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Effects of the Acellular Dermal Matrix and Fat Graft on Radiation-Induced Fibrosis in a Prosthesis-Implanted Rat Model

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Abstract

Introduction: Capsular contracture is a multifactorial process involving inflammation and fibrosis. Although Acellular Dermal Matrix (ADM) is widely used to prevent the formation of capsular contracture, it has drawbacks such as seroma formation, infection, and high costs. Clinical studies have reported that fat grafting performed prior to implantation after irradiation improves fibrosis. However, none has compared ADM and fat grafting because of difficulty in setting the control group.

Objective: This study aimed to provide an objective basis for the clinical use of ADM and fat graft by comparing the changes in soft tissue after irradiation in a prosthesis-implanted rat model with those of a control group.

Methods: Thirty adult female Wistar rats were implanted and irradiated. For the ADM group, silicone implant was wrapped before implantation. For the fat graft group, autologous fat was grafted around the implant after irradiation. Capsular thickness and expression of fibrosis-related proteins, including α-smooth muscle actin, collagen 1, and transforming growth factor β1, were evaluated.

Results: Capsules were thinner at week 12 than at week 4, and they were thickest in the control group and thinnest in the fat graft group. Fibrosis-related protein expression was lowest in the fat graft group and highest in the control group.

Conclusion: Both ADM and fat graft had positive effects on fibrotic change after irradiation on the silicone-implanted area in a rat model. Further study is needed to compare with simultaneous use of ADM and fat graft over a longer period in order to obtain more reliable results that are clinically applicable.

Keywords: Radiation fibrosis, Acellular dermal matrix; Fat graft

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Introduction

The number of implant-based breast reconstructions has surpassed autologous reconstruction in 2002, and it has grown steadily to become the most common approach used for reconstruction [1]. There is increasing evidence of the benefits of Post-Mastectomy Radiotherapy (PMRT). Some patients undergoing breast reconstruction may need adjuvant radiotherapy. Recently, guidelines published by the American Society of Surgical Oncology recommend that all patients with T1-2 breast cancer with one to three positive axillary nodes should undergo PMRT to reduce the risk of locoregional failure, recurrence, and breast cancer-related mortality [2].

However, radiotherapy, especially after immediate breast reconstruction using prosthesis only, is known to increase the risk of complications. A meta-analysis of 1,105 patients revealed that patients undergoing PMRT and breast reconstruction are about four times more likely to develop complications compared with patients not undergoing PMRT [3].

Capsular contracture is one of the common complications after prosthetic implantation, and PMRT may increase the risk. It is known as the first or second most common reason for reoperation after breast augmentation [4,5]. Capsular contracture occurs as a multifactorial process [4,6], and fibrosis has been considered as a major cause of capsular contracture [7]. Tightening of the tissue

capsule around an implant results in firmness or hardening of the breast, and if it worsens, it may squeeze the implant. Several studies have reported that the incidence decreases when Acellular Dermal Matrix (ADM) is used [8,9]. Recently, fat grafting has been introduced in the treatment of fibrosis due to breast implant surgery [5,10,11].

However, none has compared ADM and fat grafting because of difficulty in setting the control group. Assuming that both ADM and fat graft would have a positive effect on Radiation-Induced Fibrosis (RIF), this study aimed to provide an objective basis for the clinical use of these modalities by comparing the changes in soft tissues in terms of fibrosis after irradiation in prosthesis-implanted rat model with control group.

Materials and Methods

Thirty adult female Wistar rats (8 weeks of age, 300 g) were used for the animal model. The rats were divided into three groups: A control group and two experimental groups. The control group underwent implantation and irradiation, whereas experimental group 1 (ADM group) underwent ADM-wrapped implant insertion following irradiation and experimental group 2 (fat graft group) underwent irradiation after bare implant insertion and fat grafting.

Anesthesia was induced in each mouse via peritoneal injection of 5 mg/kg Zoletil® (Virbac, Carros, France) and 2 mg/kg Rompun® (Bayer, Seoul, Korea) and maintained through isoflurane inhalation (IsoFlo®; Abbott Laboratories, Abbott Park, IL, USA). After complete hair removal on the lower dorsal area, a horizontal incision was made. After pocket dissection, a 2-cm cylindrical silicon bar was inserted. For experimental group 1 the silicon bar was completely wrapped in human ADM (MegaDerm™; L&C BIO, Seongnam-Si, Gyeonggi-Do, Korea) (Figure 1a). The wound was closed with 4-0 nylon sutures.

The rats were irradiated with 40 Gy using a biological irradiator (XRAD-320; Precision X-ray, North Branford, CT, USA) with a single-dose external beam at a dose rate of 2 Gy/min on a 2 × 2-cm2 dorsal area at skin depth.

For experimental group 2 (fat graft group), on the day after the irradiation, 1 cm^3 of autologous fat tissue was harvested from the inguinal area. The fat was minced finely after removal of fascia debris and vessels and transferred evenly around the implant using a 14-gauge needle (Figure 1b).

At 4 and 12 weeks after irradiation, the dermal tissue containing the capsule and surrounding normal tissue was collected.

Histology and Immunohistochemistry

Tissue samples were fixed in 10% formalin and then processed and embedded in paraffin. Sections (4 μm) were cut for hematoxylin and eosin (Richard-Allan Scientific, Kalamazoo, MI, USA) staining and immunohistochemistry. Immunohistochemical evaluation was performed using polyclonal antibodies specific for α-smooth muscle actin (Abcam, Cambridge, MA, USA), which is an indicator of myofibroblast (Clone 1A4; Dako, Glostrup, Denmark), and for collagen I (Abcam) and Transforming Growth Factor β1 (TGF-β; Abcam).

Image analysis

Sections were imaged at x40 magnifications and analyzed using Nikon NIS Elements Advanced Research software (Nikon, Melville, NY, USA). Capsular thickness was measured from five evenly spaced measurements of the capsule (defined as the collagen fiber layer of tissue closest to the implant surface) on a representative x40 magnification image (Figure 2) using Image J (NIH, Bethesda, MD, USA).

Statistical analysis

Capsule thickness and protein expression were analyzed as mean ± standard error. One-way analysis of variance was used for statistical analysis with Bonferroni correction method for multiple-comparison. A p-value <0.05 was considered statistically significant.

Results

Capsule thickness

Capsule thickness was thickest in the control group and thinnest in experimental group 2. Capsules were thinner at week 12 than at week 4. Capsule thickness was not significantly different at 4 weeks after surgery, but after 12 weeks, there was a statistically significant difference between the control group and experimental group 1 and between the control group and experimental group 2. No significant difference was noted between experimental groups 1 and 2 (Figure 3).

Fibrosis-related protein expression

All three proteins evaluated were lowest in experimental group 2 and highest in the control group (Figure 4). At 12 weeks after surgery, a statistically significant difference was noted between the control

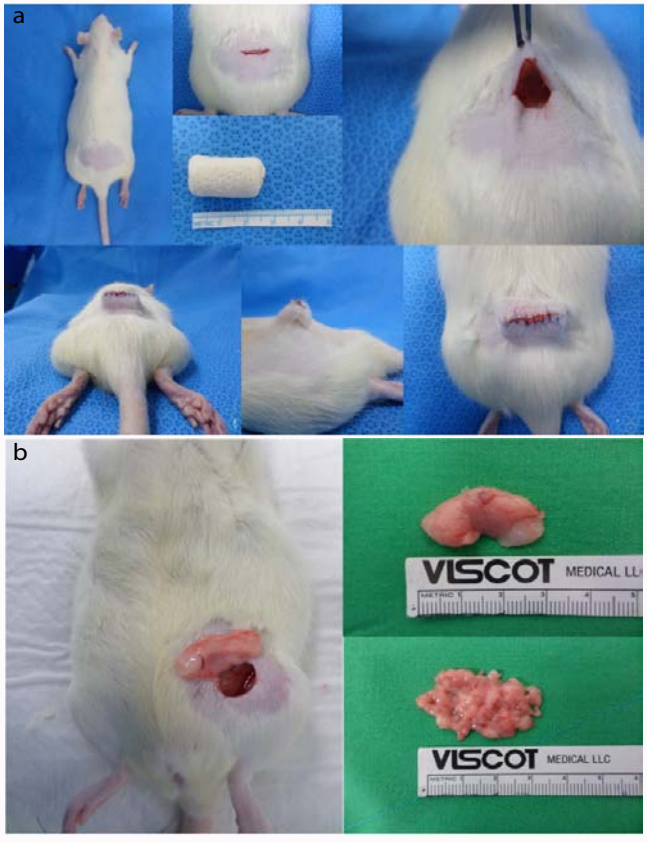

Figure 1: a) **Procedure:** After anesthesia and complete hair removal, a pocket was dissected through horizontal incision. For experimental group 1, a 2-cm-long cylindrical silicon bar was completely wrapped with human acellular dermal matrix (Mega Derm™) and implanted to the lower dorsal area. The wound was repaired with 4-0 nylon sutures. b) **Procedure:** For experimental group 2, rats were irradiated (40 Gy), and the day after irradiation, 1 cm^3 of autologous fat was harvested from the inguinal area. The fat was minced finely after removal of fascia debris and vessels and transferred evenly around the implant using a 14-gauge needle.

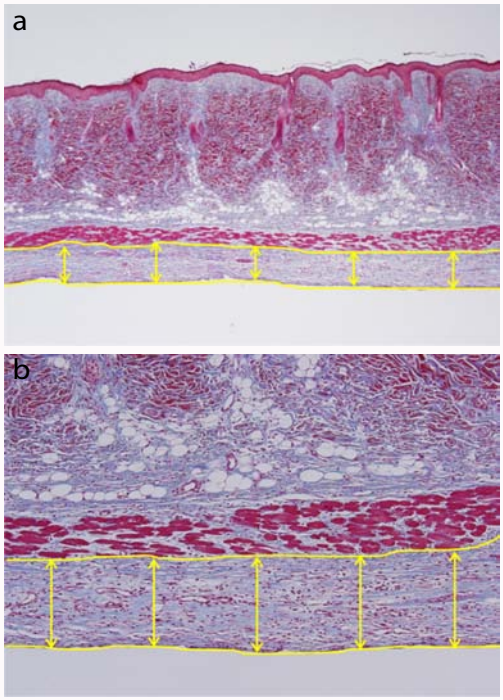

Figure 2: Capsular thickness measurement. Capsular thickness was measured from five evenly spaced measurements of the capsule (defined as the collagen fiber layer of tissue closest to the implant surface) on a representative x40 magnification image using Image J (hematoxylin and eosin; left: x40 magnification, right: x100 magnification).

group and experimental group 1 ($p<0.001$) and between the control group and experimental group 2 ($p<0.0001$).

Discussion and Conclusion

Both ADM and fat graft had a positive effect on fibrotic change after irradiation on the silicone-implanted area in rat model. Silicone is the most commonly implanted material in the human body [12]. Following implantation, tissue integration that typically accompanies foreign body response results in the formation of fibrous encapsulation [13], and it can lead to serious complications, depending on the extent. The most common presentation is capsular contracture, which causes problems such as pain and aesthetic issues. In severe cases, explanation should be considered. As a prophylactic strategy, ADMs have been used in implant-based breast reconstruction. Acting as a biological barrier, it is known to reduce foreign body reaction and inflammatory response. A recent long-term study in a rat model revealed that when ADMs were used in implantation, inflammatory and fibrotic tissue reactions were reduced [14]. At week 12, the thickness of collagenous- and myofibroblast-rich layers increased, as in the control group, but at week 52, the thickness was decreased in the ADM group. Although the mechanism of capsular contracture is not yet clear, the thickness of the collagenous layer and myofibroblasts have been reported to correlate with the level of capsular contracture [5,14]. In addition, the levels of TGF-β1, which plays an important role in fibrotic disease, and Tumor Necrosis Factor α (TNF-α), which inhibits collagen synthesis, are also reduced in the ADM group, which may be associated with this antifibrotic tendency [14-16]. Matrix Metalloproteinase's (MMPs) and tissue Inhibitors of Metalloproteinase's (TIMPs) may also play key roles in various fibrotic diseases, and a long-term study also showed a higher MMP1/TIMP1 ratio in the ADM group [17].

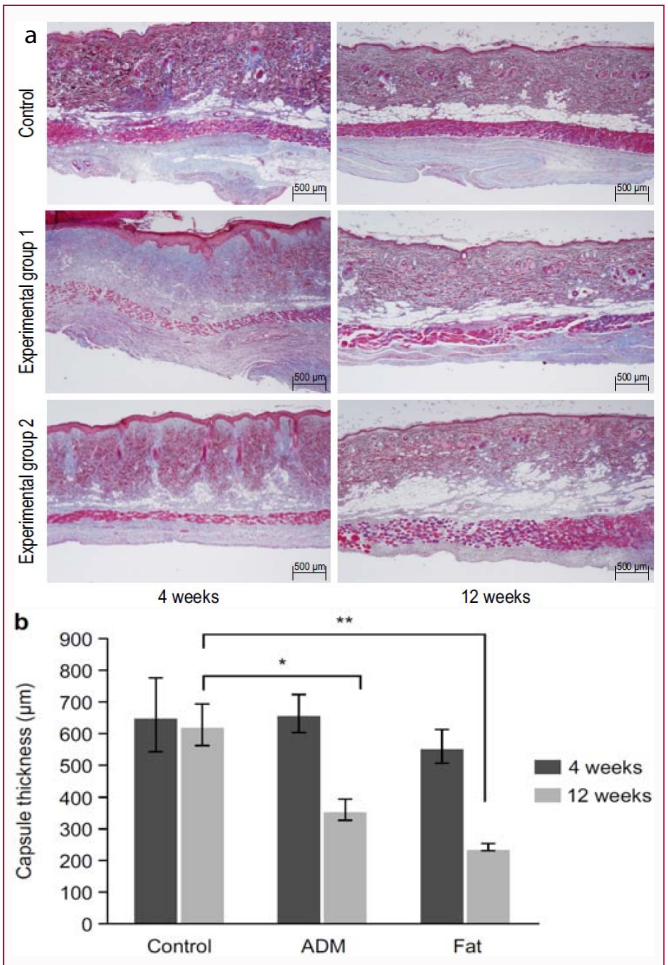

Figure 3: Comparison of capsule thickness. Capsules were thinner at week 12 than at week 4. Capsule thickness was not significantly different 4 weeks after surgery, but after 12 weeks, there was a statistically significant difference between the control group, experimental group 1 (Acellular Dermal Matrix [ADM] group) and between the control group and experimental group 2 (fat graft group) (hematoxylin and eosin; x40 magnification).
*$p<0.001$ and **$p<0.0001$.

In terms of treatment of fibrosis, some studies have used fat grafting on fibrotic adherent scars [18,19]. Fat grafting enhances tissue elasticity and maximal extension in scars [18]. The mechanism is not yet clear, but it was hypothesized to contribute to molecular changes in the microenvironment through the paracrine effect of mesenchymal stem cells in the adipocytes in the grafted fat. These adipose-derived stem cells respond to chemotactic signals associated with inflammation and tissue regeneration [20]. Moreover, they secrete various cytokines and growth factors that have both autocrine and paracrine activities, which can promote angiogenesis, anti-inflammatory properties, immunomodulation, inhibition of fibrosis, and extracellular matrix production. It is also suggested that the ischemic condition after fat grafting triggers the perivascular adipose stem cells to promote adipocyte regeneration and revascularization [18,21,22].

Although adjuvant radiation therapy is highly effective in controlling local recurrence risk after mastectomy, it changes the adjacent tissues unfavorably, with darkening of skin color and markedly reduced elasticity [23]. Tissue fibrosis occurs after ionizing injury through multiple molecular and cellular interactions. The activation and proliferation of myofibroblasts play a pivotal role in

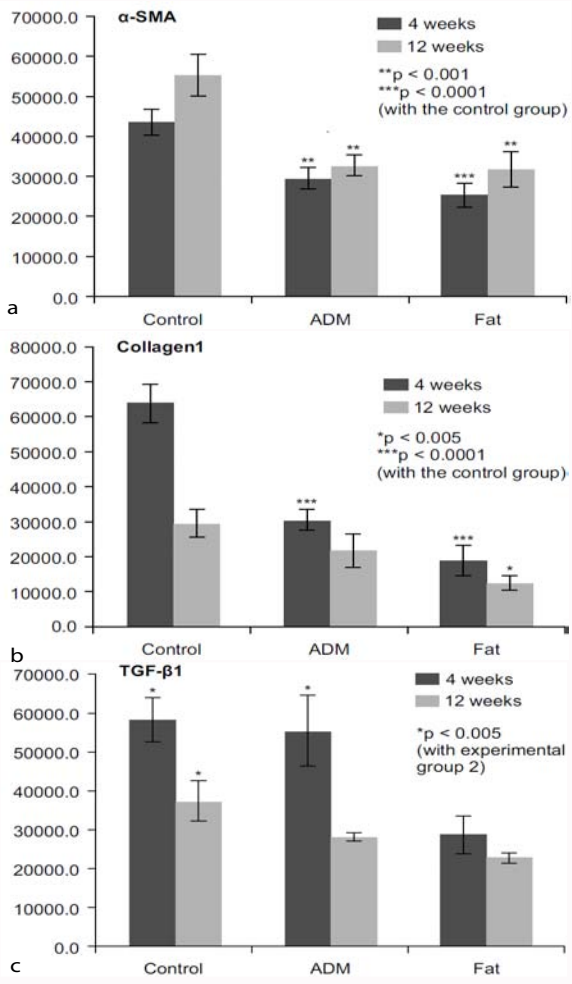

Figure 4: Fibrosis-related protein expression: α-Smooth Muscle Actin (α-SMA), collagen 1, and Transforming Growth Factor β1 (TGF-β1). All three proteins were lowest in experimental group 2 (fat graft group) and highest in the control group. At 12 weeks after surgery, there was significant statistical difference between the control group and each of the experimental groups (experimental group 1 [Acellular Dermal Matrix; ADM], p<0.001, experimental group 2 [fat graft], p<0.0001).

fibrogenesis, and the key cytokine is TGF-β1 [24]. Platelet-derived growth factor and TNF-α are also known to have potential roles in RIF [25]. Fat grafting of radiation-injured tissue improves dermal thickness, collagen content, hypovascularity, and skin elasticity in mice model [23].

To maximize both oncologic and aesthetic satisfaction in patients with breast cancer, RIF is one of the critical issues. Fibrosis may lead to severe capsular contracture, especially in patients who had undergone implant-based reconstruction, which can result in reconstruction failure and frustration. In this study, the use of ADM and fat graft showed anti-fibrotic effects in the irradiated tissue. It is premature to say that either is superior because the experimental period was short (3 months), with only a small number of animals. In future study, a group with simultaneous use of both ADM and fat graft should be included and the effectiveness of each method should be compared over a long period in order to obtain more reliable results that are clinically applicable.

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