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The Effect of Mesenchymal Stem Cells on Capsule Formation around Silicone Implant in Rats

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ABSTRACT

Objective: Capsular contracture is a common adverse effect of breast augmentation and reconstruction surgeries. In the present study we evaluate the effect of adipose tissue-derived mesenchymal stem cells on the capsular structure following breast implant surgery.

Materials and Methods: Included in the study were 16 female Wistar-Albino rats, who were divided randomly into two groups. Silicone implants were placed subcutaneously in the pectoral region of the animals in the sham group, while 1 cc of approximately 40 million adipose tissue-derived stem cells were injected into the suture gap after the placement of silicone implants in the experimental group. All experimental animals were re-operated under the same conditions at postoperative week 12 and the implants were removed. All operations were performed by the same surgeon. The capsular structure that had formed around the implants was examined volumetrically and immunohistochemically.

Results: Immunohistochemically, the presence of a thick fibrous capsule and prominent procollagen in the periphery was detected in the control group, while capsular staining was noted in the experimental group, wherein thin collagen fibers were organized with the appearance of loose connective tissue, while procollagen staining was lighter and scattered. A volumetric comparison revealed the mean capsule volume to be statistically significantly lower in the experimental group than in the control group.

Conclusion: The administration of adipose tissue-derived stem cells resulted in the development of a soft capsule and the formation of new vessels around the implant, and so is likely to have effects that may reduce the development of capsular contracture following breast silicone implant surgery.

Keywords: Adipose tissue, Breast implant, Capsular contracture, Mesenchymal stem cell.

INTRODUCTION

Silicone prostheses are widely used today for esthetic purposes. Following the application of various surgical approaches, silicone, which was developed to meet a need of the aircraft manufacturers during World War II, entered into use in breast surgery in the 1960s.[1] Since then, breast silicone implant surgery has become an important area of cosmetic surgery, both for reconstruction after breast cancer surgery and for esthetic reasons.[2] There have been reports linking silicone implants with lung cancer, rheumatoid arthritis, Sjögren's syndrome and Raynaud's syndrome, although current findings regarding the serious long-term health effects of silicone implants remain controversial.[3,4] In the short term, ruptures of saline implants and capsular contractures in silicone implants are significant adverse effects.[3,5] According to the post-approval studies database of the US Food and Drug Administration (FDA), capsular contracture occurs in 7.2% of primary breast augmentation surgeries and 12.7% of primary reconstructions, and it is the most common reason for reoperation in breast augmentation surgery.[3,6] Various studies to date have reported an incidence rate of capsular contracture ranging from 2.8–18.9%,[7-9] with a heterogeneity between studies that may be due to the differences in follow-up times, surgical techniques and the type of implants used. Nevertheless, capsular contracture is currently considered as a significant problem with a negative effect on long-term patient satisfaction in breast implant surgery.[6]

Capsular contracture is an excessive fibrotic foreign body reaction that occurs after implantation.[10] Today, various surgical techniques and methods, medications and solutions, and implants with a textured surface are used in an attempt to minimize the risk of capsular contracture development.[11] These prevention/treatment approaches have also been

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investigated in animal experiments with different results in terms of their effect on capsular contracture formation.[12-14]

Kim et al.[14] examined the effect of endothelial precursor cells derived from human embryonic stem cells on capsule development around silicone implants in an experimental rat model, and reported a significant decrease in the thickness of the peri-implant capsules in the experimental group when compared to the control group, while less inflammatory reaction was noted and the formation of more new vessels were observed in the experimental group in the early postoperative period. The authors concluded that tissue hypoxia around the implant might be a risk for capsular contracture, while the formation of new vessels may serve as a preventive method by reducing ischemia in this area.[14] Similarly, adipose tissue-derived stem cells are believed to prevent excessive collagen production during the reorganization phase of wound healing by ensuring the formation of new vessels and changing the behavior of fibroblasts in the area of application.[15,16]

MATERIALS AND METHODS

Ethical Considerations

The study was carried out after gaining the approval of the Animal Experiments Local Ethics Committee of Istanbul University (Ethics Committee Approval No: 198 and Date: 01.12.2010). The rights of the animals used as subjects in the study were protected in accordance with the principles of the Guide for the Care and Use of Laboratory Animals (www.nap.edu/catalog/5140.html), and the study was conducted in line with the principles of the Declaration of Helsinki.

Study Design and Place

This one-way, parallel experimental design study was carried out in the physical facilities of the Institute of Experimental Medicine of Istanbul University (DETAE), the Department of Histology and Embryology of the Istanbul University Faculty of Medicine, and the Department of Histology and Embryology of Yeditepe University between 01/12/2010 and 01/06/2011.

The subjects used in the study were divided into two groups in accordance with the purpose of the study. The first group was accepted as the experimental group, and received adipose tissue-derived mesenchymal stem cells; while the second group was accepted as the control group, and received no stem cells. The 16 animals used in the study were assigned equally to the two groups randomly using a computer-based random number generator.

Experimental Animals

The 16 adult female Wistar-Albino rats used in the study weighed 270–310 g (average 295 g). The experimental animals were supplied and fed by DETAE, and fed with standard feed. No subjects were sacrificed during the study.

After the experimental procedures, the animals were kept in a conventional cage system in a 12/12 h light/dark cycle at 21°C and 45% humidity for 12 weeks, with food provided ad libitum. No unexpected surgical events related to the surgery were observed in the subjects during follow-up.

Stem Cell Preparation

Adipose tissues (of 1 mg standard weight) were taken from the right inguinal region of each rat in Group II and sent for stem cell production under sterile conditions. The tissues were minced with a scalpel blade in a sterile petri dish, placed into falcon tubes containing 1% collagenase in CMF-PBS (Calcium Magnesium Free-Phosphate Buffered Saline) and shaken in a 37°C water bath for 1 hour. After filtering through a 40 μm nylon mesh (Falcon Cell Strainer, BD Biosciences, USA), the filtrate was centrifuged at 300 g for 5 minutes. The supernatant was discarded and the pellet was suspended in DMEM-F12 + 10% FCS (Fetal Calf Serum) medium. The cells were inoculated into cell culture flasks, with 2 million cells in each flask. After the cells cultured in incubators containing an air mixture with 5% CO were observed to attach to the bottom, the medium was replaced every three days and the culture was maintained until the bottom of the flasks became 3/2 confluent. Cells were harvested by treating the confluent flasks with a 0.5% trypsin solution containing EDTA (Ethylene Diamine Tetra Acetic acid). The number of collected cells was determined in the counting chamber (hemocytometer), injectors with 40 million cells/ml were prepared and stored at 37°C in the incubator until needed.

Experimental Procedures

All surgical procedures were performed in the DETAE operating room under sterile conditions and by the same researcher. The subjects were anesthetized using xylazine hydrochloride (Rompun, Bayer, Turkey, 5 mg/kg IM) and ketamine hydrochloride (35 mg/kg IM). The pectoral hair of the subjects was shaved, and the site was cleaned using 10% polyvinylpyrrolidone-iodine solution (Polyod, Drogsan, Turkey) and covered with sterile drapes. Antibiotic (Gent bulb, IR, Turkey, 3 mg/kg IM) was administered at preoperative 15 minutes and on postoperative day 1.

A horizontal 1.5 cm incision was made to the pectoral regions of the subjects, and a 1.5 x 2 cm subcutaneous pocket was created by dissection. The subjects were implanted with custom-made implants filled with liquid silicone (Nagor, GC Aesthetics, UK). The first group received no additional procedure, while 1 cc of approximately 40 million adipose tissue-derived stem cells were injected into the suture gap after the placement of the silicone implants in the second group.

All experimental animals were re-operated under the same conditions at postoperative week 12 and the implants

were removed. In the pectoral regions of the subjects, the placed implants were located by palpation, the subcutaneous tissue was reached via a 1.5 cm horizontal incision, and the implants were removed carefully, taking care not to damage the capsule around the implant. All of the removed tissues were fixed in a 10% formaldehyde solution for 24 hours. The preparations were cut transversely in 3 mm thicknesses with the capsule and implant, and were subjected to routine tissue tracking.

Morphometric and Volumetric Assessment

The capsule was sampled using physical dissector with 5 mm spaces. Each capsule structure was fixed with 10% neutral formaldehyde prepared in a 0.1 M phosphate buffer using the immersion technique and subjected to routine histological processing, and four–five tissue blocks were obtained from each subject. The blocks were sampled at a ratio of 1/2 and serial sections were taken at 10 µm thicknesses. The first section was selected according to the systematic random sampling method and sampled at a ratio of 1/50. The sampled sections were stained according to the Masson Trichrome technique.

The stained sections were examined at a stereology workstation – being a system that includes a CCD digital camera (Optronics Microfire 1600x1200P, Goleta, CA, USA), a graphics card (ATI FireGL Advance Micro Device, Camberly, UK), a computer-controlled motorized stage, (Bioprecision, Howtrone, NY, USA), on which the analysis is carried out and the images are examined, a Microcator (Heidenhein, Traunreut, Germany) and a light microscope (Leica DM 4000B, Wetzlar, Germany). Measurements were made using Sterioinvestigator 7.0.5 (Microbrightfield, Williston, VT, USA) software, while cell counts were carried out performed using a Leica C plan 10X objective.

Volumetric measurements were conducted using the Cavalieri method, and with a dotted counting scale with 200 µm distance between two dots.

Immunohistochemical Assessment

Immunohistochemical staining was applied using the Streptavidin-Biotin-peroxidase method to demonstrate the location of procollagen I fibers based on labeled polyclonal antibodies.

The 2 µm-thick sections prepared from routinely formalin-paraffin-processed blocks were placed on positively charged electrostatic slides and kept in the oven at 56°C overnight, then recovered from paraffin through immersion in Xylol for 30 minutes, in acetone for 5 minutes and in ethyl alcohol for 15 minutes, respectively and then immersed in distilled water. The slides were placed in a microwave oven in citrate buffer (pH: 6) for antigen conditioning, and then irradiated in the oven three times for five minutes each. After irradiation, the slides were allowed to cool at room temperature for 20 minutes and then immersed in distilled water. The tissues were circumscribed with a hydrophobic pen and taken back into the distilled water. Afterwards, protease (Dako Proteinase K RTU Code No: S3020) was applied to the tissues for five minutes at room temperature, and the slides were returned to distilled water and then immersed in phosphate-buffered saline (PBS) (pH: 7.6) to initiate the immunohistochemistry phase.

A peroxidase block (Peroxidase block Immunocruz Staining System, Santa Cruz Biotechnology, USA) was then dropped to suppress the endogenous peroxidase activity for 15 minutes, and the slides were then returned to the distilled water. Immediately afterwards, a serum block (ImmunoCruz Staining System, sc-2053, Santa Cruz Biotechnology, USA) was dropped and kept for 20 minutes. After 20 minutes, the serum block was aspirated from the slides and antibody (Procollagen Type I (A-17) sc-25973, Goat polyclonal Ig G, Santa Cruz Biotechnology, USA) was dropped and left for 90 minutes. Afterwards, the slides were washed with PBS for 5 minutes, and biotin-bound antibodies (Immunocruz Staining System Donkey Anti-Goat IgG, Santa Cruz Biotechnology, USA) were dropped onto the slides and kept for 30 minutes. The slides were taken back into PBS and washed. At this stage, HRP-Streptavidin (Santa Cruz Biotechnology, USA) was dropped onto the slides, kept for 30 minutes, and the slides were taken back into the PBS. Subsequently, 5 drops of the substrate buffer included in the kit, one drop of DAB chromogen and one drop of peroxidase substrate were added to 1.6 ml distilled water. The slides were taken into distilled water, and then into Mayer's hematoxylin for counterstaining, and kept there for 10 seconds. After rewashing with distilled water, the slides were coverslipped with a water-based coverslipping liquid (Aqueous-Mount AML060, ScyTek Laboratories Inc., USA).

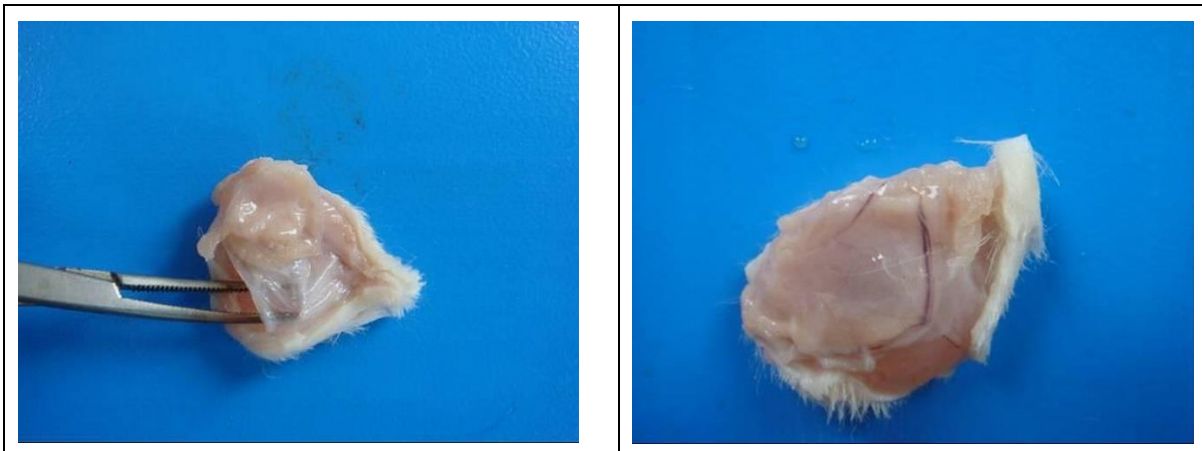
Statistical Analysis

The capsule volumes of the groups were presented as mean and standard error values. A Mann-Whitney U-test was used for the between-group volumetric comparisons. A value of $p<0.05$ was accepted as the statistical significance limit.

RESULTS

Following the placement of the silicone implants, at week 12 – the initial time of standard capsule formation – the implants were easily palpable and mobilized on a limited basis in the control group, while the implants in the experimental group were more difficult to palpate and were more mobilized. A macroscopic assessment after the removal of the implants revealed a clearly visible fascia-like capsule structure around the implants in the control group, while there was a smooth capsule structure with a visible vessel formation around the implants in the experimental group (Figure 1).

Figure 1.

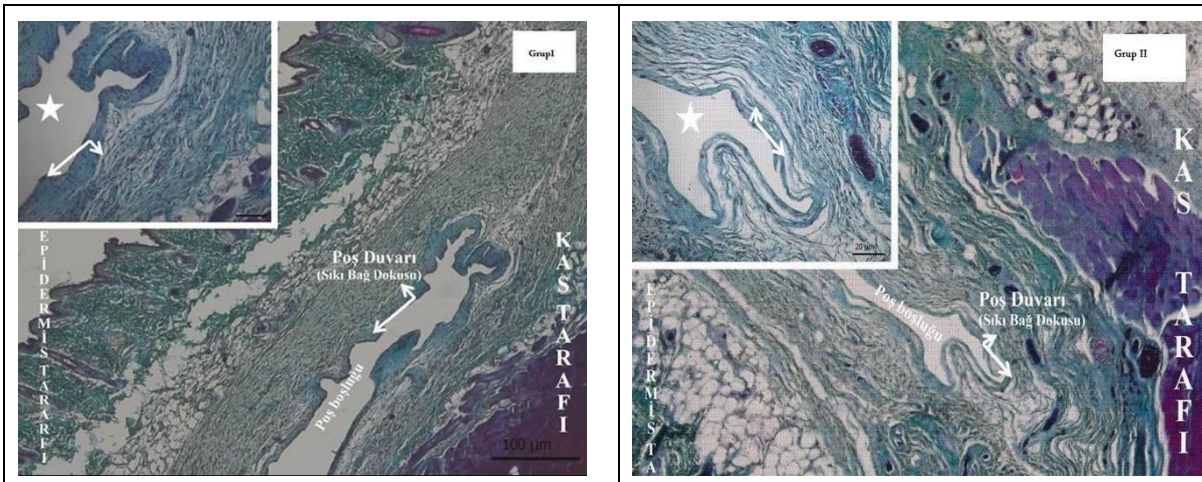


a **b**

Macroscopic view of capsule. (a) Fascia-like capsule structure around an implant in the control group, (b) A smooth capsule structure around an implant in the experimental group.

A light microscope was used to examine the vessel formation and capsule structure in the preparations prepared from the implants removed from the subjects. The capsule structure was found to be rich in dense connective tissue in the control group. A light microscopic examination of the capsule structure of the implants in the experimental group showed that the capsule structure was mucous, with many vascular structures. Masson's trichrome staining revealed the capsule structure to be organized and thicker in the control group, while the capsule structure was thin and irregular in the experimental group in comparison (Figure 2).

Figure 2.



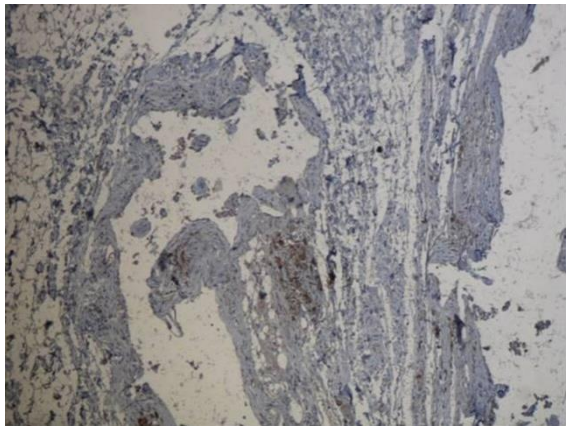
a **b**

(a) Control group and (b) Experimental group capsule samples, stained using Masson's trichrome technique. The larger image shows the muscle side, epidermis side and pouch. The white arrow indicates the capsule wall (dense connective tissue) and the white star indicates the inner pouch.

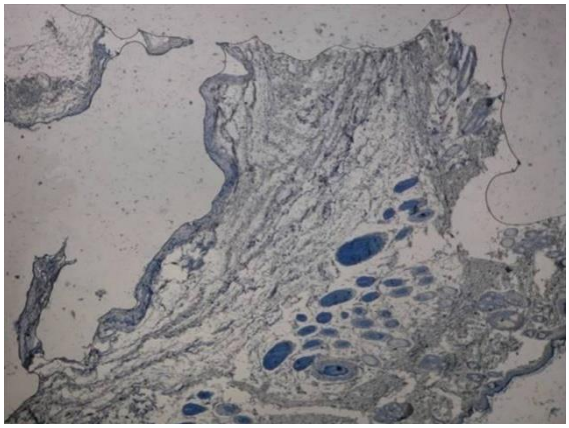
An immunohistochemical examination of the capsules revealed a thick fibrous capsule formed by dense collagen bundles on the pouch wall in the control group, as well as prominent procollagen in the periphery, which was brown in

color due to the immunohistochemical staining with diaminobenzidine. The capsule on the pouch wall in the experimental group, in turn, was thin, the collagen fibers just outside were organized in the appearance of loose connective tissue, and the procollagen staining was lighter and scattered (Figure 3).

Figure 3.



a

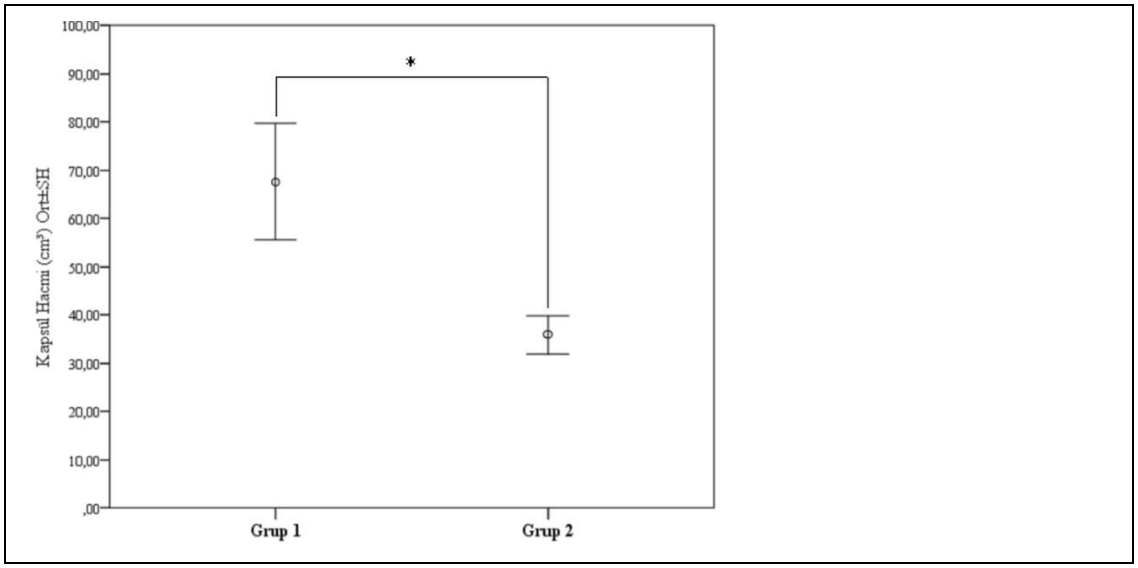


b

Immunohistochemical examination of capsules. (a) In the control group, a thick fibrous capsule was formed by dense collagen bundles on the pouch wall, and prominent procollagen in the periphery, which is brown in color due to the immunohistochemical staining with diaminobenzidine. (b) In the experimental group, the capsule on the pouch wall is thin and the collagen fibers just outside are organized in such a way that they have an appearance of loose connective tissue. Procollagen staining is observed as lighter and scattered.

A volumetric assessment revealed the mean capsule volume to be 67.58 cm^3 in the control group, and 35.88 cm^3 in the experimental group. This difference was statistically significant (p=0.032) (Table 1 and Figure 4).

Figure 4.



*Between-group comparison of capsule volumes

p<0.05

Table 1. Capsule volume comparison between groups

	Group 1 (Control group)		Group 2 (Study Group)		*p, U**
	Mean	**Standard Error**	**Mean**	**Standard Error**	
Capsule volume (cm^3)	67.58	12.12	35.88	3.89	0.032

U, Mann-Whitney U-test was used; *p<0.05

DISCUSSION

In this experimental study, in which the effect of adipose tissue-derived mesenchymal stem cells on capsule development after breast implant surgery is evaluated volumetrically and immunohistochemically, the development of a macroscopically softer capsule with the formation of new vessels was observed in the stem cell-treated group when compared to the control group. In addition, a immunohistochemical analysis revealed the presence of a thick fibrous capsule and prominent procollagen in the periphery in the control group, and capsular staining in the experimental group, wherein thin collagen fibers were organized in the appearance of loose connective tissue and procollagen staining was lighter and scattered. A volumetric comparison, in turn, revealed that mean volume to be statistically significantly lower in the experimental group than in the control group. Since a volumetric comparison was made, it was not considered necessary to measure capsule thickness.

The pathogenesis of capsular contractures is believed to be multifactorial. Previous studies have reported the immune response, in which macrophages, lymphocytes, mast cells and fibroblasts are actively involved, to be the underlying cause,[10,17,18] while other studies have suggested that the immune response is caused by hematoma or infection.[19,20] In particular, capsular contracture has been associated with biofilm formation based on bacteria found on implants in animal models.[19] That said, this mechanism is not without controversy, since washing with antibiotics and the use of systemic antibiotics have both failed to prevent capsular contracture completely, and contractures have developed also in implants without bacteria.

A histological assessment of the capsule has revealed that most of the tissue is homogeneously dispersed collagen fibers, although the severity of contracture increases with the thickening of the collagen fibers upon the impaired orientation and organization of the fibers.[25] Mast cells may be involved in the pathogenesis of capsular contracture by expressing renin, histamine and tumor growth factor β-1 (TGF-β).[18] As concluded in a previous study, myofibroblasts express estrogen receptor-α (ER-α) and estrogen receptor-β (ER-β), which are activated by 17-β-estradiol, and increase the contractile forces produced by myofibroblasts, and anti-estrogenic therapy may reduce or prevent the severity of capsular contracture.[26] Accordingly, female rats were preferred for the study. Regarding pathogenesis, it has been reported that CD4+ T cells can mediate an immune response by generating a specific profibrotic cytokine profile and activating TH1 and TH17 cells.[27,28]

In addition to investigating cellular components, it is also important to know the substances that provide cellular activation. Relationships have been reported between the severity of contracture and the cellular expression of tumor growth factor-β (TGF-β), tumor necrosis factor-α (TNF-α), matrix metalloproteinase-2 (MMP-2) and endogenous inhibitors metalloproteinase tissue inhibitor-1 and metalloproteinase tissue inhibitor-2 (TIMP-I and TIMP-2).[17,29,30]

A study by Vieira et al.[31] examining the differences in the morphological and molecular properties of the capsule induced around polyurethane-coated and textured silicone implants in rats reported the capsules around polyurethane-coated silicone implants to have a more intense expression of vascular endothelial growth factor (VEGF) than textured silicone implants, increasing vascularization. This leads to softer capsule development, which the authors indicated may reduce the occurrence of capsular contracture around breast implants.[31] These results support the findings of the present study, although it should be noted that the differences in the types of silicon used are also an important factor.

Based on the hypothesis that the prevention of increased immune response in the capsular contracture development may reduce the development of contracture, the administration of steroids was attempted in several studies, and found to be effective in some, but not in others.[32-34] Furthermore, the use of vitamin E injections to prevent capsular contracture was attempted, but its application was restricted due to the need for the prolonged use of high doses and the unproven effects.[35,36] It has been reported that topical application of Mitomycin-C during implant placement in experimental animals prevented the development of capsular contracture by decreasing fibroblast proliferation and collagen synthesis in the capsule structure.[37] Based on the assumption that hyaluronic acid may inhibit mononuclear phagocyte and lymphocyte activity, a human amniotic fluid injection was applied around the silicone implant in experimental animals, resulting in decreases in capsule thickness and cellular content.[38,39] In a recent study it was concluded that roxatidine played an important role in preventing fibrosis by inhibiting the activation of NF-κB and p38/mitogen-activated protein kinase (MAPK) signals in macrophages.[40]

Kølle et al.[41] reported that autologous adipose tissue grafts enriched with adipose tissue-derived stem cells had a longer life, less resorption and higher volume than non-enriched grafts in breast reconstruction surgery. However, the determination of the best adipose tissue source for the isolation of adipose tissue+derived stem cells, the means of maintaining mature adipose tissue volumes in breast reconstruction, the oncological safety of stem cell applications in patients with breast cancer due to the risk of cancer recurrence, the effects of adjuvant therapies on stem

cell isolation and the associated effects on implant surgery have not been fully elucidated.[42]

Hypothesizing that hypoxia is a significant factor in the development of tissue fibrosis, Kim et al.[14] sought to determine the effect of human embryonic stem cell (hESC)-derived endothelial precursor cells (EPC), which have strong angiogenic potential, on capsule development around the silicone implant in a rat model, and reported a significant decrease in capsule thickness, less inflammatory reaction and the greater formation of new vessels in the early postoperative period in the experimental group than in the control group. While these results are similar to those of the present study, a review of literature revealed no study investigating the effect of adipose tissue-derived stem cell administration on capsule structure in breast implant surgery. In attempting to explain the findings of the present study, we believe that the formation of new vessels prevents potential hypoxia, and that adipose tissue-derived stem cells transform into endothelial cells and change the behavioral pattern of fibroblasts responsible for capsule formation.

In the light of this information, the present study sought to evaluate the effect of adipose tissue-derived mesenchymal stem cells on the capsular structure after breast implant surgery. To this end, an experimental rat model was designed in which volumetric and immunohistochemical comparisons were made between stem cell-treated and non-treated rat groups.

CONCLUSION

The mechanism behind capsular contracture development after breast implant surgery can be compared to a web-like tangle of cellular and molecular relationships. The present study has shown that the administration of adipose tissue-derived stem cells around the implant has an antifibrotic effect, and we believe that it has the potential for use as means of reducing the development of capsular contractures after breast silicone implant surgery, although larger-scale studies are required.

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