Original Article

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Porcine Dermal Collagen Prevents Seroma Formation After Mastectomy and Axillary Dissection in Rats

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ABSTRACT

Objective: Seroma occurs as a result of accumulation lymphovascular liquid in the dead space forming after tissue dissection. It is the most common complication after breast surgery. Collagens are the common component of extracellular matrix and have an important role in wound healing. In this study, we aimed to investigate the efficiency of the Porcine Dermal Collagen in preventing Seroma.

Materials and methods: Eighteen young female Wistar rats were used and divided into three groups. Mastectomy and axillary dissection were performed in each group. No other procedures were performed in Group 1 (Control group). Porcine dermal collagen was applied to 50% of the mastectomy field in Group 2 and to 100% of the mastectomy field in Group 3.

Results: Seroma volume was significantly decreased in Group 3 in contrast to Groups 1 and 2 ($p<0.001$) and in Group 2 in contrast to Group 1 ($p<0.001$). Vascular proliferation, granulation tissue formation and congestion were significantly increased in Group 3 ($p<0.05$).

Conclusion: We conclude that the use of Porcine Dermal Collagen reduces the formation of seroma in the model of experimental mastectomy and axillary dissection. As the amount of Porcine Dermal Collagen applied increases the formation of seroma reduces.

Keywords: Axillary Dissection, breast, mastectomy, porcine dermal collagen, seroma

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Introduction

Seroma occurs as a result of leakage of the lymphovascular fluid into the dead space forming after tissue excision or dissection. It is the most common complication after breast cancer surgery. The incidence varies between 10-50% (1-3). In breast cancer surgery, it occurs most frequently after modified radical mastectomy and axillary lymph node dissection (4).

It can cause many problems such as prolonged hospitalization time, wound infection and delay adjuvant treatment. There is no agreement on the prevention of seroma in breast cancer surgery.

Porcine Dermal Collagen is a non-allergenic, nontoxic material which is structurally similar to human dermis and is often used for the repair of the abdominal wall defects. It is produced from acellular pig matrix and does not cause foreign body tissue reaction. Its 3-dimensional collagenous architecture increases fibroblast infiltration, fibroblast growth and neovascularization (5-8). It has been shown that in contrast to other materials, seroma occurs less frequently in abdominal wall repair when Porcine dermal collagen-containing materials are used (6, 9).

Porcine dermal collagen temperately increases inflammatory process. After implantation, granulocytes are dominant in first 48 hours. In 7 days, monocytes/macrophages and a lesser number of natural killer cells become dominant in the infiltrate. These cells activate expression of cell surface adhesion molecules such as intercellular adhesion molecule 1 (ICAM-1) and CD11b. It is known that these adhesion molecules have a critical role in increasing inflammatory response (6).

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In this study, we aimed to show the effect of Porcine Dermal Collagen on preventing Seroma through its characteristics such as 3-dimensional structure, being a component of normal human tissue and being inert and increasing adhesion.

Materials and Methods

The approval of the study was obtained from the local Ethical Committee of the Experimental Animal Laboratory. Eighteen female Wistar rats at the average weight of 200-250 grams (g) were used in the study. We divided the rats into 3 groups and each group included 6 rats. All the rats were fed with standard laboratory diet and tap water, and observed under controlled conditions of temperature (22±2 centigrade degree) and light with 12 hours day/night cycle.

Unilateral (right) mastectomy and axillary dissection were performed in all rats by the method described by Harada et al. (10). Prophylactic antibiotics were used in none of the groups. For anesthesia, 50 milligram (mg), kilogram (kg) intraperitoneal ketamine (Ketalar®, Parke Davis and Co. Inc., Detroit, Michigan, United States of America) and 5 mg/kg intramuscular xylazine (Rompun®, Bayer, Leverkusen, North Rhine-Westphalia, Germany) were used. After that, the anesthesia rats were fixed to operation desk with plaster. Their anterior chest wall and right axillary region were shaved with lancet and cleaned with 10% povidone-iodine solution.

After a vertical incision extending from the jugular notch to the xiphoid, a flap formed by the cutaneous and subcutaneous tissue was lifted from the chest wall. The major pectoralis muscle was dissected up to the level of the latissimus dorsi muscle, freed, and then excised. All the axillary lymph nodes were excised while preserving the integrity of the axillary artery, veins, and nerves.

The dissection area was measured (Figure 1, 2). The average dissection area was found to be 3 cm². After mastectomy and axillary dissection, no additional procedures were performed in the control group (Group 1). Porcine dermal collagen was postoperatively applied to 50% of mastectomy area in Group 2 and to 100% of mastectomy area in Group 3 (Figure 3, 4, 5, 6). After hemostatic control was established, the skin was closed with 2.0 silk sutures in all groups.

Figure 1. Measuring of dissection area after mastectomy and axillary dissection (Average dissection area is 3 cm²)

The rats were observed for 10 days after the operation. On post-operative Day 10, seroma fluid was aspirated with sterile injectors from the surgical site under ketamine anesthesia, and the total volume in milliliters was recorded. Tissue samples were obtained from the skin, axilla and chest wall in the surgical area for histopathologic examination and all the samples were placed in 10% formaldehyde solution. The rats were sacrificed with high dose ether anesthesia at the end of all procedures.

The tissue samples were embedded in paraffin blocks after the tissue processing, 5-micrometer sections were taken from each block and stained with hematoxylin-eosin (HE). Hemorrhage, edema, conges-

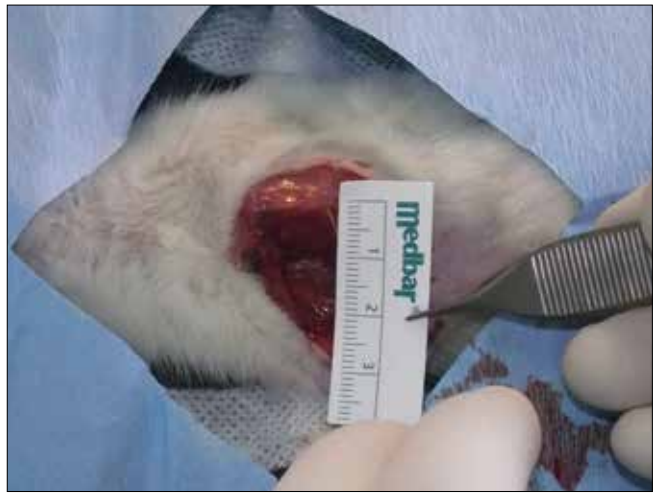


Figure 2. Measuring of dissection area after mastectomy and axillary dissection (Average dissection area is 3 cm²)

Figure 3. Cutting the porcine dermal collagen to cover 50% of the dissection area

Figure 4. Cutting the porcine dermal collagen to cover 100% of the dissection area

Figure 5. Applying porcine dermal collagen to 50% of dissection area

Figure 6. Applying porcine dermal collagen to 100% of dissection area

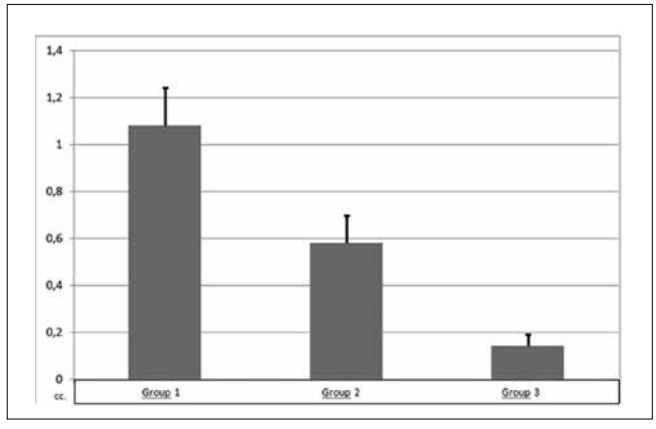
tion, polymorphonuclear leukocytes, growth of fibrous tissue, fibroblasts, lymphocytes, macrophages, granulation tissue, vascular proliferation and necrosis were evaluated qualitatively under light microscope. Cellular and histopathological data were scored semi-quantitatively in 0 to 3+scale as 0=absent, 1=mild, 2=moderate, and 3=marked.

Statistical analyzes were performed with Microsoft Windows Excel 2007 program. The volumes of seroma were statistically evaluated by ANOVA and Multiple Comparison Procedures (Holm-Sidak method). Histopathological parameters were statistically evaluated by Kruskal-Wallis One Way Analysis of Variance on Ranks method and Tukey tests.

Results

Neither complications such as wound infection, wound clefting nor flap necrosis were seen in any rats and nor any of them died during the course of the experiment. The mean seroma volumes were 1.083 cc (±0.16 cc) in the control group (Group 1), 0.583 cc (±0.11 cc) in Group 2 (50% porcine dermal collagen mesh implantation group) and 0.142 cc (±0.05 cc) in Group 3 (100% porcine dermal collagen mesh implantation group) respectively. Seroma volume was significantly lower in Group 3 in contrast to Group 1 and 2 ($p<0.001$) and in Group 2 in contrast to Group 1 ($p<0.001$) (Graphic 1).

Vascular proliferation and granulation tissue formation were significantly higher in Group 3 in contrast to Group 1 and 2 ($p<0.05$); there was no significant difference between Groups 2 and 1 ($p>0.05$)(Figure 7). The congestion was found to be significantly higher in Group 3 in contrast to Group 1 and 2 ($p<0.05$). There was no significant difference in hemorrhage, macrophage, neutrophil, lymphocyte parameters among all the groups. Despite the fact that we observed more severe



Graphic 1. Mean seroma volumes in each groups. Group 1 = 1.083cc. (±0.16 cc.); Group 2 = 0.583cc.(±0.11 cc.); Group 3= 0.142 cc. (±0.05 cc.). $p < 0.001$

fibroblasts and fibrous tissue formation in Group 2 and 3, these parameters did not show significance in between all groups.

Discussion and Conclusion

In the literature, several methods have been recommended to reduce seroma formation by this time. These methods can be separated into 2 groups. The first group of these methods intends to prevent the dead space occurring after surgery.

Aitken et al. (11) concluded that seroma formation was reduced with the method of flap fixation by sutures. In the study where external

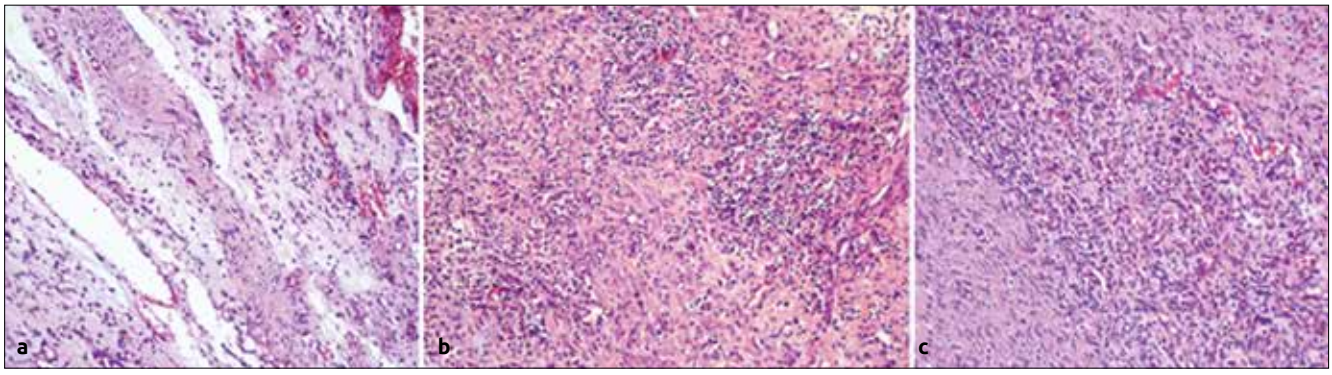


Figure 7. Vascular proliferation and granulation tissue in Group 1(a), Group 2(b) and Group 3(c)

Table 1: The experimental studies in which sclerosing agents were used

Researchers	Experimental subject	The agent used	Mean seroma volume in control group	Mean seroma volum in study group
Silverman et al.	Sprague-Dawley rat (280-320 gr)	Poliethylene oxide dimethacrylate	3.25 cc	0.37 cc
Wang et al.	Sprague- Dawley rat (280-320 gr)	Light-activated Fibrin glue	4.2±2.9 cc	1.1 ±1.6 cc
Lindsey et al.	Sprague- Dawley rat (280-320 gr)	Fibrin glue	1.1-4.3 cc	1.8-2.4 cc
Menon et al.	Sprague- Dawley rat (280-320 gr)	Albumin-Gluteraldehyde	5.19±3.65 cc	0.25±0.43cc.
Koçdor et al.	Sprague- Dawley rat (280-320 gr)	Corynebacterium parvum	1.4 cc	0.3 cc
Eroglu et al.	Male guinea pigs (480-900 gr)	Fibrin glue	4- 6.8 cc	1.3 cc
Koçdor et al.	Wistar rat (215.2gr)	Fluorouracil	1.04 cc	0.3 cc

compression method and flap fixation method were compared after breast surgery, Kuroi et al. (12) showed that seroma formation was lower in the group of patients for whom flap fixation method was applied by using sutures. Bonnema et al. (13) compared low pressure vacuum system and high pressure vacuum system and found that body mass index was directly related to seroma formation but negative pressure vacuum and postoperative arm and shoulder movement restrictions were not related to seroma formation.

The second group of methods intends to reduce the formation of seroma. The agents used for this purpose are as follows: sclerosing agents (Fibrin glue, Tetrasycline, Hidrocloric acide, Microporous Polysaccharide spherules, Iodized talc), anti-inflammatory drugs (Acetyl salicylic acid, Nonsteroidal anti-inflammatory drugs), chemotherapeutic agents (Fluorouracil, Mytomycin-C) and radiotherapy.

These sclerosing agents were used in some clinical and experimental studies. Burak et al. (14) observed that 37% of patients given bovine thrombin showed seroma formation and 40% of the control patients showed seroma formation. In the study by Berger et al. (15), the ratio of seroma formation was 39% in the group that used fibrin glue and 42% in control group. Waddenburn et al. (16) used same agent and found the ratio of seroma formation as being 64% in the study group and 53% in control group.

In the literature, there are also experimental studies in which sclerosing agents such as fibrin glue, Poliethylene oxide dimethacrylate, albumin gluteraldehyde, cornybacterium parvum, 5- Fluorourasil, Microporous Polysaccharide spherules, Myhtilus edilus protein were used. These studies are summarized in Table 1. Experimental studies in which sclerosing agents were used had better outcomes than clinical studies.

After reviewing the relevant literature, it was seen that the studies using sclerosing agents published more successful outcomes than the studies aiming to reduce dead space after surgery. For this reason, a material which could partially reduce dead space and increase fibrosis when it was applied to dissection area was decided to be used in this study.

Porcine dermal collagen is an agent which is used for repair of abdominal wall defects, treatment of burns, pelvic reconstructions, head and face reconstructions. It is a non-allergenic and non-toxic material which is structurally similar to human dermis. Its 3-dimensional structure increases fibroblast infiltration and neovascularization (5-8). In repair of abdominal wall defects, it was seen that seroma formation was lower when porcine dermal collagen was used (6, 9). According to these clinical observations, porcine dermal collagen was chosen in this experimental seroma model.

The significant decrease in seroma volume seen in our study groups is similar to the results of some sclerosing agents such as fibrin glue used by Harada et al. (10), mytilus edilus protein used by Chung et al. (3) and albumin glutaraldehyde used by Menon et al. (17).

The inverse ratio between the seroma volume and the amount of porcine dermal collagen applied to the tissue in our study was also seen in the study of Sanders et al. (18) in which fibrinogen and thrombin concentration was used.

In histopathological examinations, we investigated whether there was any difference in tissue healing between control and study groups and evaluated the effect of porcine dermal collagen on the process of tissue healing.

In pathological samples, no microorganisms were seen, which proved not only that we provided the sterile operation conditions but also that no microscopic wound separation occurred in rats.

Increase in congestion, granulation tissue and neovascularization were seen in Group 3 in contrast to other groups ($p<0.05$). According to the studies in the literature, it was predicted that the 3-dimensional architecture of the porcine dermal collagen would increase fibroblast infiltration into the mesh and in this way there would be increase in fibrous tissue in pathological samples (6-9). But no significance was found in the increase of fibrous tissue ($p>0.05$).

In our study, we detected that the effect of reduced seroma occurred with the increase of neovascularisation and granulation tissue, not with the increase of fibrous tissue as in the studies in which sclerosing agents were used such as the study by Wang et al. (19) who used light-activated fibrin glue and the study by Egeli et al. (20) who used microporous polysaccharide spherules.

The major limitations of our study are as follows: having a limited number of rats in working groups and not having compared the effectiveness of porcine dermal collagen with other agents or acellular dermal matrix derived from human dermis.

In the literature, it was seen that neovascularization was not emphasized in the studies related to seroma. In the light of our results, we are convinced that drugs that increase neovascularization can also be used to repress seroma. Additional experimental and clinical studies are needed to prove this point.

In conclusion, the use of Porcine Dermal Collagen reduces the formation of seroma formation after breast surgery, as the amount of Porcine Dermal Collagen applied is increased the formation of seroma is reduced in parallel. Porcine Dermal Collagen shows its effect on reducing seroma formation by filling in the dead space, increasing the adhesion, neovascularization, granulation tissue and accelerating the wound healing. However, our findings are based on experimental animal research; further clinical studies with using porcine dermal collagen and/or acellular human dermal matrix are needed.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Dokuz Eylül University Faculty of Medicine (30.03.2012/20.2012).

Informed Consent: Informed consent was not requested for this study.

Peer-review: Externally peer-reviewed.

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Conflict of Interest: No conflict of interest was declared by the authors.

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