

# The Effect of Barrier Gel and Free Fat Graft on the Formation of Epidural Fibrosis in the Laminectomy Model

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**Introduction:** This study aims to compare the efficacy of ADCON-L and free fat graft used to prevent epidural fibrosis in the experimental laminectomy model in rabbits.

**Materials and Methods:** Thirty adult male New Zealand-type white rabbits weighing 2500-3000 grams were used. Three groups of ten were formed. New subjects and all subjects who replaced subjects who died during the study and follow-up period were sacrificed at the end of the 12th week. Group 1 was determined as the control group. Free fat grafts were placed into the laminectomy areas in group 2. In group 3, ADCON-L was injected into the surgical area. Subjects were sacrificed at week 12. After decalcification, laminectomy zones were taken into routine tissue follow-up for histopathological examination.

**Results:** When group-1 and group-3 were compared regarding fibroblastic proliferation, there was a statistically significant difference ( $p < 0.001$ ). When group-2 and group-3 were compared, it was determined that fibroblastic proliferation was statistically higher in group-2 ( $p < 0.001$ ). When group-1 and group-3 were compared in terms of collagen amount, the amount of collagen in group-3 was significantly less ( $p < 0.001$ ). When the groups were evaluated statistically regarding the adhesion of the scar tissue to the dura, there was a significant difference between group-1 and group-2 ( $p < 0.01$ ). Adhesion was significantly less in group-3 compared to group-2 ( $p < 0.001$ ).

**Conclusion:** We concluded that ADCON-L did not cause FBS syndrome. So, ADCON-L is more successful than free fat graft in the prevention of epidural fibrosis after laminectomy in spinal surgery.

**Keywords:** Laminectomy; epidural fibrosis; free fat grafts; ADCON-L; adhesion

## Introduction

Met Fibrotic tissue formation, a physiological part of wound healing after all surgical interventions, can become a problem when intense (1, 2). Undesirable density fibrotic tissue that develops in the epidural space, dura surface, and around the nerve root after laminotomy/laminectomy applied in patients

such as narrow spinal canal (NSC), lumbar disc herniation (LDH) who do not benefit from conservative treatment and have surgical indications for Failed Back Surgery (FBS) syndrome (3, 4). In order to prevent epidural fibrosis or the resulting fibrosis from reaching the spinal dura mater, it is necessary to create

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an anatomical or physiological barrier between the dura mater and the m. erector spinae. For this purpose, various biological and non-biological materials have been used for many years. These include fat grafts as solid barriers, fascia grafts, kiel bone grafts, polyvinyl alcohol hydrogel membrane, polytetrafluoroethylene membrane, polylactic acid membrane, vicryl mesh, hyaluronate as the viscous solution, gel as fibrinolytic agent, recombinant plasminogen activator, urokinase gel, hemostat. Sponge, microfibrillar collagen, methylprednisolone, triamcinolone, prednisolone, ketoprofen as an anti-inflammatory agent, dexamethasone, CO<sub>2</sub> laser, and Antiadhesion Barrier Gel (ADCON-L), a carbohydrate polymer developed in recent years for the prevention of epidural fibrosis, were used (5-10). However, there is no adopted standard treatment protocol to prevent or minimize occurrence of epidural fibrosis (11-13).

It is well known that ADCON-L, a carbohydrate polymer developed in recent years, is highly effective in preventing epidural fibrosis, and this is proportional to clinical improvement (14). Frederickson showed in vitro that astrocytes respond to damage by forming a gliotic scar, that this gliotic scar creates a barrier against the spread of neuritis in the brain, and that proteoglycans, a component of this scar, can inhibit the spread of neuritis (15). Studies with proteoglycans and related molecules have shown that this substance can act as a barrier against fibroblasts; this formed the scientific logic in the production of ADCON-L (16, 17). It has been reported that with further tests on the proteoglycan molecule, it has been determined that the main responsible for the barrier effect is the carbohydrate content, and ADCON-L was developed with this basic logic (15, 18).

This study aims to compare the efficacy of ADCON-L and free fat graft used to prevent epidural fibrosis in experimental laminectomy model in rabbits.

## Materials and Methods

### *Study Design*

This study was carried out within the facilities of Erciyes University Faculty of Medicine (E.U.T.F) Hakan Çetinsaya Experimental and Clinical Research, Orthopedics and Traumatology and Pathology departments.

### *Ethical Statement*

Ethics Committee approval was obtained from the Erciyes University, Faculty of Medicine, the experimental research ethics Committee (Approval No: 02.02.43, 2003). Our study used 30 adults (1-year-old) male New Zealand-type white rabbits weighing 2500-3000 grams. Three groups of ten were formed. New subjects and all subjects who replaced subjects who died during the study and follow-up period were sacrificed at the end of the 12th week.

Group I (n: 10) control group: The group that underwent laminectomy but no material was placed in the distance.

Group II (n:10) free fat graft group: The autologous free fat graft was placed in the distance after laminectomy.

Group III (n:10) ADCON-L group: The group injected with ADCON-L gel into the (Gliatech, Cleveland, OH, USA) laminectomy distance after laminectomy.

The subjects were taken into operation after fasting for 4 hours. Anesthesia was provided to the subjects by intramuscular injection of 40 mg/kg ketamine hydrochloride (Ketalar 50 mg/ml, 10ml vial, Eczacıbaşı İlaç Sanayi İstanbul, Turkey). Cefazolin sodium (Cefazol I.M. vial 1000 mg, Mustafa Nevzat) at 70 mg/kg was administered intramuscularly for prophylactic purposes. The lumbar region was shaved, and the hair was removed. Subjects were fixed in the prone position on the operating table prepared for this study. After determining the distance in the lumbar region for the operation area, a skin incision of approximately 4 cm was made over the spinous processes from the midline. The

iliac wings were palpated, and the L<sub>5</sub> level was determined. The lumbar fascia was opened from the midline, and the paravertebral muscles were stripped bilaterally by blunt dissection. After the spinous process was removed with a rongeur, a total laminectomy was performed at the L<sub>5</sub> level with the help of a drill.

Bleeding was stopped by applying a compress and using Surgicell. Surgicell was removed after bleeding control. After the operation area was washed with physiological saline, the necrotic paravertebral muscles were deprived. No other action was taken in the operation area of Group I. Autologous free fat graft was placed in the laminectomy distance of Group II, covering the dura mater. The free fat graft was obtained by releasing the adipose tissue in the epidural space. 0.5cc of ADCON-L was injected into the laminectomy distance of Group III (19).

Subjects were sacrificed at week 12. After the skin was opened in all subjects, the prevertebral region was reached with a transverse incision approximately 2 cm distal to and 2 cm proximal to the L<sub>5</sub> level. The vertebral column, which was cut with bone scissors, was removed as a whole and sliced at 5mm intervals. It was detected in 10% formaldehyde solution in the Department of Pathology for 48-72 hours. After fixation, they were decalcified in 10% EDTA solution. After decalcification, laminectomy zones were taken into routine tissue follow-up for histopathological examination. After decalcification, laminectomy zones were taken into routine tissue follow-up for histopathological exam. Sections of 3-4 micron thickness were obtained from the blocked tissues with a microtome and stained with Hematoxylin-Eosin (H&E) and Masson Trichrome (MT). The criteria of He et al. (20) were used in the histopathological evaluation of the obtained preparations by light microscopy (Table 1).

### Statistical Analysis

Statistical Package for Social Sciences (SPSS) for Windows 16 program was used for statistical

analysis. According to the Kolmogorov Smirnov test distribution analysis, the "Kruskal Wallis Variance Analysis Test" was used for the statistical evaluation of the data obtained in this study, and the "Man Whitney U Test" was used for the comparison of subgroups. The results were evaluated at the 95% confidence interval and the significance level of  $p < 0.05$ .

**Table 1.** Histopathological Evaluation Criteria (50)

|                                              |
|----------------------------------------------|
| A. Fibroblastic Proliferation                |
| 0: No fibroblastic proliferation             |
| 1: Fibroblastic proliferation minimal        |
| 2: Moderate fibroblastic proliferation       |
| 3: Fibroblastic proliferation is severe      |
| B. Amount of Collagen                        |
| 0: No collagen                               |
| 1: Collagen minimal                          |
| 2: Collagen moderately                       |
| 3: Collagen is advanced                      |
| C. Adhesion of Scar Tissue to the Dura Mater |
| 0: No adhesion                               |
| 1: Adhesion is minimal                       |
| 2: Moderate adhesion                         |
| 3: Adhesion is severe                        |

## Results

### Macroscopic

After sacrifice, the entire vertebral column was separated from the surrounding soft tissues and sliced at 5mm intervals. Macroscopically, an increase in fibrous tissue was observed in the laminectomy areas. Large spaces were observed between the spinal cord, dura mater, and fibrous tissue formed macroscopically in the subjects using ADCON-L. The other two groups had no significant difference (Table 2).

### Microscopic - Fibroblastic Proliferation

When the subjects sacrificed at the end of the 12th week were evaluated regarding fibroblastic proliferation, six advanced, three moderate, and one minimal fibroblastic proliferation were observed in group-1. In group-2, 3 had severe, 5 had moderate fibroblastic proliferation, and 2 had minimal fibroblastic proliferation.

In group-3, moderate fibroblastic proliferation was observed in one and minimal fibroblastic proliferation in 4, while fibroblastic proliferation was not detected in 5. In addition, we observed fibroblastic proliferation was more intense in the parts adjacent to paraspinal muscles (Table 2). When the groups were compared regarding fibroblastic proliferation, no difference was observed between group-1 and group-2. A difference was found when group-1 [3(1-3)] and group-3 were compared [0.5(1-2)] ( $p<0.001$ ). When group-2 and group-3 [0.5 (1-2)] were compared, it was determined that fibroblastic proliferation was statistically higher in group-2 [2 (1-3)] ( $p<0.001$ ) (Table 2).

**Table 2.** Comparison of fibroblastic proliferation

| Groups  | n  | Median | Min-Max |
|---------|----|--------|---------|
| Control | 10 | 3      | 1-3     |
| Fat     | 10 | 2      | 1-3     |
| ADCON   | 10 | 0.5    | 0-2     |

Kruskal Wallis= 16.608,  $p<0.001$

### Collagen Synthesis

All the groups were evaluated regarding collagen synthesis. In the group-1, advanced collagen synthesis was observed in five and moderate collagen synthesis in five. In group-2 (Fat), moderate collagen synthesis was detected in five and minimal collagen synthesis in seven, while collagen synthesis was not detected in two (Figure 1a). In group-3 (ADCON), moderate collagen synthesis was detected in one, minimal collagen synthesis in four, and no collagen synthesis in five (Figure 1b). When the groups were compared in terms of collagen amount, no statistical difference was found between group-2 [1.0 (0-2)] and group-3 [0.5 (0-2)] ( $p>0.05$ ). The amount of collagen was found to be more intense in group-1 [2.5 (2-3)] than in group-2 [1.0 (0-2)] ( $p<0.001$ ). When group-1 and group-3 were compared in terms of collagen amount, the amount of collagen in group-3 was less ( $p<0.001$ ) (Table 3).

**Table 3.** Comparison of the amount of collagen

| Groups  | n  | Median | Min-Max |
|---------|----|--------|---------|
| Control | 10 | 2.5    | 2-3     |
| Fat     | 10 | 1.0    | 0-2     |
| ADCON   | 10 | 0.5    | 0-2     |

Kruskal Wallis: 19.462,  $p<0.001$

### Adhesion of Scar Tissue to the Dura Mater

When evaluated regarding the adhesion of the scar tissue to the dura mater, four severe, four moderate, and 2 minimal adhesions were observed in group-1. In group-2, moderate adhesion was observed in four, minimal adhesion was observed in two, and no adhesion was observed in four (Figure 2a). None of the group-3 had scar tissue adhesion to the dura mater (Figure 2b).

When the groups were evaluated statistically regarding the adhesion of the scar tissue to the dura, there was a significant difference between group-1 and group-2, and adhesion was less in group-2 ( $p<0.01$ ). Adhesion was significantly less in group-3 compared to group-2 ( $p<0.001$ ). When group-1 and group-3 were compared in terms of the adhesion of the scar tissue to the dura, the adhesion was significantly less in group-3 ( $p<0.001$ ) (Table 4).

**Table 4.** Comparison of scar adhesion

| Groups  | n  | Median | Min-Max |
|---------|----|--------|---------|
| Control | 10 | 2      | 1-3     |
| Fat     | 10 | 1      | 0-2     |
| ADCON   | 10 | 0      | 0-0     |

Kruskal Wallis: 19.345,  $p<0.001$

In the samples obtained from the control subjects, it was observed that there was intense scar tissue in the laminectomy area, and the scar tissue progressed toward the spinal canal and adhered to the dura mater. The scar tissue was an extension of the scar tissue under the paraspinal muscles rather than originating from the inside of the spinal canal. Adhesion was higher in the parts of the laminectomy defect close to the bone in the free fat graft group. In addition, in some samples, it was observed that the fat graft was not wholly absorbed and

dispersed in the form of islets (Figure 1a, 2a). In cross-sections of the distances using ADCON-L, gaps were observed between the dura mater around the medulla spinalis and surrounding muscle layer and nerve roots (Figure 3). No residual ADCON-L was found in any subjects, and ADCON-L was resorbed. No foreign body reaction to ADCON-L was observed in any subjects (Figure 3). In some subjects, foreign body type inflammatory granulation tissue consisting of multinuclear giant cells, fibroblasts, capillary vessel proliferation, and a small number of PMNL (Polymorph Core Leukocyte) that phagocytized them was seen around suture material used in some subjects.

## Discussion

In spinal surgery, epidural fibrosis, which develops on the dural sac and around the nerve root, especially after decompression and LDH surgeries, causes neurological symptoms with the effect of traction or compression, causes the patient's complaints to persist, and/or causes additional complaints, is one of the problems that still awaits a solution in spinal surgery. Research on the causes, prevention, and treatment of epidural fibrosis is ongoing, and opinions differ. Epidural fibrosis is one of the causes accepted to cause FBS syndrome, and it is reported to cause clinical complaints at a rate of 6-25% (4, 21, 22).

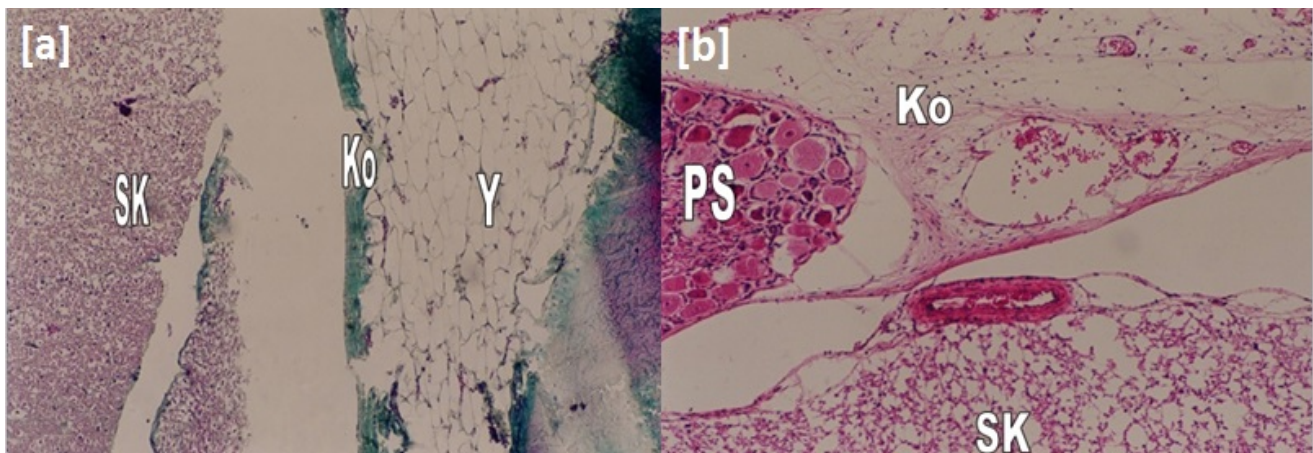


**Figure 1a:** A section from the fat group. Minimal amount of collagen is seen. SK: Spinal cord, Ko: Collagen, Y: Fat graft (MTx100). **b:** A section from the ADCON-L group. Minimal collagen formation is seen around the nerve root and spinal cord. SK: Spinal cord, PS: Peripheral nerve, Ko: Collagen synthesis (HEX100).

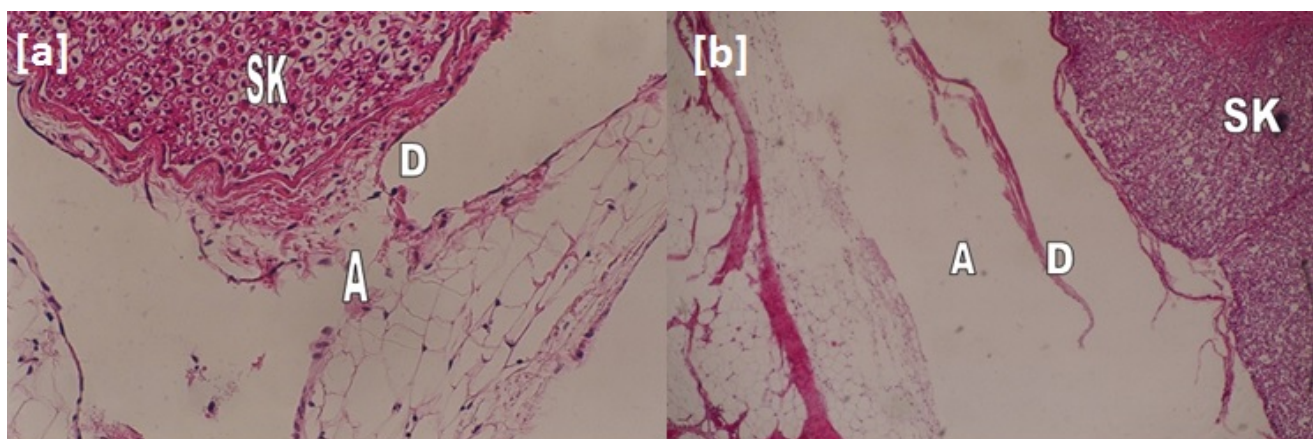


**Figure 2a:** A cross-section from the fat graft group. Moderate adhesion to the Duramater is seen. SK: Spinal cord, D: Dura, A: Adhesion (HEX100). **b:** A section from the ADCON-L group. There is no adhesion to Duramater. SK: Spinal cord, D: Dura mater, A: Adhesion (HEX40).

In a study, he et al. examined rats by sacrificing them on the 8<sup>th</sup>, 15<sup>th</sup>, 30<sup>th</sup>, and 90<sup>th</sup> days (20). They reported that the laminectomy membrane started to appear on the 15<sup>th</sup> day, the laminectomy site was closed after 30 days, and after 90 days, massive scar tissue was located on the defect and adhered to the dura mater (20). Songer et al. reported that the adhesion maturity or strength was not different at the 12<sup>th</sup> and 26<sup>th</sup> weeks in their examination in dogs at the 2<sup>nd</sup>, 4<sup>th</sup>, 12<sup>th</sup>, and 26<sup>th</sup> weeks (8). Our study aimed to see the final state of scar tissue formation by sacrificing all subjects at the end of the 12<sup>th</sup> week.

Gerszten et al. found in their study on dogs that the laminectomy defect was covered with scar tissue originating from the paraspinal muscles and advancing toward the epidural region (25). Yang et al. (26) and Sobti et al. (27) found similar results in similar studies. Our study found that fibroblastic proliferation was more in the parts close to the paraspinal muscles. This finding can be interpreted as a substance that will be placed in the laminectomy or laminectomy area and will prevent the progression of the scar and may block the laminectomy membrane. When groups were evaluated regarding fibroblastic proliferation, there was no significant difference between the control and fat graft groups. In contrast, the ADCON-L group's fibroblastic proliferation was considerably lower than the other two groups.

La Rocca et al., in their study on dogs, found that the laminectomy defect was covered with scar tissue originating from the paraspinal muscles and advancing toward the epidural region. (28). Keller et al. and Vaquero et al. found similar results in similar studies. (29, 30). Our study found that fibroblastic proliferation was more in the parts close to the paraspinal muscles. This finding can be interpreted as a substance that will be placed in the laminectomy or laminectomy area and will prevent the progression of the scar and may

block the laminectomy membrane. When the groups were evaluated regarding fibroblastic proliferation, there was no significant difference between the control and fat graft groups. In contrast, the ADCON-L group's fibroblastic proliferation was considerably lower than the other two groups.

The primary building material of epidural fibrosis or scar tissue is collagen. Fibroblasts form collagen synthesis during wound healing. Barbera et al. determined that the main component originating from the dorsal spinal muscles and filling the laminectomy defect is collagen, and its amount is directly proportional to the size of the scar tissue (6). Massie et al. determined that main component originating from the dorsal spinal muscles and filling the laminectomy defect is collagen, and its amount is directly proportional to the size of the scar tissue (31). Ding et al. reached the same conclusion in a similar study (32). Our study observed that the amount of collagen in the scar tissue showed parallelism with fibroblastic activity in the control and ADCON-L groups but not in the fat graft group.

Autogenous fat grafts are the most effective substance in preventing epidural scarring among the substances (33, 34). VEDIAPPAN et al. showed in their study that even if the fat graft is alive, it does not prevent the formation of fibrosis in the anterior/antero-lateral regions of the nerve root emerging from the spinal cord (35). In many cases, the spinal cord was compressed posteriorly by the fat graft. The fat graft may compress the dura posteriorly. In our study, we found moderate scar formation and, in parallel, moderate perineural adhesions in the subjects who used fat grafts. The results were significant compared to the control.

It is known that ADCON-L, a carbohydrate polymer developed in recent years, is highly effective in preventing epidural fibrosis, and this is proportional to clinical improvement (11, 36). Kazanci et al. applied ADCON-L to rats after

laminectomy and evaluated the scar gross anatomically by microdissection, sacrificing eight weeks later. They reported that ADCON-L reduced epidural fibrosis compared to the control group (37). Our results were statistically significant, and no adhesion was observed in any subjects using ADCON-L. Dermirel et al. applied ADCON-L to rats after laminectomy, sacrificed the subjects after 2, 4, 8, 16 and 26 weeks, and investigated the effect of ADCON-L gross anatomically and histologically (11). The surgical area contains minimal scarring, and the dura can be easily seen; In the histological study reported that ADCON-L was resorbed in the fourth week, and there was minimal or no foreign body and inflammatory response to ADCON-L. Kasimcan et al. also reported that the absorption period of ADCON-L was four weeks (38). In our study results, no foreign body reaction or inflammatory response was observed in any of the subjects using ADCON-L, no residual ADCON-L was found, and it was thought to be completely resorbed. In order to prevent epidural fibrosis, the substance to be used must be resorbed over time because the substance placed for a long time increases the risk of infection.

## Conclusion

Epidural fibrosis, a physiological consequence of wound healing after laminectomies, occurred in varying degrees except in ADCON-L injected subjects. We concluded that free autogenous fat grafting does not prevent epidural fibrosis formation at an acceptable level. We concluded that ADCON-L would not cause FBS syndrome because there are no side effects associated with its use, it is absorbed over time, it is easy to apply, the risk of infection is low, and it is an effective substance in the prevention of epidural fibrosis and the second operation can be performed more comfortably and safely in patients who need it. Due to the limited number of studies in the literature, our study makes an essential contribution to the literature. Since the

relationship between epidural fibrosis and findings has not been fully demonstrated, we think comparative clinical studies should support the efficacy of ADCON-L.

## Conflict of Interests

The authors declared no conflicts of interest concerning financial resources, the authorship, and the publication of this article.

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