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Effect of platelet-rich fibrin on epidural fibrosis and comparison to ADCON® Gel and hyaluronic acid

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ABSTRACT

Objective: In this experimental study, PRF (Platelet Rich Fibrin), HA (Hyaluronic Acid) gel and ADCON® Gel were compared in terms of preventing epidural fibrosis.
Methods: Twenty-eight Sprague–Dawley rats (mean weight, 400–450 g) were divided into 4 groups. L3-L4 laminectomy was performed in each group. Following laminectomy, Adcon® Gel, HA gel and PRF were applied onto the surgery site locally in Group 1, 2 and 3, respectively. Group 4 was maintained as control without any local application. After five weeks, L3-L4 vertebrae were removed totally and taken to histopathological evaluation for epidural fibrosis, acute inflammatory cell density, chronic inflammatory cell density, hemorrhage, angiogenesis and new bone formation.
Results: Acute inflammation cell density, angiogenesis, and new bone formation levels were comparable among the study groups ($p > 0.05$). However, new bone formation was higher in the PRF group. Epidural fibrosis and chronic inflammatory cell density were significantly lower in the PRF group ($p < 0.05$).
Conclusion: We concluded that PRF contributed to hemostasis and prevented epidural fibrosis.

Introduction

Epidural fibrosis is one of the most important causes of recurrent radicular pain after spinal surgery and is a result of the natural healing process. However, it largely results in adhesion between tissue layers and pressure on nerve roots and thus in undesirable consequences.[1] Epidural fibrosis increases the risk of nerve root compression and tension, which in turn leads to recurrent pain after surgery. Epidural fibrosis has been shown to be responsible for 8–24% of cases of failed back surgery syndrome.[2,3] In a study of 1500 patients undergoing unilateral single-level open microdiscectomy, the incidence of epidural fibrosis was reported between 18 and 37%, depending upon the technique.[4]

Numerous local or systemic chemical agents and physical barriers have to date been studied in order to prevent epidural fibrosis. Unfortunately, there is no standard treatment for this disorder. This study compared platelet-rich fibrin (PRF), used to prevent epidural fibrosis, with Adcon-L® and Hyaluronic acid (HA), with proven efficacy against epidural fibrosis.

PRF is a second-generation platelet concentration which accelerates wound healing due to ingredient growth factors. It is routinely used in dental surgery, maxillofacial surgery, and plastic surgery for its beneficial effects on soft tissue and bone regeneration.[5]

Adcon® gel and Hyaluronic acid are both agents that have some effect on epiduaral fibrosis. These two agents were used as a control study group for epidural fibrosis after laminectomy as spinal surgey.

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Materials and methods

Approvals for the study were obtained from the Atatürk University Animal Experimentation Local Ethics Committee (HADYEK) and the Atatürk University Center for Medical Experimentation and Research (ATADEM). Thirty two male Sprague–Dawley rats (12 months old, average weight, 400–450 g) were randomly divided into four groups (n = 8 in each). In this study, only male rats were used for avoiding the potential factors of gender relationship with epidural fibrosis via to sex hormones. All animals were allowed ad libitum access to standard rat chow and tap water. All animals were kept under standard laboratory conditions (12-hour light:dark cycle, 55 ± 10% humidity, and 21 °C ± 2 °C ambient temperature). PRF was obtained from 16 cc blood samples taken from rats by intracardiac puncture. These samples were placed into two empty biochemistry tubes (8 cc each) with red caps and centrifuged at 3000 rpm for 13 min (Illustration 1). PRF were prepared according to international guidelines on literature.[6]

All ethical forms were applied, signed and received. Anesthesia was induced with combination of xylazine 10 mg/kg and ketamine 40 mg/kg administered by intramuscular injection into the hind limbs. The lumbar region was shaved, and the operation site was cleaned with povidone iodine. All 32 animals then underwent L3-L4 partial laminectomy. The control group received no local administration after laminectomy, while the other groups received local PRF, HA or Adcon® gel to the laminectomy site, respectively (Illustration 2).

One day postoperatively, one rat from the control group and one from the HA group died due to anesthesia complications. These two animals were excluded from the analysis. One rat from the Adcon® group was also excluded due to abscess development in the operative site 10 days after surgery. In order to equalize the number of rats in all groups, one randomly selected rat was also excluded from the PRF group on postoperative tenth day. The study was thus completed with seven rats in each group. After six weeks, all rats were sacrificed using high dose thiopental. The L3-L4 vertebral blocks were then removed. Rats were available for our laboratory conditions. Partial laminectomy were applied in the light of textbook technique approaches.[7]

Collected tissue samples were fixed in 10% buffered neutral formalin and decalcified in 6% nitric acid solution for 13 days. They were then embedded in paraffin following dehydration by ethanol and cleaning with xylene. Five-millimeter sections were next taken from the paraffin embedded material. Sections were stained with Hematoxylin–Eosin and Masson's trichrome. Epidural fibrosis was assessed using Masson's trichrome staining. Stained sections were examined under microscope (Olympus BX51, Japan) for analysis of epidural fibrosis density, acute inflammatory cell density, chronic inflammatory cell density, hemorrhage, angiogenesis and new bone formation, and the four groups were then compared. All specimens were objectively evaluated by one pathologist by light microscope. Epidural fibrosis evaluation was based on the classification described by He et al[1,5] This classification is as following classification:

G0: Dura mater free of fibrotic tissue.
G1: Only a thin fibrous band present between the dura and scar tissue.

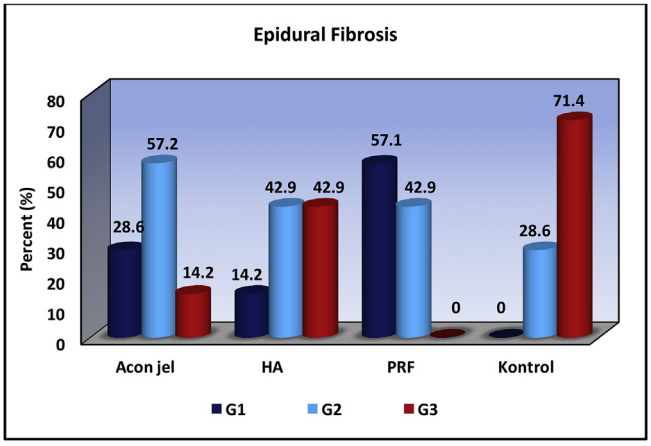


Fig. 1. Comparison of epidural fibrosis in study groups.

Illustration-1. Platelet rich fibrin (PRF).

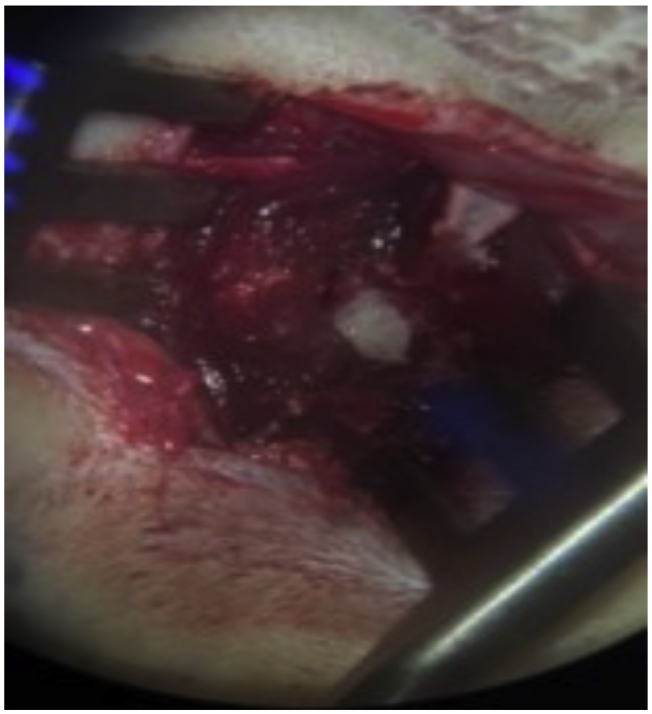
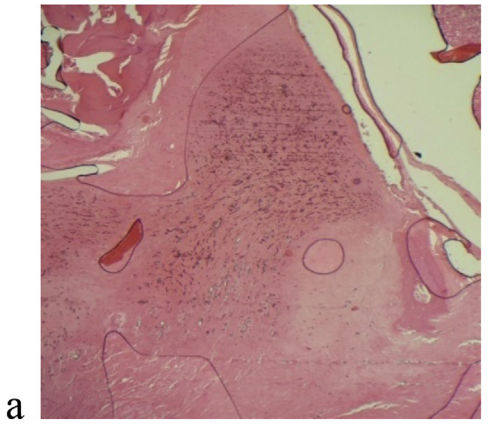
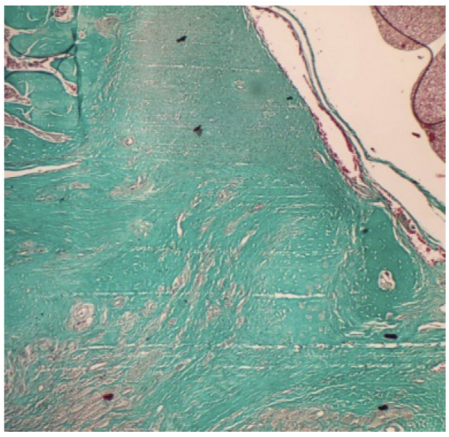


Illustration-2. PRF application onto laminectomy site.



a



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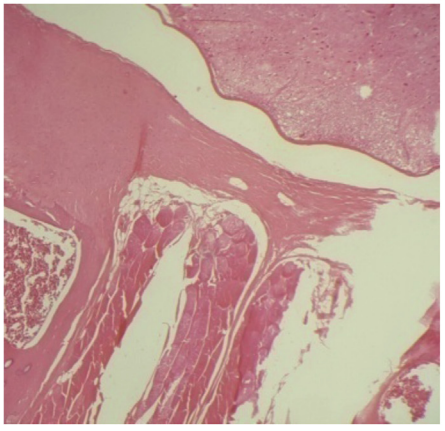
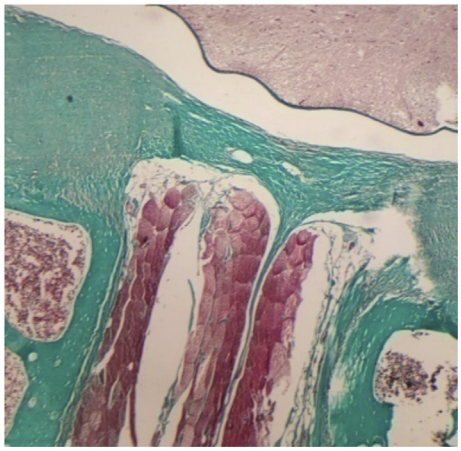


c



d

G2: Continuous adherence observed, but in less than two-thirds of the laminectomy defect.
G3: Significant scar tissue adherence involving more than two-thirds of the laminectomy area, and/or extending to the nerve root.

Acute inflammatory cell density, chronic inflammatory cell density, angiogenesis, hemorrhage, and new bone formation were evaluated in two classes: G0: no exist, and G1: exist.

Statistical analyses were carried out in NCSS (Number Cruncher Statistical System) 2007 (Kaysville, Utah, USA) software. Descriptive statistical methods were used (mean, standard deviation, median, frequency, ratio, minimum and maximum values). Qualitative comparisons were performed using the Fisher-Freeman-Halton test and Fisher's Exact test. Significance was set at $p < 0.05$.

Results

All animals were sacrificed at the sixth week, and the tissues were subjected to histopathological examination.

Epidural fibrosis differed significantly among the study groups ($p = 0.048$; $p < 0.05$). Post-hoc pairwise comparisons revealed a significantly lower level of epidural fibrosis in the PRF group than in the control group ($p = 0.016$; $p < 0.05$) (Fig. 1) (Illustration 3).

Chronic inflammation cell densities differed significantly different among the study groups ($p = 0.044$; $p < 0.05$). Post-hoc pairwise comparisons revealed significantly lower chronic inflammation cell density in the PRF group than in the control group ($p = 0.021$; $p < 0.05$). No significant differences were observed in other pairwise comparisons ($p > 0.05$) (Tables 1–3).

Acute inflammation cell density, angiogenesis, and new bone formation levels were comparable among the study groups ($p > 0.05$). However, new bone formation was higher in the PRF group. Hemorrhage levels were lower in the PRF group than in the other study groups. Epidural fibrosis was significantly lower in the PRF group than in the controls in our study. Levels of epidural fibrosis were lower in the PRF group than in the Adcon® gel group. The level of epidural fibrosis was lower in the PRF group than in the HA group.

Discussion

Epidural fibrosis was first described by Key and Ford, who concluded that damage to the annulus fibrosis was the principal factor involved.[8] Released anti-inflammatory cytokines, such as IL-6, IL-8, and TNF, lead to scar formation. Fibrosis can be developmental at fourth weeks according to clinical and laboratory findings. Early inflammatory phase can be seen prior weeks.[9] The most important mechanism in the pathogenesis of epidural fibrosis is migration of fibroblasts from perivertebral muscle tissue and the systemic circulation to the site of laminectomy, leading to adhesion.[2] Studies have therefore focused on potential barriers between epidural fibrous tissue and neural structures, hemostatic agents which might reduce bleeding, and local or systemic agents which might improve the quality of tissue healing and reduce fibrosis.[2,10,11]

The exact effect mechanism of PRF on wound healing is unclear. The ingredient growth factors and cytokines may accelerate cell proliferation and stimulate apoptosis. These may improve the

Illustration-3. a) Epidural fibrosis in a control group rat (hematoxylin-eosin × 40) b) Epidural fibrosis in a control group rat (Masson's trichrome × 40) c) Epidural fibrosis in a PRF group rat (hematoxylin-eosin × 40) d) Epidural fibrosis in a PRF group rat (Masson's trichrome × 40).

Table 1
Evaluations of the groups.

		[1]Adcon gel	[2]HA	[3]PRF	[4]Control	p
		N (%)	n (%)	n (%)	n (%)	
EpiduralFibrosis	G1	2 (28.6)	1 (14.2)	4 (57.1)	0 (0)	[a]0.048[c]
	G2	4 (57.2)	3 (42.9)	3 (42.9)	2 (28.6)	
	G3	1 (14.2)	3 (42.9)	0 (0)	5 (71.4)	
Acute inflammation cell density	Present	1 (14.2)	2 (28.6)	0 (0)	2 (28.6)	[b]0.708
	Absent	6 (85.8)	5 (71.4)	7 (100)	5 (71.4)	
Chronicinflammation cell density	Present	4 (57.1)	3 (42.9)	2 (28.6)	7 (100)	[b]0.044[c]
	Absent	3 (42.9)	4 (57.2)	5 (71.4)	0 (0)	
Angiogenesis	Present	5 (71.4)	7 (100)	5 (71.4)	7 (100)	[b]0.283
	Absent	2 (28.6)	0 (0)	2 (28.6)	0 (0)	
Hemorrhage	Present	2 (28.6)	2 (28.6)	1 (14.2)	4 (57.1)	[b]0.491
	Absent	5 (71.4)	5 (71.4)	6 (85.8)	3 (42.9)	
New bone formation	Present	3 (42.9)	6 (85.7)	4 (57.1)	6 (85.8)	[b]0.306
	Absent	4 (57.1)	1 (14.3)	3 (42.9)	1 (14.2)	

G1: Only thin fibrous band between dura and scar tissue.
G2: Continuous adherence observed but less than two-thirds of laminectomy defect.
G3: Scar tissue adherence large, more than two-thirds of laminectomy area, and/or extending to nerve root.
[a]Fisher Freeman Halton Test. [b]Fisher's Exact Test. [c]$p < 0.05$ (Statistically significant).
[1]Adcon gel, [2]HA, [3]PRF, [4]Control group.

Table 2
Pairwise comparisons of the groups.

	p^{1-2}	[b]p^{1-3}	[b]p^{1-4}	[b]p^{2-3}	[b]p^{2-4}	[b]p^{3-4}
EpiduralFibrosis	[a]0.632	[a]0.598	[a]0.152	[a]0.174	[a]0.591	[a]0.016[c]
Acute inflammation	1.000	1.000	1.000	0.462	1.000	0.462
Chronic inflammation	1.000	0.592	0.192	1.000	0.070	0.021[c]
Angiogenesis	0.462	1.000	0.462	0.462	–	0.462
Hemorrhage	1.000	1.000	0.592	1.000	0.592	0.266
New bone formation	0.266	1.000	0.266	0.559	1.000	0.559

[a]Fisher Freeman Halton Test. [b]Fisher's Exact Test. [c]$p < 0.05$.

Table 3
Fibroblast cell counts as to hinton criteria.

Control 1	Grade 3
Control 2	Grade 3
Control 3	Grade 3
Adcon 1	Grade 1
Adcon 2	Grade 1
Adcon 3	Grade 2
Adcon 4	Grade 1
Adcon 2	Grade 2
PRF 1	Grade 1
PRF 2.	Grade 2
PRF 3.	Grade 1
PRF 4	Grade 2
HA 1	Grade 2
HA 2	Grade 1
HA 3	Grade 1

Grade 1: less than 100.
Grade 2: 100–500.
Grade 3: in excess of 150.

quality of wound healing. PRF has been described as effective in chronic leg ulcers, non-healing wounds and bone defects.[12–14]

Platelet-rich fibrin (PRF) belongs to a new generation of platelet concentrates, with simplified processing and without biochemical blood handling. During PRF processing, leucocytes could also secrete cytokines in reaction to the hemostatic and inflammatory phenomena artificially induced in the centrifuged tube. We therefore undertook to quantify 5 significant cell mediators within platelet poor plasma supernatant and PRF clot exudate serum: 3 proinflammatory cytokines (IL-1β, IL-6, and TNF-α), an antiinflammatory cytokine (IL-4), and a key growth promoter of angiogenesis (VEGF). Our data are correlated with that obtained in plasma (nonactivated blood) and in sera (activated blood). These initial analyses revealed that PRF could be an immune regulation node with inflammation retrocontrol abilities. This concept could explain the reduction of postoperative infections when PRF is used as surgical additive.[15]

Due to its membranous structure, PRF acts as a barrier and reduces adhesion. It also controls collagenase release and regulates collagen synthesis. PRF induces hemostasis of wound tissue and prevents fibroblast migration from blood to the wound site. It is also tissue-compatible and does not lead to alergic reaction. The leukocytes it contains also help to prevent infection and inflammation.[12,14,16] Chronic inflammation cell density was significantly lower in the PRF group than in the control group in this study. We think that PRF established hemostasis and prevented the first step in inflammation, thus reducing chronic inflammation. Previous studies have shown that hemostatic agents in spinal surgery reduce inflammation and fibrosis.[17]

PRF exhibits beneficial effects on hemostasis through platelets and fibrin. It also improves osteoblast proliferation, angiogenesis and tissue healing through cytokines and growth factors released from platelets, such as platelet derived growth factor (PDGF), transforming growth factor beta (TGFβ), vascular endothelial growth factor (VEGF), epidermal growth factor, (EGF), and insulin-like growth factor (IGF).[14] PRF use in orthopedic surgery is becoming most popular. PRF has many growth factors and cytokines such as platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF), transforming growth factor-β (TGF-β), insulin like growth factor-I (IGF-I), interleukin-1β (IL-1β), interleukin-4 (IL-4), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α).[18] And also another study empahised that slow polymerization during centrifugation and fibrin-based structure makes PRF a better healing biomaterial than PRP and other fibrin adhesives.[19]

Adcon® gel provides a physical barrier to inter-tissue adhesions and inhibits fibroblast migration on and around neural tendinous structures. Adcon® gel prevents adhesion formation. Resorbable Adcon® gel contains a polyglycan ester, combined with absorbable gelatine in phosphate-buffered saline, which is gradually resorbed over a period of 4 weeks. Adcon® gel were used in clinical applyings since 1996. And also Adcon® gel is a cost effective agent.[20]

Hyaluronic acid is high molecular weighted, negative charged linear polisaccarid that is found in the extracellular matrix of soft connective tissue. It contains glycosaminoglycan that is the most simple and non-sulfate-free of the connective tissue proteins. The use of Adcon® gel may provide a simple means of preventing of

postoperative peritendinous adhesions, thereby offering a beneficial effect on tendon repair. Cross-linked high-molecular-weight HA had positive effects on the prevention of epidural fibrosis and the reduction of fibrotic tissue density. The efficacy of this agent should also be verified in further experimental and clinical studies.[21]

Patients who are re-operated for epidural fibrosis have an increased risk of scar formation due to dural damage, nerve root damage, epidural hematoma and infection. The principal aim of studies on this subject has therefore been to prevent epidural fibrosis before it occurs. Cotton particles in the surgery site, compression of epidural veins, manual traction of nerve roots, and bipolar coagulation have also been implicated in the etiology of epidural fibrosis.[22] Epiduroscopy demonstrates that the prevalence of severe epidural fibrosis after FBSS is substantially higher than is generally reported in MRI evaluations. Severe epidural fibrosis is an underlying pathology in most patients with FBSS.[23] Paraspinal muscle dissection and bone removal lead to fibroblast migration from periosteum and muscle tissue to the site of laminectomy. Hematoma resulting from surgical manipulation also transforms into granulation tissue, which is in turn converted into epidural scar tissue.[22,24,25] Songer et al suggested that epidural fat tissue extends from the posterior epidural compartment to the intervertebral foramen, and that with its semi-viscous nature it replaces epidural fibrosis tissue, which compresses the dural sac and nerve roots.[26]

Adherence around the nerve roots and scar formation are inevitable due to epidural fibrosis after laminectomy and disc surgery. This undesirable condition mostly results in nerve compression and intractable pain after surgery. Further surgery may also be required in some cases in order to reduce adherence and nerve compression.[27]

Vaquero et al showed that fibrin-containing gels reduced epidural fibrosis within the first two weeks after laminectomy.[28] Anitua et al reported that growth factor-containing platelet concentrate reduced fibrotic tissue by enhancing reperfusion of damaged muscle tissue.[29]

Another woundered subjects about PRF was that gender. Endogenous estrogen could have an effect on epidural fibrosis formation after lumbar laminectomy in rats.[30] Another subject were the using of PRF in other health branches. Some previous studies have shown that PRF increases dermal and gingival fibroblast proliferation, and also increases inflammation through cytokines. These characteristics may be considered to increase fibrosis, although PRF has also been used to treat hypertrophic scars.[12,31] And also, some studies established that PRF were used in sports medicine and orthopedics as a topical agent.[32]

Fibrin in PRF functions as a support for wound tissue and guides the transformation of mesenchymal stem cells in PRF into various cell types in the wound sites.[33] PRF also stimulates osteoblast proliferation.[13] It thus exhibits regenerative effects in bone defect and soft tissue wounds. Landi et al showed that platelet gel exhibited some beneficial effects on fusion in patients undergoing posterolateral fusion.[34]

Adcon® gel (Adhesion Control in a Barrier Gel) is a barrier gel used to prevent epidural fibrosis. Some studies have reported that it reduces epidural fibrosis, whereas others have described it as ineffective in reducing epidural fibrosis and in low back pain after laminectomy.[3,10,35] Our Adcon® gel group was not significantly superior to the other groups in terms of reducing epidural fibrosis.

High molecular-weight cross-linked HA has been shown to reduce epidural fibrosis in some studies.[36–38] In our study, no superiority was observed in the HA group in terms of reducing epidural fibrosis.

In the present study, new bone formation was higher in the PRF group than in the other groups, although this was not statistically significant, and no neural tissue compression was observed. In this study, PRF can be defined as satisfying agent as compared other potential inhibitors of epidural fibrosis. The findings of this study considered together with the existing literature, suggest that local PRF may reduce epidural fibrosis in patients that were applied laminectomy.

Limitations

Immunohistochemical studies, PCR, electronmicroscopy could be used in the aim of demonstratation of preventing effect of PRF from tissue adhesion after the spnal surgery. Using light microscopy is a limitation factor for our study. The statistically non-significant results elicited at pair-wise comparisons of HA vs PRF and Adcon® gel vs PRF may be due to the small number of animals in each group. This represented a limitation of our study.

Informed consent

Informed consent was obtained from all individual participants included in the study.

Conflict of interest

The authors declare that they have no conflict of interest.

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