A 3D-printed local drug delivery patch for pancreatic cancer growth suppression. *J Control Release* 2016;238:231-241.

국 문 요 약

배경: 본 연구에서는 실리콘 보형물을 이용한 동물 모델에서 전신 면역억제제에 따라 피막 형성이 달라진다는 가설을 세웠다.

재료 및 방법: 이 연구는 근육 위에 삽입한 실리콘 보형물 삽입술을 받은 18 마리의 SD rat 으로 실험을 디자인 하였다. 그들은 치료되지 않은 대조군인 그룹 1(n = 6), Tacrolimus 을 투여 받은 그룹 2 (n = 6), Dexamethasone 을 투여 받은 그룹 3 (n = 6)의 네 그룹으로 나뉘었다. 수술 후 15 일 간 투약하였고, 수술 3 개월 후 조직학 및 면역화학 염색을 한 피막 조직을 분석하였다.

결과: Tacrolimus 와 Dexamethasone 의 전신 투여는 피막 두께와 염증을 성공적으로 감소시켰다. 덱사메타손과 타크로리무스는 피막 주변의 CD3 (T 림프구), CD68 (조직구) 양성 세포의 응집을 감소 시킴을 확인하였다.

결론: 본 연구에서 Tacrolimus 과 Dexamethasone 의 전신 사용은 임플란트 주변의 피막 형성과 염증을 성공적으로 감소시킴을 확인하였다. Tacolimus 는 유방 보형물 수술 시 피막 구축에 대한 잠재적인 예방 효과를 가질 수 있는 후보 약물로써 그 가능성을 입증하였다. Tacrolimus 효과를 평가하기 위한 추가 연구, 인간 연구 및 국소 약물 전달 연구 등을 통해 추가적 효과 입증에 대한 연구가 필요하다.