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# Hemostasis vs. epidural fibrosis?: A comparative study on an experimental rat model of laminectomy

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ABSTRACT

*Aim:* The aim of this study was to evaluate the histopathological and biochemical impact and effectiveness of two hemostatic agents, Ankaferd blood stopper (ABS) and Microporous Polysaccharide Hemospheres (MPH), on epidural fibrosis in an experimental rat laminectomy model.

*Material and methods:* Twenty adult Wistar albino rats were divided into MPH-treated ($n = 6$), ABS-treated ($n = 6$) and control ($n = 8$) groups. Laminectomy of the lumbar spine was performed in all animals and treatment groups were exposed to MPH and ABS while closure was applied in control group as per usual. Epidural fibrosis was evaluated in all groups macroscopically, histopathologically, biochemically and with electron microscopy four weeks later.

*Results:* Statistically, it was found that MPH-treated group had significantly less epidural fibrosis compared to ABS-treated and control groups.

*Conclusion:* We compared two hemostatic agents for their propensity to cause adhesions in the present study. Our results show that MPH significantly reduces epidural scar formation and dural adhesion in a rat model of laminectomy while ABS increases postoperative fibrosis.

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## 1. Introduction

In spinal surgery, uncontrolled bleeding may result in loss of neurological function and intractable complaints. Therefore, hemostasis is one of the most important phase of these surgeries. Specific features of the spine prevents rendering mechanical methods of haemostasis such as direct pressure and ligature. For such a long time, bipolar cautery has been the essential apparatus for coagulation of small vessels with

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minimizing effect on adjacent tissue. However, chemical haemostatic agents were suggested preferable to bipolar cautery in spinal procedures because of some drawbacks. The complete occlusion of the vessel lumen compromising the perfusion of the neural tissue, thermal injury to adjacent structures induced by dissipiation of heat from the tips of the bipolar forceps and insufficiency in controlling the diffuse capillary bleeding were attributed to bipolar cautery and local haemostatic agents were recommended especially for diffuse capillary oozing which characterizes most intraspinal pathologies [1].

Epidural fibrosis, which is characterized as increased granulation formation and adhesive properties, continues to be a major cause of poor results in spinal surgery [2]. Scar formation increases the risk and technical difficulty of subsequent procedures in addition to persistent pain. Epidural fibrosis prevents nerve roots from gliding within the spinal canal and results in pain postoperatively. Nerve fibers are subject to compression which leads to impaired axoplasmic transport and restricted arterial supply and venous return [3,4].

A great deal of effort has been made to prevent the development of scar formation fibrosis. A variety of materials such as autogenic fat [5], Adcon-L [6], omentum graft [7], amniotic membrane [8], polytetrafluoroethylene [9] and many others have been investigated to prevent or reduce epidural fibrosis. However, results of these studies remain unsatisfactory. It is now widely accepted that, invasion of the postoperative hematoma by the dense fibrotic tissue results with epidural fibrosis. Since residual material of any nature, including blood can serve as nidus for epidural fibrosis, haemostatic agents may play a different role in pathogenesis with a dual mechanism unlike other materials or drugs. For, their presence would reduce the effect of remnants of blood while they act as foreign bodies which provoke scar formation and fibrosis.

Thus, the present study aimed to investigate histopathological and biochemical impacts of Microporous Polysaccharide Hemospheres (HaemoCer™) and Ankaferd blood stopper® on extent of spinal epidural fibrosis in the rats.

## 2. Material and methods

### 2.1. Experimental design

Twenty adult Wistar albino rats weighting 300–350 g were used in this study. The study was conducted at the Maltepe University, Faculty of Medicine, Laboratory for Experimental Animals with the approval of Animal Experiments Local Ethics Committee. All rats received human care as outlined in the ''Guide for the care and use of laboratory animals'' (National Research Council). The rats were divided in three groups randomly: MPH-treated group ($n = 6$), ABS-treated group ($n = 6$) and control group ($n = 8$). They were subjected to food deprivation 24 h before surgery, but were allowed free intake of water. Prophylactic antibiotics were not applied pre- or postoperatively.

### 2.2. Surgical procedure

All rats were anesthetized via the intramuscular route by 60 mg/kg ketamine (Ketalar, Pfizer, Istanbul) and 10 mg/kg xylazine (Rompun, Bayer, Istanbul). After the animals were stabilized on the operation table in prone position, surgical area was shaved and cleaned with povidone iodine solution (Drogsan, Istanbul). Following a vertical midline incision from Th11 to L3 to expose L1 vertebra, the lumbar fascia was opened bilaterally from the midline and paravertebral muscles were detached in a subperiosteal manner. L1 total laminectomy was performed under the operating microscope (Möller-Wedel, Wedel, Germany). Exposure of dura mater was carried out by the removal of ligamentum flavum and epidural fat tissue. Hemostasis was obtained with the topical application of MPH, ABS and saline in first, second and third groups respectively. Similar appearance of the surgical area was detected in all rats with regard to hemorrhage. The closure of the wound was achieved with 3/0 vicryl as per usual. The rats were sacrificed at the 30th postoperative day (4th week) with intra-peritoneal high dose (75–100 mg/kg) of Tiopental Sodium (Pentothal Sodium, Abbott, Italy). Finally, each vertebral column was resected in an en bloc fashion.

### 2.3. Macroscopic assessment

Macroscopic assessment was performed blindly by selecting samples randomly. Animals with dural tear, nerve root injury and infection were excluded from the study. The results were classified according to the Rydell classification (Table 1) [10].

### 2.4. Histopathological examination and grading

Histopathological examinations were done in Medical Biology and Histology Department Laboratory of Cerrahpasa Medical Faculty at Istanbul University. Tissue samples were fixed in 10% buffered formalin solution for 4 days and decalcified with 5% hydrochloric acid solution for 5 days. Three consequent sections from middle, proximal and distal parts of laminectomy region were taken and placed in sampling casettes. After they were washed with tap water for 3 h to eliminate acidic remnants, routine follow-up process was carried out. Afterwards, each segment was embedded in paraffin blocks and sectioned in 3 μm thick coronal slices by microtome. Hematoxylin–Eosin (H&E) and masson trichrome dye were performed for histopathological examination and sections were evaluated blindly by the histopathologist with Olympos BX61 light microscope (Olympos, Tokyo, Japan) and photographed with Olympos camera DP71 (Olympos, Tokyo, Japan). Evaluation of epidural fibrosis was performed and graded according to the definitions of He et al. [11] as summarized in Table 2. Many studies that evaluate the efficacy of the drugs or materials against epidural fibrosis, histopathological grading. The interrater reliability of this classification was tested and it

**Table 1 – Macroscopic evaluation according to Rydell classification [10].**

| | |
|---|---|
| Grade 0 | No scar tissue in the duramater |
| Grade 1 | Scar tissue in the duramater but dissected easily |
| Grade 2 | Scar tissue in the duramater, difficult dissection together with impaired duramater |
| Grade 3 | Adhered scar tissue in the duramater and cannot be dissected |

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