MEMBRANE BARRIERS TO PREVENT DURAL ADHESIONS AND VALIDATION OF A NOVEL APPROACH TO REPAIRING DURA

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INTRODUCTION

Over 2 million operations were carried out on the central nervous system each year in the United States. These include over 100,000 operations where the skull, brain, and cerebral meninges are incised and over 125,000 laminectomies exposing the spinal cord (National Center for Health Statistics, 2002). Many of these surgeries require dural repair. Although suturing the dura is often sufficient, duroplasty or dural patches may be necessary to provide water-tight closures (Foyt et al. 1996). Adhesive scarring between the dura and surrounding tissues, as well as tethering of the spinal cord to the dura is a serious surgical complication of spinal cord surgery (Quist et al. 1998; Llado et al. 1999; Llado et al. 1999).

Neurosurgeons have long used autologous human membranes to repair the dura. These include posterior atlanto-occipital membrane (Tubbs et al. 2002), periosteum (Vrankovic et al. 1992), or muscle sheath (Thammavaram et al. 1990; Kudo et al. 2000). Heterologous human materials are also popular. For example, some neurosurgeons use acellular human dermis Alloderm® (Warren et al. 2000). Many use chemically treated human cadaver dura (Sharkey et al. 1958; Macfarlane and Symon 1979; Baglaj et al. 1992; Laus et al. 1992; Filippi et al. 2000; Dufrane et al. 2002; Dufrane et al. 2003; Caroli et al. 2004). Cadaveric dura mater, however, have been associated with Creutzfeldt-Jakob disease (Bartosz and Vasterling 1994; Brown et al. 2000; Croes et al. 2001; Hannah et al. 2001; Kimura et al. 2001; Lang et al. 2001; Munoz Guerra 2001; Blattler 2002; Nishida et al. 2002; Boutoleau et al. 2003; Hamada et al. 2003; Mochizuki et al. 2003), bacterial infections (Jallo et al. 1999), and immune responses (Johnson and Thompson 1981).

Neurosurgeons sometimes use animal materials for dural repair, i.e. porcine intestinal submucosa (Cobb et al. 1996; Cobb et al. 1999), peritoneum (Xu et al. 1988), pericardium (Gok et al. 1995), and dermis (O'Neill and Booth 1984), as well as ovine (Parizek et al. 1996) and bovine pericardium (Parizek et al. 1989). Dura-Guard® is a non-absorbable biomaterial derived from bovine pericardium. Many dural substitutes are collagen-based (Harat et al. 1989; Collins et al. 1991; Pietrucha 1991; Bidzinski et al. 1993; Laquerriere et al. 1993). Collagen attracts fibroblasts (Unsal et al. 1999) but some biosynthetic collagen (Mello et al. 1997) and propylene-treated collagen (Maher et al. 2003) do not. Hyaluronic acid may reduce peridural or epidural scarring (Abitbol et al. 1994). Dura-Guard® from Synovis Life Technologies is a collagen patch. Integra recently launched DuraGen Plus™, a collagen matrix graft for cranial and spinal dural repair.

The most popular commercial non-degradable synthetic dural substitute is Gore-Tex® or expanded polytetrafluoroethylene membrane ePTFE (Yukna 1992; Yamagata et al. 1993; Inoue et al. 1994; Park and Tator 1998; Aliredjo et al. 1999; Vinas et al. 1999). However, ePTFE has low adhesiveness to fibrin glue and must be sewed. Gore-Tex® sutures reduce water leakage through suture holes (Yamamura et al. 1999). Absorbable polyglycolic acid mesh soaked with fibrinogen fluid placed on suture lines helps prevent CSF leakage (Nagata et al. 1999; Nagata et al. 1999). Spraying fibrin onto suture margins also may help (Terasaka et al. 1994). Ion-beam irradiation increases adhesiveness of ePTFE (Takahashi et al. 2003) and allows it to be glued. Some neurosurgeons repair the dura first and then place a ePTFE sheet between the repaired dura and surrounding tissues (Kawaguchi et al. 2003). Recent studies suggest that epidural abscesses may develop under ePTFE duroplasties (Uemura et al. 2002). Commercial ePTFE products include Preclude® Dura Substitute (Mohsenipour et al. 1998; Barbolt et al. 2001).

Other permanent materials have been used for dural repair. For example, neurosurgeons have used polyurethane-polysiloxane-carbonate block copolymer (Sakas et al. 1990), Teflon (Teng and Papatheodorou 1963), Vinyon (Teng 1961), silicon-coated Dacron or Durafilm, and vicryl mesh (Maurer and McDonald 1985; Keller et al. 1989; Nussbaum et al. 1989; Verheggen et al. 1997). Polypropylene mesh (Marlex®) is a mainstay in general surgery for hernia repair but may lead to delayed inflammation and meningioma formation when used to repair dura (Cohen et al. 1989). Neuro-Patch® is a polyester urethane (Auque et al. 2000; Raul et al. 2003), Vicryl Collagen is a resorbable mesh of polyglactin 910 coated with bovine collage (Meddings et al. 1992; Van Calenbergh et al. 1997) but cause postoperative infections (Gudmundsson and Sogaard 1995).

Earlier products include Polyactive membrane sheets (Cook et al. 1994). ZenoDERM (now called AcryDerm) formerly produced by Ethicon was an absorbable wound dressing that neurosurgeons used to repair dura (Harat et al. 1989). Durafilm is silicon-coated Dacron (Fisher and Six 1983; Ongkiko et al. 1984). Silastic was once a popular dural patch material (Miyamoto et al. 1983; Boop and Chadduck 1991; Thompson et al. 1994). Although silastic is well known to be associated with delayed hemorrhagic complications (Banerjee et al. 1974; Miyamoto et al. 1983; Simpson and Robson 1984; Misra and Shaw 1987; Ng et al. 1990; Awwad et al. 1991; Gondo et al. 1991; Berrington 1992; Fontana et al. 1992; Nixon et al. 1994; Ohbayashi et al. 1994; Thompson et al. 1994; Robertson and Menezes 1997) and fibrosis (Siccardi and Ventimiglia 1995), it is still being used for repair of spinal dysraphism (Ohe et al. 2000).

Recent studies suggest that resorbable materials can be used to repair the dura. Bioresorbable artificial dura mater made from two L-lactic acid-epsilon-caprolactone (50% L-lactic acid, 50% epsilon caprilactone (Yamada et al. 1997; Yamada et al. 2002) is eventually replaced by collagenous tissue. Another promising material is a composite of polyglycolic acid (PGA) mesh, collagen sponge, and gelatin sponge (Matsumoto et al. 2001). A bioabsorbable composite sheet made from L-lactic acid-epsilon-caprolactone (50:50) copolymer and a poly glycocolic acid non-woven fabric apparently produced excellent regeneration of rabbit dura without infection, cerebrospinal fluid leakage, adhesion or damage to underlying cortex, or calcification (Ikada 1998). The Codman ETHISORB® dural patch is an absorbable polyglactin 910 and polydioxanone (Barbolt et al. 2001). Likewise, Neuroplast® is the biodegradable elastin-fibrin material (San-Galli et al. 1996).

The optimal dural graft should be able to provide watertight closure, not adhere to the underlying neural or surrounding tissues, and encourage growth of normal dura. These qualities are contradictory. If one used an inert material that does not adhere to tissues, the material will not stick to tissues, may be difficult to manipulate, and may not form watertight seals. However, if one used material that sticks to tissues, adhesive scarring forms between the material and surrounding tissues. Permanent materials may wear away or damage tissues around them, causing inflammation and hemorrhage. Resorbable material must encourage cellular replacement or else the dural repair is likely to fail. No currently available dural graft material satisfies all these conditions.

Repair of the dura after penetrating lesions of the spinal cord is an important problem both in the clinic and laboratory. In the clinic, surgeons are reluctant to operate on the spinal cord for fear of producing dural adhesions and tethering of the spinal cord. In the laboratory, epidural adhesion to the laminectomy scar is probably the main reason that so few laboratories have published studies of cell transplants into spinal cords more than 2 weeks after injury. Despite great interest and pressure to do such experiments, few efforts to transplant cells into chronically injured spinal cord injury have been successful. This is due to the difficulty of re-exposing the chronically injured spinal cord more than 2 weeks after injury because of scarring at the laminectomy site. Also, many laboratories use transect and hemisect the spinal cord but do not repair the dura. Leaving the dura open allows invasion of fibroblasts and establishment of a dense collagenous scar in the spinal cord, a major obstacle to regeneration in the spinal cord. Therefore, identification of graft materials that prevent dural adhesions will have a major impact on both clinical and laboratory practice.

METHODS

We studied over 100 Long-Evan's hooded rats in three sets of experiments. The first set assessed biomaterials to prevent spinal dural adhesion at 2 weeks after a 12.5 mm weight drop cord contusion. The second set examined the ability of the thin resorbable PCA/PLA fabric to prevent long-term (6-week) dural adhesions after a 12.5 mm contusion. The third set of experiments utilized the thin resorbable fabric to repair a dural opening after a three-quarter section of the spinal cord. The rats were examined histologically 6 weeks after injury.

Experiments

Table 1 summarizes the experiments that were carried out.

- Experiment 1. Comparison of three epidural barriers at 2 weeks. We assessed spinal cords histologically at 2 weeks after a laminectomy (n=2/barrier type), 12.5 mm (n=4/barrier type), and 25.0-mm (n=4/barrier type) weight drop contusion of the T13 cord, and placed three types of biomaterials on the dura:
  - *Non-resorbable fabric*: a thin (100μ) porous, non-biodegradable polyvinylidene fluoride copolymer fabric made by electrostatic spinning.
  - *Thin resorbable fabric*: a thin (100μ) porous, 60/40 PLA/PCL (polylactic acid/polycaprolactone) absorbable fabric made by electrostatic spinning.
  - *Thick resorbable film*: a thicker (120-150μ), non-porous, biodegradable 60/40 PLA/PCL absorbable film made by compression moding.
- Experiment 2. Effect of thin resorbable PCA/PCL fabric on dural adhesion at 2-6 weeks. We initially studied 10 rats and found that placing a piece of autologous fat on the epidural barrier suppressed scar formation. We studied three groups of 10 rats, contused the T13 cord with a 12.5 mm weight drop, placed a thin PCA/PCL resorbable fabric epidurally with a piece of autologous fat tissue on top, and assessed the rats histologically at 6 weeks after a 12.5-mm contusion.

- Experiment 3. Use of thin resorbable PCA/PLA fabric for dural repair. We initially studied a group of 10 rats with laminectomy and dural repair only, to develop and optimize the sandwich dural repair method. Resorbable barriers were placed epidurally and subdurally to repair a surgical dural opening after a three-quarter section of the T10 spinal cord. We then studied 10 rats, doing a laminectomy, surgically exposed the spinal cord, did a three-quarter section of the spinal cord, used the sandwich dural repair method to close the dura, and assessed the rats histologically at 6 weeks after injury.

Spinal Cord Injury Methods

We used two methods to injure rat spinal cords.

Spinal Cord Contusion. In the first and second sets of experiments, we anesthetized Long-Evan's hooded rats (77±1 day old) with intraperitoneal pentobarbital (45 mg/kg female, 65 mg/kg male), exposed the T13 spinal cord with a T9-10 laminectomy, dropped a 10 gram weight 12.5 (mild) or 25mm (severe) onto T13 cord using the Impactor. The T13 spinal cord is located at T9-10 vertebral level. After establishing hemostasis and carefully washing the epidural surface with sterile saline, we placed three types of biomaterials onto the dorsal dural surface of the spinal cord: thin non-resorbable polyvinylidene fabric (NF), thin resorbable PCA/PCL fabric (RF), and a thick resorbable PCA/PCL film barrier (RB). In the first set of experiments, we placed the material on the dural surface and examined the rats histologically at 2 weeks after injury. In the second set of experiments, we placed a thin resorbable fabric on the dural surface, placed a piece of fat on top of the membrane, and assessed the rats histologically at 6 weeks after injury. In addition, we assessed barrier adhesion to the dura at 2- and 4-weeks after injury.[1]

Three-Quarter Section of the Spinal Cord. In the third set of experiments, we did a T9-10 laminectomy to expose T13 spinal cord, cut a U-shaped dural flap with microscissors, and then cut three-quarter of the spinal cord with a sharp razor blade fragment. After establishing hemostasis, we placed a thin resorbable membrane between the spinal cord and dura, lowered the dural flap, and placed another thin resorbable membrane on top of the opening. Leaving a quarter of the spinal cord prevented the separation of the cord. We then placed a piece of autologous fat to hold the barrier in place. Paravertebral muscle was closed with two silk stitches rostral and caudal to the dural repair, to avoid putting undue pressure on site.

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[1] Re-exposing the Spinal Cord and Removing of Epidural Barrier. At 2 and 4 weeks after injuring the spinal cord and placing the resorbable fabric, we anesthetized the rats with isoflurane (5% induction, 2% maintenance with a nose-cone), surgically re-exposed the laminectomy site by excising any laminectomy scar, and gently removed the epidural barrier. The surgeon recorded the difficulty of removing the epidural barrier: 0=no difficulty, 1=slight adhesion, 2=barrier removable with some dissection, 3=barrier not removable without tearing the dura. After exposing the spinal cord, we transplanted cells (olfactory ensheathing glia) into the spinal cord, placed a thin resorbable membrane epidurally, closed the laminectomy site, and evaluated the rats for locomotor recovery and 12-week histological appearance of the spinal cord.

Histology

Perfusion-fixation, sectioning, and staining. We anesthetized and perfused the rats at 2 or 6 weeks after injury with 4% paraformaldehyde (300 ml over 10 minutes after an initial saline wash). The fixed spinal cords and columns were removed, decalcified, embedded in paraffin, serially sectioned in the saggital plane, and stained with Mallory trichome, which colors nuclei dark brown, cytoplasm red, and collagen blue. The sectioning and staining were done by Neuroscience Associates (Nashville, TN). Mallory trichome stains scar tissue or collagen (blue), cells (red), including membranes (dark blue) and inflammatory infiltrates (bright red). The biomaterials used in this study do not take up stain and manifest as clear unstained areas or grayish-blue although degrading PLA/PCA membranes can acquire a reddish-brown color by 6 weeks.

Imaging. The microscope slides were examined on a microscope and then scanned with a Nikon scanner at 4000 dpi resolution. The images were stored on hard disk and reviewed by unbiased observers who examined and scored five representative images for epidural and subdural adhesions, and for the presence of dural incorporation and inflammatory infiltrates. In all the figures shown in this report, the images show saggital sections with rostral (head) side of the spinal cord on the left and caudal (tail) side on the right. Inset frames indicate areas that are enlarged and shown in the same figure. The animal (experiment) code is given in the figures. Although no scale bars are given in the figures, distances can be estimated from the 3.5 mm diameter of the spinal canal at the T9-10 vertebral level. The laminectomy are typically 8-12 mm wide, depending whether the rat is male or female.

Dural Adhesion Score (DAS). To quantify dural adhesion, we devised an ordinal scale to represent unique combinations of observations concerning epidural adhesions, subdural adhesions, dural scaring, and scar extension into the cord. Epidural and subdural adhesions have four grades: none (0%), occasional (<10% of area below the laminectomy), extensive (10-50%), and severe (>50%). The percentages refer to the widths of adhesions relative to the width of the laminectomy site, i.e. adhesions that exceed over 50% of the width of the laminectomy would be "severe". Dural scarring refers to the presence of collagenous scar above and below the dura. Cord involvement refers to collagenous scar extending into the spinal cord. The Dural Adhesion Scale (DAS) combines all subscores into a single 18-point (0-17) scale which assumes that subdural adhesions are worse than epidural adhesions, that dural scarring and scar invasion into the spinal cord represent the worst forms of the condition.

Dural Adhesion Scale

Subscores	#	
A0, B0, C0, D0	0	Normal (no adhesions)
A1, B0, C0, D0	1	Occasional epidural adhesions with no subdural adhesions
A0, B1, C0, D0	2	Occasional subdural adhesions with no epidural adhesions
A1, B1, C0, D0	3	Occasional epidural and occasional subdural adhesions
A2, B0, C0, D0	4	Extensive epidural adhesions with no subdural adhesions
A2, B1, C0, D0	5	Extensive epidural adhesions with occasional subdural adhesions
A0, B2, C0, D0	6	Extensive subdural adhesions with no epidural adhesions
A1, B2, C0, D0	7	Occasional epidural adhesions with extensive subdural adhesions
A2, B2, C0, D0	8	Extensive epidural adhesions with extensive subdural adhesions
A3, B0, C0, D0	9	Severe epidural scarring with no subdural adhesions
A3, B1, C0, D0	10	Severe epidural scarring with occasional subdural adhesions
A3, B2, C0, D0	11	Severe epidural scarring with extensive subdural adhesions
A0, B3, C0, D0	12	No epidural scarring with severe subdural adhesions
A1, B3, C0, D0	13	Occasional epidural adhesions with severe subdural adhesions
A2, B3, C0, D0	14	Extensive epidural adhesions with severe subdural adhesions
A3, B3, C0, D0	15	Severe epidural adhesions and severe subdural adhesions
A3, B3, C0, D1	16	Severe epidural & subdural scarring without extending into cord
A3, B3, C1, D1	17	Severe epidural, subdural scarring extending into the cord

Explanation: The Dural Adhesion Scale has 18 categories (0-17). The subcategories A and B refers to epidural and subdural scarring respectively: 0=none (0%), 1=occasional (1-10%), 2=extensive (11-50%), 3=severe (51-100%). The percentages refer the width of the laminectomy site. For example, an adhesion that involves over 50% of the width of the laminectomy site would be "severe". Absence and presence of cord involvement are indicated by C0 and C1 respectively, while absence and presence of dural scaring are indicated by D0 and D1 respectively. The scale assumes that subdural adhesions are worse than epidural adhesions. Dural scarring indicates the presence of "severe" collagenous scar above and below the dura. Cord involvement refers to extension of collagenous dural scar into the spinal cord.

Scoring. Observers scored five representative saggital sections from each animal for the presence and severity of epidural and subdural adhesions. To adapt the DAS score for multiple images, a grade of 4 (severe) required that adhesions were present in at least 3 images and occupied over 50% of the laminectomy site in each of the three images. A score of 3 (extensive) required that adhesions were present in at least 2 images and occupied 11-50% of the laminectomy site. A score of 2 (occasional) required that adhesions occupied 1-10% of laminectomy site in at least one image. A DAS score 16 (dural scarring) means collagenous scar (blue) encasing dura. A DAS score of 17 means collagenous scar extending into the spinal cord.

Statistical Analysis. We used analysis of variance (ANOVA) to assess whether significant differences of DAS were present among the treatment groups. We then used the Bonferroni-Dunn post-hoc tests to compare pairs of treatment groups. In the analysis, we sought to determine whether dural adhesion was related to injury severity, whether the different barriers reduced adhesion compared to matched control injured groups, and whether adhesions at 6 weeks were better or worse than adhesions at 2 weeks, The post-hoc analyses therefore focused on the following ten comparisons of treatment groups. The codes below give the weeks after injury (2w, 6w), the biomaterials used (CO=control, NF=Nonresorbable Fabric, RB=Resorbable Barrier, RF=Resorbable Fabric), spinal cord injury severity in mm weight drop (@12.5, @25.0):

1. More severe spinal cord injuries are associated with higher DAS scores, i.e. control (CO) spinal cords injured with a 12.5 mm and 25.0 mm weight drop at 2 weeks, i.e. 2wCO@12.5 vs. 2wCO@25.0.
2. Non-resorbable fabric (NR) reduces DAS scores after 12.5 mm contusions, i.e. 2wCO@12.5 vs. 2wNF@12.5
3. Non-resorbable fabric (NR) reduces DAS scores at 2 weeks after 25.0 mm contusions, i.e. 2wCO@25.0 vs. 2wNF@25.0.
4. Resorbable barrier (RB) reduces DAS scores at 2 weeks after 12.5 mm contusions, i.e. 2wCO@12.5 vs. 2wRB@12.0
5. Resorbable barrier (RB) reduces DAS scores as 2 weeks after 25.0 mm contusions, i.e. 2wCO@25.0 vs. 2wRB@25.0.
6. Resorbable fabric (NF) reduces DAS scores at 2 weeks after 12.5 mm contusions, i.e. 2wCO@12.5 vs. 2wNF@12.5.
7. Resorbable fabric (NF) reduces DAS scores at 2 weeks after 25.0 mm contusions, i.e. 2wCO@25.0 vs. 2wNF@25.0.
8. Resorbable fabric (NF) reduces DAS scores at 2 weeks after 25.0 compared to 12.5 mm, i.e. 2wNF@12.5 vs. 2wNF@25.0.
9. Resorbable fabric (NF) reduces DAS scores at 6 weeks compared to 2 weeks after 12.5 mm contusion, i.e. 2wCO@12.5 vs. 6wNF@12.5.
10. Resorbable fabric (NF) reduces DAS scores at 6 weeks compared to 2 weeks after 12.5 mm contusion, i.e. 2wNF@12.5 vs. 6wNF@12.05.

To reduce the probability of false positives due to multiple comparisons, we adjusted the criterion for significance by a Bonferroni coefficient of 10 from $p<0.05$ to $p<0.005$ (i.e. $p=0.05/10$). A commercial program "Statview 5.0" was used to carry out the ANOVA and post-hoc analyses. Due to the small numbers of animals in the laminectomy control groups, they were not included in the analysis. Likewise, because the dural adhesion scores were designed for contusion injuries, we did not include the scores of the dural repair experiments in the analysis.

Post-injury Care

The rats were maintained postoperatively on a temperature-controlled environment. We had less than 10% mortality in the experiments.

1. *Dehydration.* Rats that show evidence of dehydration (tested by pinching the skin and observing how rapidly the skin fell back) receive 5-10 ml of saline subcutaneously.
2. *Bladder paralysis.* All the rats received twice daily bladder expression and a broad-spectrum cephalosporin antibiotic (kefazolin 30 mg/kg subcutaneously) daily for one week to prevent urinary tract infections. Bladder expression was continued as necessary until the rats had <1 ml of urine in the morning.
3. *Recurrent bladder infection.* In addition to Kefazolin, rats that develop urinary tract infections (identified from cloudy hemorrhagic urine) are segregated from the remainder of the colony, and received the fluoroquinolone antibiotic Baytril (8 mg/kg/day) for 7-10 days. If the rat does not respond to this treatment, it is euthanized.
4. *Autophagia and autotomy.* Rats were examined daily for evidence of any lesions below the injury site. Rats that bite themselves below the injury site were scored: 1=hair loss and skin inflammation, 2=penetration of dermis exposing subcutaneous layer, 3=penetration of subcutaneous layer exposing muscle, 4=penetration of muscle exposing internal organs. Autophagia refers to licking and biting of skin in the dermatomes close to the injury site. Autotomy refers to biting of the toes or feet. Rats that show any evidence of autophagia or autotomy were treated with daily oral acetaminophen ("Baby Tylenol Solution", cherry-flavored, 64 mg/kg) until the skin lesions are completely healed.

Functional Measures.[2] Two observers scored the rats weekly after injury, using the Basso-Beattie-Bresnahan (BBB) locomotor scale {Basso, 1996 #123}. This 0-21 ordinal scale assesses open-field locomotor performance of rats. The first third of the scale (0-7) represent non-locomotor movements of the hindlimbs. The second third of the scale (9-14) represent progressively coordinated weight-supported locomotion. The remainder of the scale (15-21) indicates better foot placement and balance. The rats are placed in an open-field and observed for four minutes during which each observer assigns separate scores for the left and right legs. The final BBB score represents a consensus by the two observers.

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[2] Because some rats are still being evaluated for functional recovery, we do not present the functional outcomes in this report but the description of the method is included so that the results can be added later.

RESULTS

The results will be described in discussed in four sections. The first section describes short-term (2-week) dural adhesions that occur after laminectomy and spinal cord injury produced by a 12.5 mm and 25.0 mm weight drop. The second describes the effects of placing four types of epidural barriers on epidural and subdural adhesions after 12.5 and 25.0 mm weight drop contusion of the T9-10 spinal cord, at two weeks after injury. The third section describes the long-term effects (6 weeks) of placing a thin resorbable epidural barrier and a piece of autologous fat at the laminectomy site. The fourth section describes the use of thin resorbable membrane to repair the dura and prevent epidural adhesions at 6 weeks after a three-quarter section of the spinal cord. The implications of the results will be discussed in each section because results of each experiment influenced the design of the subsequent experiments.

Short-Term (2 weeks) Dural Adhesions after Spinal Cord Contusion

Laminectomy alone produced a dense collagenous scar by 2 weeks. The scar can be readily seen as dark blue tissue on Mallory trichrome stained saggital sections of the spinal column. **Figure 2** shows a section of a spinal column at 2 weeks after laminectomy without spinal cord injury. Closer inspection of the dura interface at the laminectomy site shows occasional epidural adhesion to the laminectomy scar, particularly at the laminectomy edges. However, a thin layer of loose tissue matrix interposed between the laminectomy scar and dura, suggesting that the adhesion was not tight. This is consistent with our surgical experience indicating little or no difficulty removing the laminectomy scar when the spinal cord has not been injured. The spinal cord appears to be normal, with no evidence of cell loss. No subdural adhesions were present.

A 12.5 mm contusion produced extensive (11-50% of the laminectomy width) epidural adhesions in 3 rats and occasional epidural adhesions (1-10% of laminectomy width) in 1 rat at two weeks after injury. One rat had extensive and 3 out of 4 rats had occasional subdural adhesions. **Figure 3** shows one of the spinal cord with extensive epidural adhesions and occasional subdural adhesions. Spinal cord damage was restricted to the contusion site. **Figure 4** shows the worst case, a spinal cord contused with a 12.5-mm weight drop resulting in extensive epidural and extensive subdural adhesions. The dura adhered tightly to the blue laminectomy scar and the underlying spinal cord. **Figure 5** shows two other spinal cords injured at 2 weeks after a 12.5 mm contusions. One case had extensive epidural and occasional subdural while the other had occasional epidural and subdural adhesions.

A 25-mm contusion injury resulted in severe (3 rat) or extensive (1 rats) epidural adhesions and extensive (1 rat) or occasional (3 rats) subdural adhesions at 2 weeks after injury. **Figure 6** shows an example of a spinal cord injured with a 25.0 mm weight drop. The dura adhered tightly to the dense collagenous (blue) laminectomy scar. Arachnoid proliferation and loose subdural adhesion were present. As expected, spinal cord damage was more extensive than after 12.5 mm contusions with Wallerian degeneration of spinal tracts extending well beyond the contusion site. **Figure 7** shows another example where the dura adhered to the laminectomy scar. Note the extensive tissue damage and the presence of small syringomyelic cyst (an expansion of the central canal).

In summary, laminectomy alone produced occasional epidural adhesions and no subdural adhesions (i.e., mean±sem DAS of 1.00±0.00) in 2 rats at 2 weeks. A 12.5 mm weight-drop contusion caused extensive epidural adhesions in 3 of 4 rats and occasional subdural adhesions in 1 of 4 rats (DAS 3.25±0.85). A 25.0 mm weight drop contusion produced extensive epidural adhesions in all 4 rats and occasional subdural adhesions in 3 rats (DAS 4.75±0.25). So, injury severity increased dural adhesions.

Comparison of Epidural Barriers

Non-resorbable epidural barriers produced severe epidural adhesions in 4 out of 4 rats injured with a 12.5 mm weight drop contusion and occasional subdural adhesions in one rat. **Figure 8** shows a non-resorbable epidural barrier on a spinal cord at 2 weeks after a 12.5 mm contusion. A red-stained border of inflammatory cells surrounded the barrier. The rostral end of the barrier had folded where the dura adhered to the laminectomy scar. The dura also adhered to the inflammatory layer around the barrier. Subdural adhesions were minimal. **Figure 9** shows a spinal cord injured with a 12.5 mm contusion. A red-stained inflammatory border surrounded the non-resorbable barrier. Dura adhered to the inflammatory border, occupying over 50% of the laminectomy width, indicating severe epidural adhesions.

The non-resorbable epidural barrier likewise caused severe epidural scarring in 4 out of 4 rats contused with a 25.0 mm weight drop. One rat had extensive and the rest occasional subdural adhesions. **Figure 10** shows a spinal cord contused with a 25.0 mm weight drop. An inflammatory infiltrate surrounded the non-resorbable barrier. A cyst had developed under the barrier. The dura adhered to the underside of the cyst. Scar tissue had crept around the rostral edge of the barrier. The injury caused widespread Wallerian degeneration and a syringomyelic cyst in the rostral cord with microcystic changes in the caudal cord.

A thick resorbable barrier also did not prevent epidural adhesions after 12.5 mm contusions. Three of the 4 rats had severe epidural adhesions and one had occasional epidural adhesions. Two had occasional subdural adhesions. Because the membrane was thick, it compressed the spinal cord. **Figure 11** shows a spinal cord injured with a 12.5 mm contusion and covered with a thick resorbable epidural barrier. The thick barrier was clearly visible as unstained material at the laminectomy. A red-stained inflammatory infiltrate was present on the dorsal surface of the barrier. Blue collagenous scar tissue had crept around both rostral and caudal edges to envelope the barrier. Dura adhered to the collagenous scar on the ventral surface of the barrier. The barrier compressed the spinal cord. **Figure 12** shows another spinal cord with a thick resorbable barrier enveloped in collagenous scar. Dura adhered to the scar. An inflammatory infiltrate was present on the dorsal surface of the barrier. Again, the bulky barrier was compressing the spinal cord. **Figure 13** also showed a 12.5 mm contused cord with thick resorbable membrane enveloped by the laminectomy scar. Dura adhered to the ventral barrier surface. Unlike the previous two examples, no inflammatory infiltrate was present on the dorsal surface of the barrier.

The thick resorbable barrier did not prevent epidural adhesions in rats injured with a 25.0 mm weight drop. Two of 4 spinal cords had extensive and two had occasional epidural adhesions while two rats had occasional and 2 rats had extensive subdural adhesions. The thick barrier compressed the cord in two rats. **Figure 14** shows a thick resorbable barrier protruding into the spinal canal. Dura adhered partly to the barrier. The cord showed extensive Wallerian degeneration in the rostral and caudal cord. **Figure 15** shows a thick resorbable barrier compressing the cord. A long syringomyelic cavity extended 3 segments in rostral cord. The cord had complete cell loss at the contusion site with extensive Wallerian degeneration in the rostral and caudal cord.

The thin resorbable barrier reduced epidural adhesions of spinal cords contused with a 12.5 mm weight drop. Three of 4 spinal cords had occasional epidural adhesions with minimal subdural adhesions. One rat had extensive epidural adhesions but no subdural adhesions. **Figure 16** shows a spinal cord contused with a 12.5 mm weight drop and then covered with a thin epidural resorbable barrier. The undulating barrier spanned the laminectomy site. A loose acellular matrix separated the dura from the barrier. An inflammatory infiltrate was present on the dorsal surface of the barrier. Subdural adhesions were minimal while the cord damages was limited to the injury site. **Figures 17-18** show two examples where the thin resorbable epidural barrier prevented adhesion. Note the absence of Wallerian degeneration in all the spinal cords.

The thin resorbable epidural barrier also reduced epidural adhesions after 25 mm contusions. Collagenous scar had crept around barrier edges, allowing extensive epidural adhesions in 2 rats and occasional epidural adhesions in 2 rat with minimal or occasional subdural adhesions. **Figure 19** shows a spinal cord with extensive epidural adhesions to collagenous scar below a resorbable epidural barrier. The cord showed extensive Wallerian degeneration rostral and caudal to the contusion site, as expected from a 25 mm weight drop contusion. However, as shown in **figure 20**, in other sections of the same spinal cord (JJ01-G3m) where the barrier had covered the laminectomy site and scar tissue had not crept around the barrier edge, the dura did not adhere tightly to the barrier. A thin layer of red surrounding the barrier, representing a thin layer of inflammatory macrophages. There were no subdural adhesions.

In summary, the non-resorbable barrier became coated with an inflammatory layer of cells and dura adhered to this layer after 12.5 mm (DAS 9.25±0.25) and 25.0 mm contusions (DAS 9.75±0.25). Thick resorbable barriers compressed the spinal cord and increased epidural adhesions after 12.5 mm (DAS 8.00±1.68) and 25.0 mm contusions (DAS 6.25±0.75). Thin resorbable barriers, however, reduced epidural adhesions after 12.5 mm (DAS 2.50±0.50) and 25.0 mm contusions (DAS 3.25±0.48). The thin resorbable membrane appears to be the best material for preventing both epidural and subdural adhesions.

Thin Resorbable Barriers at 6 weeks after 12.5 mm contusion

In the preceding experiments, collagenous scar tissue crept around the barrier edges to coat the barriers, resulting occasional or even extensive epidural adhesions. We therefore carried out experiments to find ways to prevent this situation. Tucking the barrier under the laminectomy edge did not always prevent the collagenous scar formation below the barrier. However, when we placed a piece of autologous fat on the dorsal surface of the epidural barrier, it not only held the barrier in place but also prevented the formation of the laminectomy scar above the barrier. We used this approach to prevent dural adhesions for 6 weeks after a 12.5 mm contusion in 10 rats.

At 6 weeks after a 12.5 mm contusion, the thin resorbable barrier with a piece of autologous fat reduced but did not completely prevent epidural adhesions in 8 of 10 experiments. **Figures 21-37** show both saggital sections of spinal cords, except for **Figure 35** which show the cord in horizontal sections. One cord had occasional epidural adhesions (DAS=1). Four cords had occasional subdural adhesions with no epidural adhesions (DAS=2). Five cords had occasional epidural and subdural adhesions (DAS=3). The mean DAS score was 2.40±0.22.

Mean dural adhesion scores (DAS) suggest that the resorbable fabric reduced dural adhesions at 2 and 6 weeks. **Figure 38** shows a graph of the mean DAS scores. ANOVA indicated significant differences of scores among treatment groups ($F=22.45$, $p<0.0001$). The mean scores matched subjective impressions that more severe contusion injuries were associated with more dural adhesions (DAS +4.25, $p=0.0002$). The nonresorbable fabric (NF) aggravated dural adhesions after 12.5 mm (DAS +4.00, $p=0.0004$) but not after 25.0 mm contusions (DAS +0.25, $p=0.8072$). The thick resorbable PLA/PLC barrier (RB) had variable dural adhesion scores after 12.5 mm contusions because the thick material compressed the cord but the scores suggest that it reduced adhesions after 25 mm contusions (DAS -3.25, $p=0.0030$). The resorbable PLA/PLC fabric (RF) appeared to reduce adhesion (-2.75, $p=0.0107$) for 12.5 mm contusions although this did not reach our significance criterion of $p<0.005$). Finally, 6-week DAS scores in rats treated with the resorbable fabric were significantly (-2.85, $p=0.020$) from DAS scores of untreated rats at 2 weeks after 12.5 mm contusion.

In summary, the thin resorbable fabric reduced but did not completely prevent epidural and subdural adhesions after a 12.5 mm contusion. Nine of the 10 spinal cords studied at 6 weeks continued to have occasional subdural adhesions. Four of the spinal cords had no epidural adhesions and the rest had occasional epidural adhesions. None of the rats had extensive or severe epidural adhesions that would prevent re-exposure of the spinal cord.[3] The dural adhesion scores confirmed subjective impressions that 25 mm contusions produced significantly more adhesions, that non-resorbable fabric increased adhesions, that thick resorbable barrier may reduce adhesions but compressed the cord, and that the resorbable fabric prevented adhesions at 2 and 6 weeks after 12.5 and 25.0 mm contusions.

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[3] In separate experiments, we placed the resorbable PCA/PCL fabric in two groups of 10 rats, re-exposed the laminectomy sites at 2 weeks and 4 weeks after a 12.5 mm contusion. In all the rats, the laminectomy scar could be easily removed and exposed the spinal cord with no difficulty, confirming that the thin resorbable fabric epidural prevented adhesions that would have interfered with re-exposing the spinal cord at 2 and 4 weeks after surgery. From past experience, we know that we cannot expose the spinal cord at 2 weeks after a 12.5 mm contusion without tearing the dura or injuring the spinal cord. Note that once we removed the barrier, histological analysis would not have told us very must. We therefore elected to transplant olfactory ensheathing glial cells into these rats, place another epidural fabric, and observed locomotor recovery and do long term histological analysis of the rats at 12 weeks after injury. Although these experiments are still ongoing and therefore double-blinded, none of the treatment groups have worse locomotor scores than expected.

Sandwich Dural Repair Experiments

We systematically tested approaches to using the thin resorbable fabric to repair dural openings. In 10 rats, we placed a resorbable fabric barrier either epidurally or subdurally and found that neither sealed the dura. In all cases, fibroblasts had invaded into the spinal cord by 2 weeks, producing dense collagenous scars with severe epidural and subdural adhesions. However, when we inserted a resorbable fabric underneath the dura and "sandwiched the dura" with an epidural barrier, this worked. We experimented with several shapes of dural flap, as opposed to a linear cut of the dura. The shape of the dural flap was important for placement of the subdural membrane.

We decided to do a three-quarter section of the spinal cord for several reasons. First, a transection of the spinal cord would have allowed the two ends of the spinal cord to separate. Second, a three quarter section can be definitively confirmed neurologically shortly after the surgery, by the presence of an intact supraspinal responses to ipsilateral hindpaw pinch and absence of such responses from pinching the contralateral hindpaw, as well as flexion reflexes from both hindlimbs indicative of intact lumbosacral cord. Rats recover walking with a three-quarter section and this would confirm that a quarter of the spinal cord was left. Third, a three-quarter section would still be a severe injury and should have produced marked Wallerian degeneration of spinal tracts in rostral and caudal spinal cord. Fourth, the Mallory trichrome stain should absence or presence of collagenous scar invasion into the spinal cord. In short, this would be a rigorous and severe test of the ability of the dural closure method.

Figure 39 shows the method that we used to repair the dura. We did a laminectomy to expose the dura, used microscissors to cut a dural flap, cut three-quarters of the spinal cord, slipped a resorbable fabric barrier under the dura, brought the flap back, placed a piece of resorbable fabric on the dura, tucking it into the laminectomy edges, and then placed a piece of autologous fat to hold the fabric in place and to retard formation of the laminectomy scar. Paravertebral muscle was closed with two stitches rostral and caudal to the laminectomy. Skin was closed with stainless steel clips. As soon as the rats awake from this procedure, we confirmed that they are hemiplegic. We pinched the hindpaw ipsilateral to the cut and observed vocalization or supraspinal responses indicating that one spinothalamic tract was intact and the other was not. All the rats had intact withdrawal reflexes on both legs. Within 10 days after this lesion, 9 of the 10 rats engaged in open-field weight-supported coordinated ambulation with both hindlimbs.

At six weeks after surgery, histological assessment of the spinal cords indicated that the sandwich method successfully repaired the dura, prevented collagenous scar formation in the spinal cord, and minimized spinal cord damage. **Figure 40** shows a saggital view and a close-up of the sandwich repair site. The resorbable fabric was still present. The epidural barrier was more prominent than the subdural barrier, although both could be see most of the time. As in the previous set of experiment, the piece of autologous fat prevented the formation of a laminectomy scar. Cystic fatty tissue occupied the dorsal surface of the epidural membrane. Dura can be seen to enter the sandwich. A loose acellular matrix was present between the spinal cord and the subdural barrier. No collagen was present at either barrier. The spinal cord lesion itself was a circumscribed cyst with no loss of cellular substance (red) around the cyst and no Wallerian degeneration of spinal tracts. **Figure 41-42** show additional views of the same spinal cord, including one section where the cut of the cord can be seen to traverse the depth of the cord, indicating that a three-quarter section had indeed been carried out.

Collage scar can invade into the sandwich. **Figure 43** shows an example where the epidural and subdural barriers appeared to separate. The caudal edge of the epidural barrier had buckled up and blue collagenous material had crept between the two layers, more prominent in the caudal side than the rostral side of the sandwich. However, the scar tissue did not cross the dura. A non-collagenous acellular matrix occupied the space below the subdural barrier. The cord immediately surrounding the injury site did not appeared remarkably intact and showed minimal Wallerian degeneration of tracts. This suggests that even if the two layers were to separate, the dura appeared to have been successfully repaired, preventing invasion of collagenous scar into the spinal cord. **Figure 44** shows a spinal cord where the barrier was mostly resorbed but the dura appears to be intact and the spinal cord around the injury appears to be remarkably undamaged.

The subdural membrane appears to degrade faster than the epidural membrane. **Figure 45** shows a successful dural repair where intact dura clearly crossed the sandwich. A spinal root is trapped in the space between the subdural barrier and the spinal cord but subdural adhesion was minimal. Note that a laminectomy scar formed over the fat implant and the epidural barrier did not reach the rostral edge of the laminectomy but there was no scar invasion into the cord. **Figure 46** shows another successful dura repair where the subdural barrier was almost gone. The Epidural barrier was still present but beginning to break up. It is not clear why the subdural membrane would degrade faster than the epidural one.

Subdural adhesions were highly variable. **Figure 47** shows a successful dural repair with occasional subdural dural adhesions. While the dura was clearly sandwiched by the two barriers, the barriers did not appear to be adherent to the overlying fat. Note that this is another example of how a laminectomy scar formed over the fat. The underlying spinal cord shows little evidence of having had a three-quarter section, with only a small area of the cell loss. **Figure 48** shows a successful dural repair but with extensive subdural adhesions. The repaired dura was clearly intact and the underlying spinal cord was well preserved. Again, a blue laminectomy scar had formed on top of the piece of fat. While the epidural barrier did not span the laminectomy site, no scar tissue had invaded into the area of the dural repair.

The dural repair failed in only one animal out of ten. **Figure 49** shows an spinal cord where the epidural barrier was degrading and bunched up. Blue collagenous scar had invaded the ventral surface of the sandwich into the spinal cord. Dura cannot be seen in the scar tissue that had formed in the area. Contrast this with the spinal cord shown in **Figure 50** where the sandwich repair had clearly worked and continuous dura was present within the sandwich. Extensive subdural adhesions were present but the spinal cord appeared remarkably undamaged. In **Figure 51**, the sandwich repaired the dura but both the epidural and subdural fabrics had buckled. Collagenous scar tissue had invaded into the space between the barriers but not into the spinal cord. **Figure 52** shows another successful dural repair. Although a loose tissue matrix was present beneath the subdural barrier, a spinal root on one end of the sandwich was not tethered and the spinal cord appeared remarkably intact. None of the animals showed cysts, hematomas, inflammatory infiltrates, or other evidence of tissue damage around the fabric.

In summary, we used two PCA/PLA electrostatically spun fabric to "sandwich" a dural flap and successfully achieved dural repair in 9 out of 10 rats. In all but one rat, intact dura could be clearly seen within the sandwich. In several rats, collagenous scar tissue had invaded into the sandwich but did not enter the spinal cord. In the one animal where the dural repair clearly failed, there was extensive collagenous scar invasion into the spinal cord. This rat did not recover locomotion. The rest of the rats did not show invasion of collagenous scar into the spinal cord and recovered bilateral weight-supported and coordinated hindlimb locomotion. Spinal cord morphology was remarkably intact without Wallerian degeneration that would be usually associated with a three-quarter section of the cord. The PCA/PLA fabric is appears to be non-inflammatory and well tolerated in the highly inflammatory environment of spinal cord injury. This approach to repairing the dura is likely to be generalizable to a variety of surgical situations.

DISCUSSION

Our results indicate that the 60/40 PCA/PCL electrostatically spun fabric effectively reduces epidural adhesions associated in spinal cord injury in rats. This porous resorbable fabric is superior to non-porous molded film made from the same material, as well as a non-biodegradable electrostatically spun fabric made from a polyvinylidene fluoride copolymer. The fabric was easy to handle. However, scar tissue sometimes crept around the edges. Placing a piece of fat tissue on top of the barrier not only held the material in place but also suppressed formation of the laminectomy scar. The combination of the epidural resorbable fabric and an autologous fat implant prevented long-term (6-week) epidural adhesions after spinal cord contusion. Finally, the absorbable fabric can be used in a sandwich method to achieve cell-tight repair of dura with remarkable preservation of underlying injured spinal cord. These will be discussed below.

When we started these experiments, it was not clear that the 60/40 PCA/PCL electrostatically spun fabric would be the best material for preventing epidural adhesions. After all, it is porous and releases organic acid as it degrades. We were worried that the porosity of the fabric and organic acid may attract inflammatory cells. In theory, an inert and non-biodegradable material may be better. For that reason, we compared it to the PCA/PCL molded in a non-porous film and also a similar electrostatically spun fabric made of non-biodegradable polyvinylidene fluoride copolymer. The results of the comparison are clear. The non-resorbable fabric seemed to attract inflammatory cells and became coated with them (**Figure 8-10**). Dura adhered to the inflammatory layers. The PCA/PCL molded film, although made from the same material, was too stiff and thick. Scar tissues crept around the edges to coat the material and fostered epidural adhesions (**Figure 11-13**). The bulkiness of the membrane also compressed the spinal cord, aggravating the tissue damage. However, it occasionally prevented epidural adhesions (**Figure 14-15**) and may be useful for some situations.

The 60/40 PCA/PCL fabric was flexible and could be easily placed on surfaces and tucked beneath laminectomy edges to discourage scar tissue from creeping around the edges (**Figure 16-18**). But, clearly, it was not just the mechanical properties or else the non-resorbable fabric would have worked as well. Even when collagenous material did coat the PCA/PCL fabric, a loose extra cellular matrix often interposed between the dura and the fabric (**Figure 19-20**). Placing a piece of fat on the PCA/PCL fabric helped. The soft fat did not place undue pressure on the spinal cord but held the fabric in place and suppressed formation of the laminectomy scar (Figure **21-37**).

PCA/PCL resorbed relatively slowly. At 2 weeks, both the fabric and the molded film were definitely intact. However, by 6 weeks, the PCA/PCL fabric was breaking down. This can be seen in the forms of breaks in the fabric (**Figure 21**) and the fabric began to take up a reddish-brownish stain, due in part to cells (probably macrophages) that have infiltrated into the fabric. It is also curious that the subdural fabric appeared to degrade faster than the epidural fabric (**Figure 39-52**). This may be because the injury produces a strong inflammatory environment with many macrophages that secrete enzymes and contribute to faster breakdown of the fabric. But, the membrane clearly lasted 6 weeks, more than long enough for dura to heal.

The sandwich approach to repairing dura has several advantages over existing methods. First, it is quick, efficient, and does not require stitching. Stitches introduce openings for cerebrospinal fluid leakage. Second, membrane substitutes are inherently limited. For example, if a resorbable material is used, it must eventually replaced by a material, preferably cells, if it is to last for years. If the material is not resorbable, it must be compatible with tissue and be strong enough and flexible enough to last for years. In contrast, placing the dura between two resorbable layers should direct growth of the dura within the space. Eventually, when the layers do resorb, one should be left with a living membrane. Third, the layers should protect the dural repair site from adhesion to surrounding tissues.

We, however, did not expect the sandwich method to work as well as it did. In 9 of 10 rats, the dural repair resulted in an intact dura that prevented scar tissue invasion into the spinal cord. Even when scar tissue invaded below the epidural barrier, it did not penetrate into the spinal cord. While we had hoped that it would reduce fibroblast invasion into the spinal cord, we did not anticipate that it would be this effective, particularly since this was our first effort. The combination of the epidural layer and the fat implant may have contributed to these impressive results. In about half of the rats, the presence of the fat implant prevented the formation of the laminectomy scar altogether. In the rest, the laminectomy scar formed on top of the fat.

The remarkable aspect of our results, however, was the clear preservation of spinal cord under the dural repair. In these experiments, we had cut 3/4 of the spinal cord. This should have produced large cystic lesions with Wallerian degeneration of spinal tracts extending into the rostral and caudal cord. For example, contusion injury produces extensive cell loss at some distance from the impact site. However, none of the spinal cords with successful dural repairs showed tissue loss beyond the immediate cystic cavity at the cut. This requires further investigation.

One disappointing aspect of the sandwich method is the presence of adhesions between the spinal cord and the subdural fabric. We had hoped that it would prevent subdural adhesions. While it may have done so in some cases, a loose tissue matrix filled the subdural space. The origin of this matrix is unclear. While Mallory trichrome does stain collagen and some of the loose matrix is slightly bluish, it would be important to do immunohistology to confirm that few or no fibroblasts have invaded into the spinal cord and also to find out what cells are present in the matrix. It would be important to know whether the subdural adhesions develop as a result of the sandwich method. Longer-term studies should be done. For example, the subdural adhesions may clear out when the subdural membrane degrades completely.

Our results suggest many interesting new directions to go. First, because the two sides of the sandwich layer serve different purposes, it would be of interest to assess membranes with a cell-repellant side and a cell-attractive side. Second, we should consider using two different materials for the subdural and epidural layers. For example, an one experiment that can be done would be to use the non-resorbable fabric as the inner layer and the PCA/PCL fabric as the outer layer. Third, it should be possible to determine what fat does to prevent the scar formation. Although surgeons have long used fat to prevent adhesions in wounds, it is not clear whether what component of the fat is responsible for retarding scarring. Finally, it would be of interest to combine the dural repair with various drug treatments, such as steroids and anti-inflammatory drugs. Although we did not see much inflammatory infiltrates, many patients may take such drugs and it would be important to know if they have a beneficial or deleterious impact on the repair.

In conclusion, we have obtained exciting results supporting a novel method to preventing epidural adhesions and to repairing dura. The research addresses a significant unmet need in a very large market. Over 100,000 operations are carried out every year in the United States alone that require incision and repair of the dura. While neurosurgeons use a huge variety of synthetic and organic membranes as dural patches, all the available methods have significant weaknesses and have not yet achieved dominance in the field. The data strongly support the use of resorbable PCA/PLA fabric for preventing epidural adhesions and for repairing dural openings. Finally, our experiments establish robust animal models for rigorously evaluating novel biomaterials for preventing epidural adhesion and dural repair.

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Description	Barriers	Variable	Outcome Measures
Comparison of three epidural barriers on dural adhesion at 2 weeks after spinal cord injury	• Control (no barrier)	• Laminectomy only (n=2) • 12.5 mm contusion (n=4) • 25.0 mm contusion (n=4)	• 2-week histology
	• Thin nonresorbable fabric (100μ thick polyvinylidene fluoride copolymer electrostatically spun fabric)	• Laminectomy only (n=2) • 12.5 mm contusion (n=4) • 25.0 mm contusion (n=4)	• 2-week histology
	• Thick resorbable barrier (120-150μ thick PLA/PCL compression molded film)	• Laminectomy only (n=2) • 12.5 mm contusion (n=4) • 25.0 mm contusion (n=4)	• 2-week histology
	• Thin resorbable fabric (100μ thick PLA/PCL porous electrostatically spun fabric)	• Laminectomy only (n=2) • 12.5 mm contusion (n=4) • 25.0 mm contusion (n=4)	• 2-week histology
Effect of a thin resorbable epidural barrier & autologous fat at 6 weeks after injury	• Thin resorbable fabric placed epidurally with a piece of autologous fat on top	• laminectomy only (n=10) • 12.5 mm contusion with removal at 2 weeks (n=10) • 12.5 mm contusion with removal at 4 weeks (n=10) • 12.5 mm contusion (n=10)	• 6-week histology • Barrier removal at 2 weeks • Barrier removal at 4 weeks • locomotor BBB scores
Sandwich repair of surgical dural opening with two thin resorbable fabric membranes	• Thin resorbable fabric placed subdurally and epidurally with autologous fat placed on top	• Dural repair (n=10) • Dural repair plus three-quarter section of T10 spinal cord (n=10)	• 6-week histology • locomotor BBB score

Figure 1. Three sets of experiments. The first set compared 3 types of epidural barriers after laminectomy, laminectomy plus a 12.5 mm contusion, and laminectomy plus a 25.0 mm contusion. The second assessed the effects of a thin resorbable epidural barrier with autologous fat on dural adhesions at 2, 4, and 6 weeks after a 12.5 mm contusion. The third set used thin resorbable fabric to repair a dural opening with and without a three-quarter section of the underlying spinal cord.

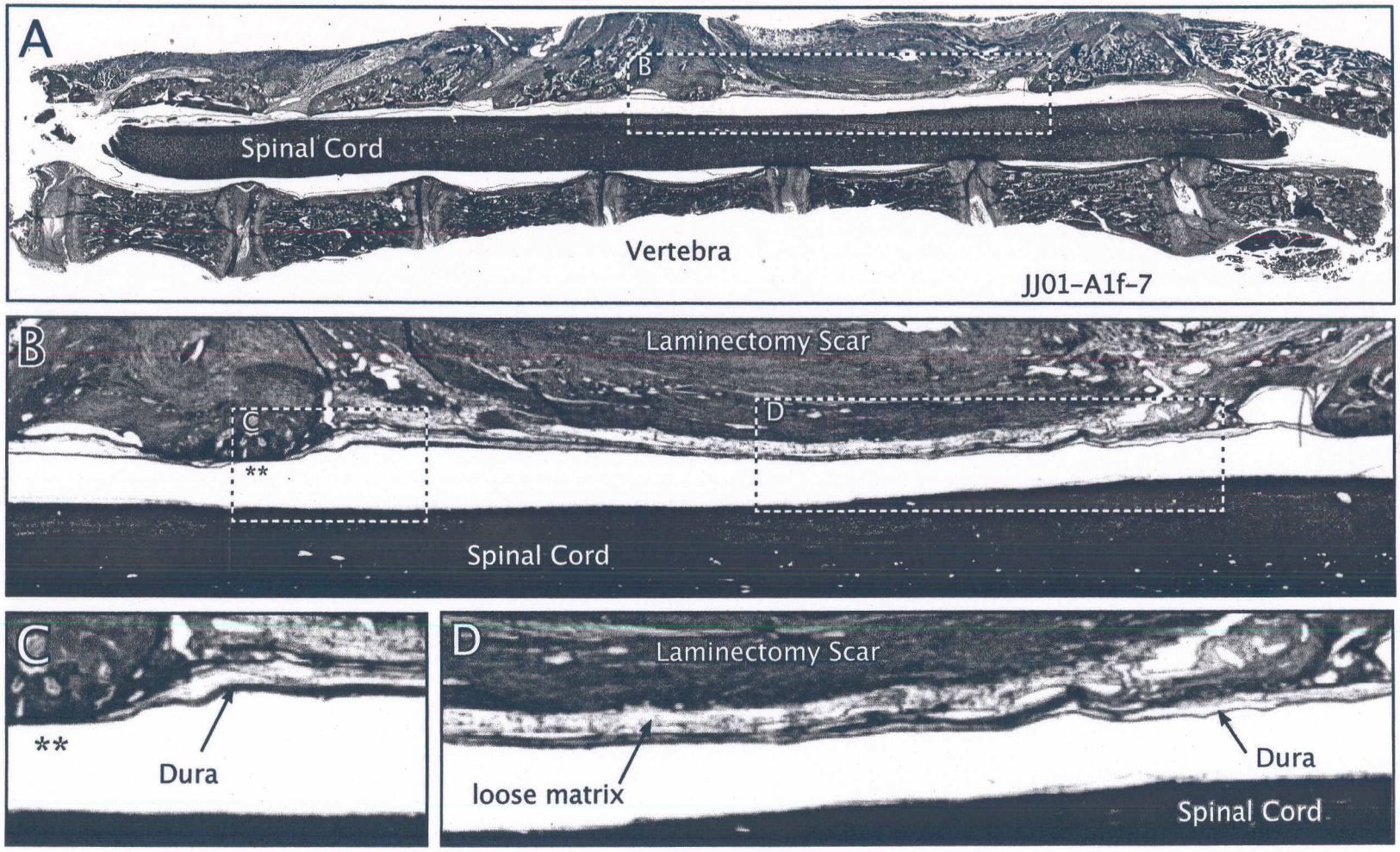


Figure 2. A control laminectomy without spinal cord injury. The top panel (A) shows a low-power picture of the spinal column, the middle panel (B) shows an enlargement of the laminectomy site, the bottom panels (C & D) shows higher magnifications of the laminectomy interface. Epidural adhesions (**) were occasionally present, particularly at the laminectomy edges.

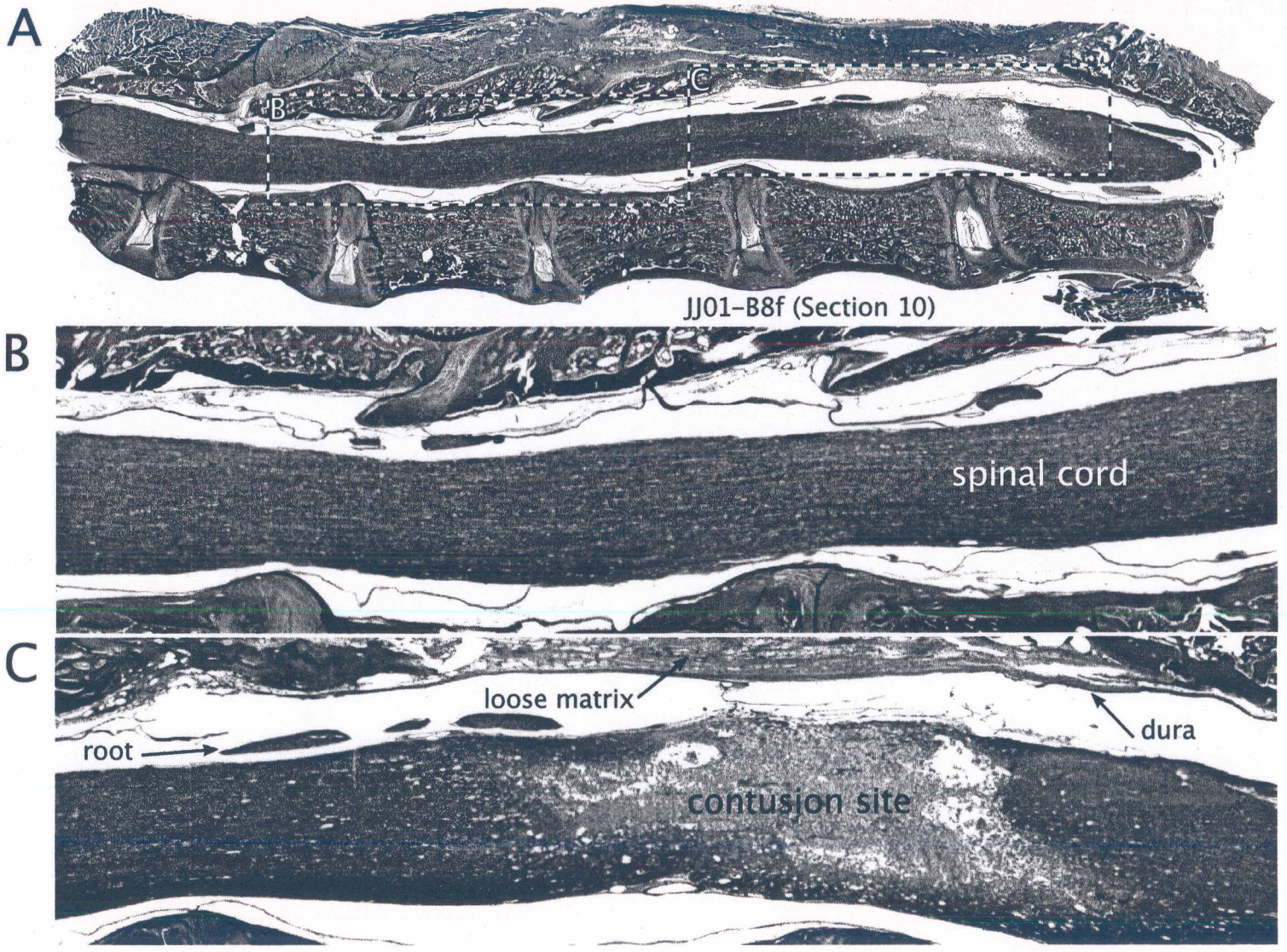


Figure 3. Saggital sections of a rat spinal cord (JJ01-B8f, section 10) at 2 weeks after a 12.5 mm weight drop contusion. Panel A shows a low-power view of the spinal . The dura was free in surrounding cord (Panel B) but was adherent (**) to the laminectomy scar (Panel B). There was minimal subdural adhesions.

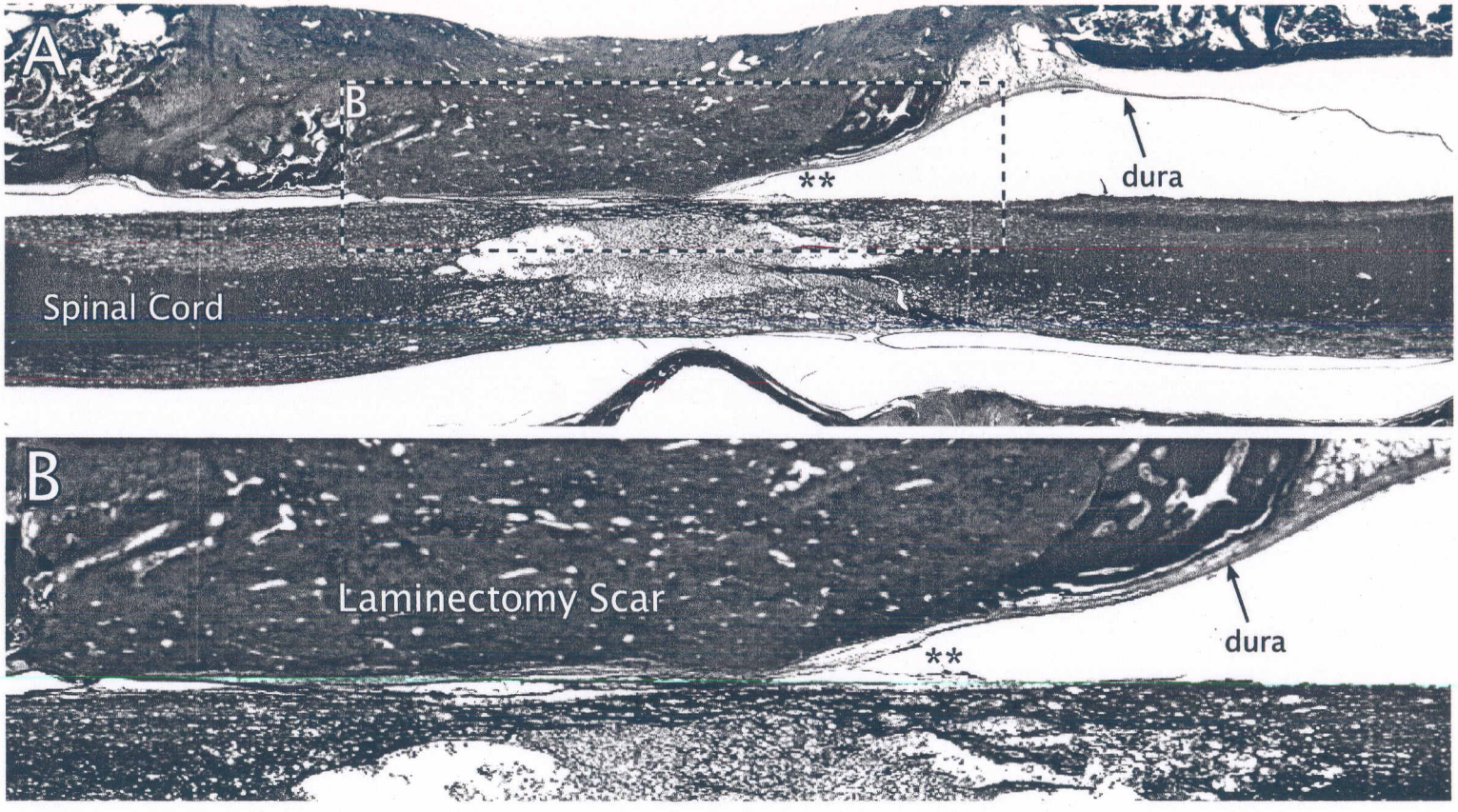


Figure 4. Control 12.5-mm weight drop contusion at 2 weeks. Extensive epidural and subdural adhesions (**) were present. The bottom panel is an enlargement of the inset area from the top image. Note the blue laminectomy scar, the cavitation in the spinal cord at the contusion site, and the preservation of a rim of cytoplasm (red) at the injury site.

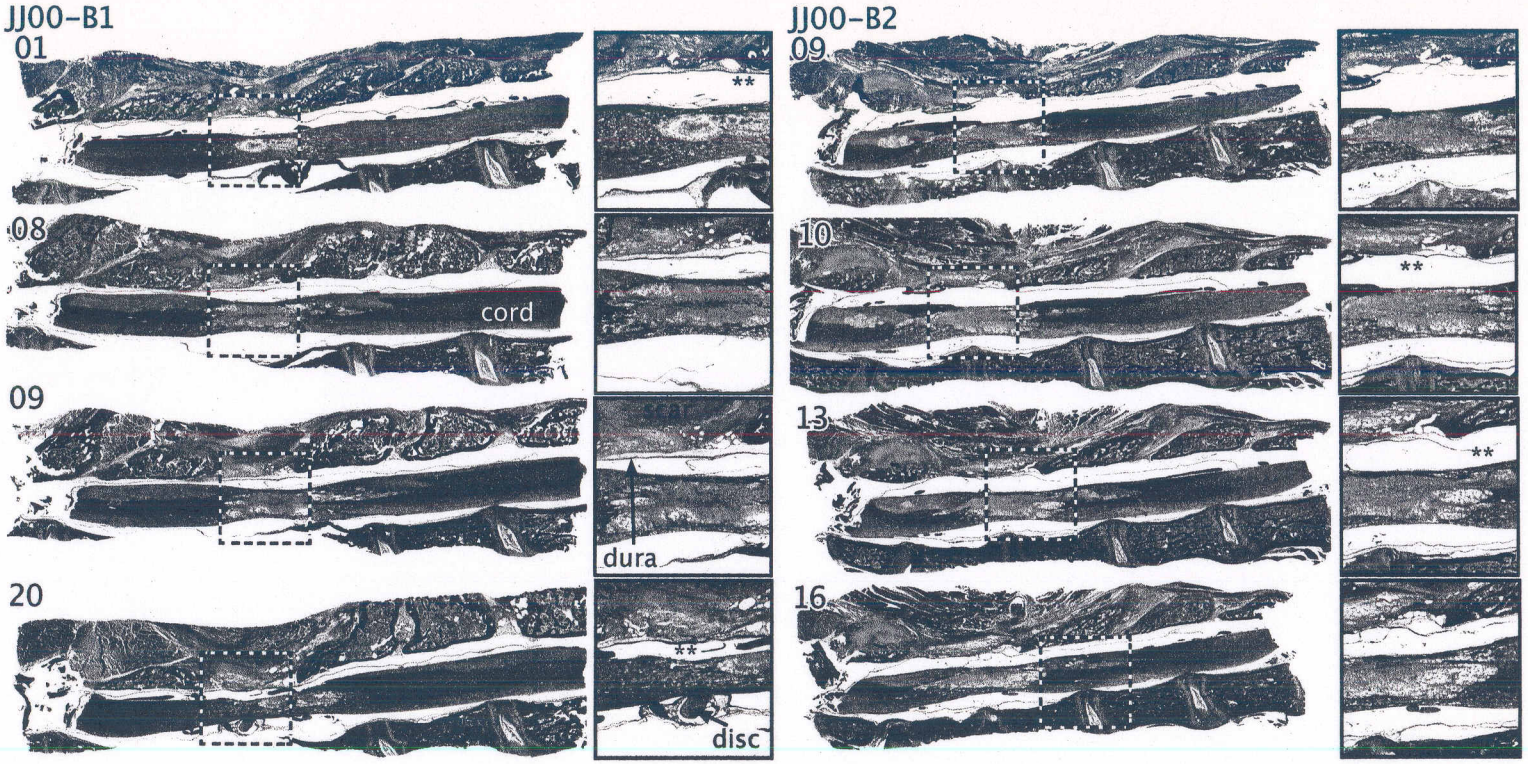


Figure 5. Control 12.5 mm contusion at 2 weeks. Representative sections from two rat spinal cords (JJ00-B1 and JJ00-B2) at 2 weeks after a 12.5-mm weight drop. No biomaterials were placed on the dural surface. Enlargements of the injury site are placed on the right on each section. Both spinal cords had occasional subdural adhesions and occasional epidural adhesions (**). A herniated disc may be compressing the spinal cord (JJ00-B1, section 20, bottom frame on left).

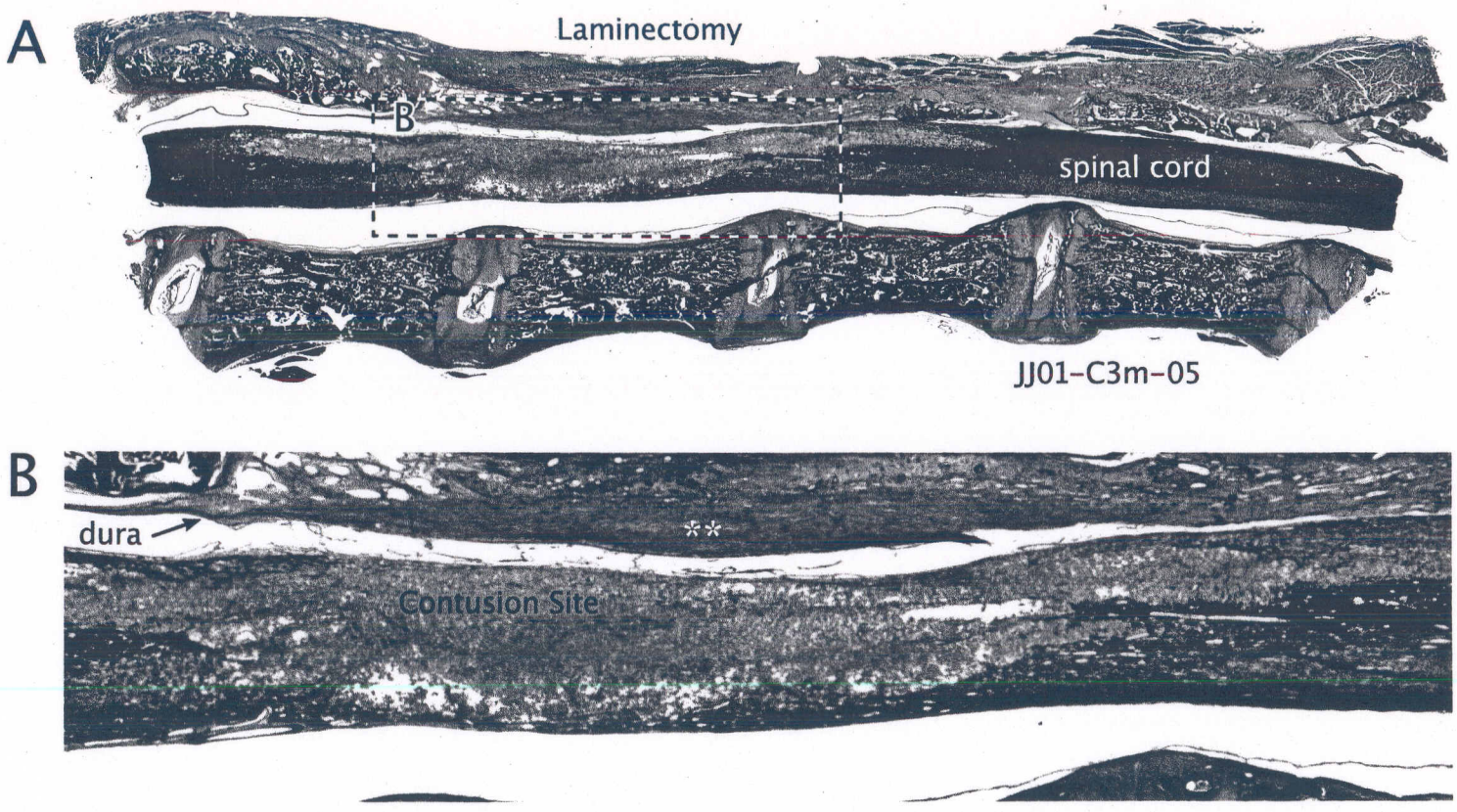


Figure 6. Control 25.0-mm contusion at 2 weeks after injury. The top panel shows a low-power sagittal view of a rat spinal cord at 2 weeks after a 25.0-mm weight drop contusion. Note severe dural adhesion to the overlying laminectomy scar (**), occasional subdural adhesions, and the widespread damage to the spinal cord with Wallerian degeneration extending into both rostral and caudal cord.

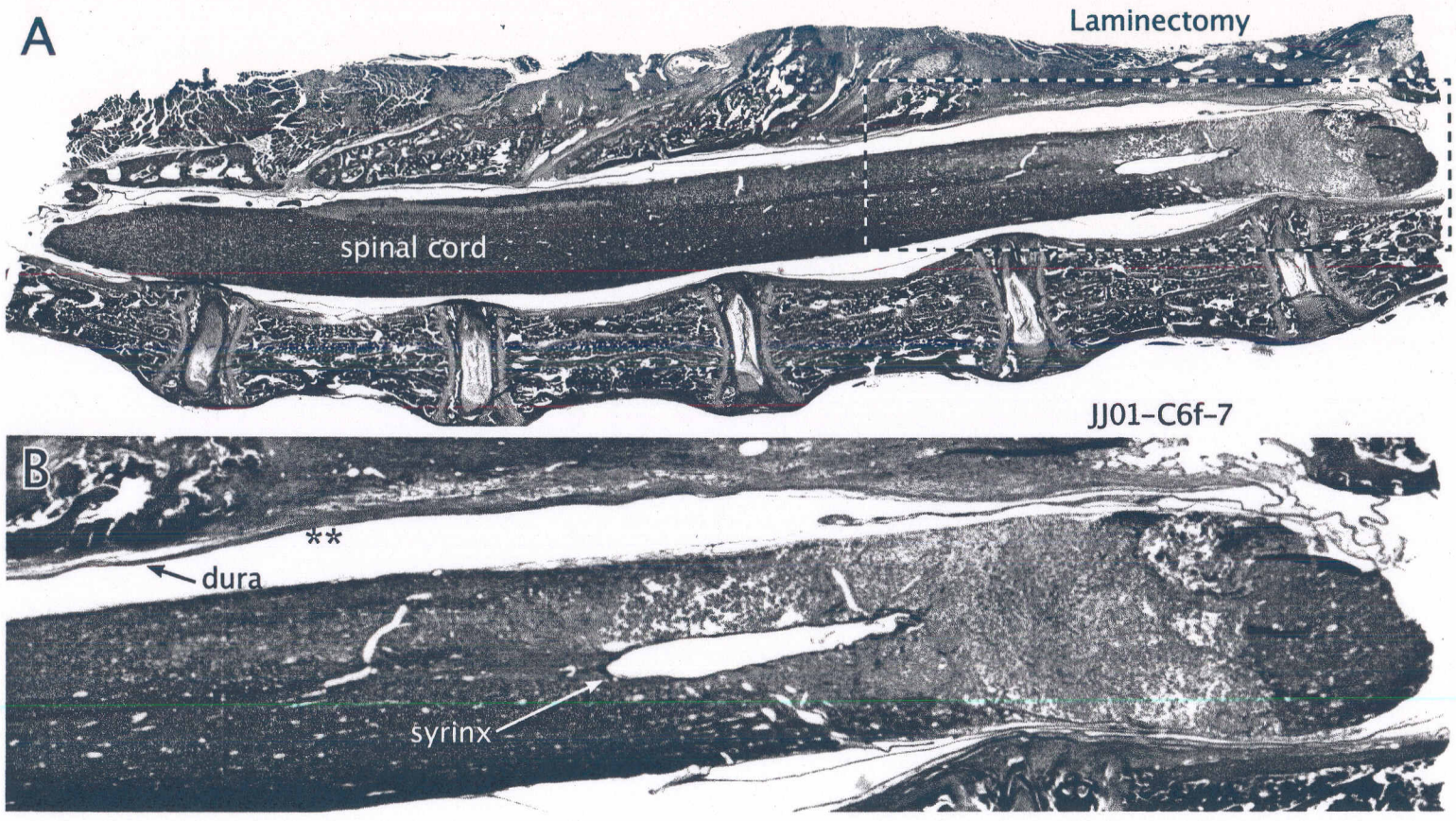


Figure 7. Control 25-mm contusion at 2 weeks after injury. The top panel shows a sagittal view of the spinal cord with the injury at one end and the bottom panel is an enlargement of the injury site. The dura adhered to the overlying scar spanning the width of the laminectomy (severe). Occasional subdural adhesions were present. Note the destruction of the spinal cord due to the contusion and a small syringomyelic cyst (syrinx).

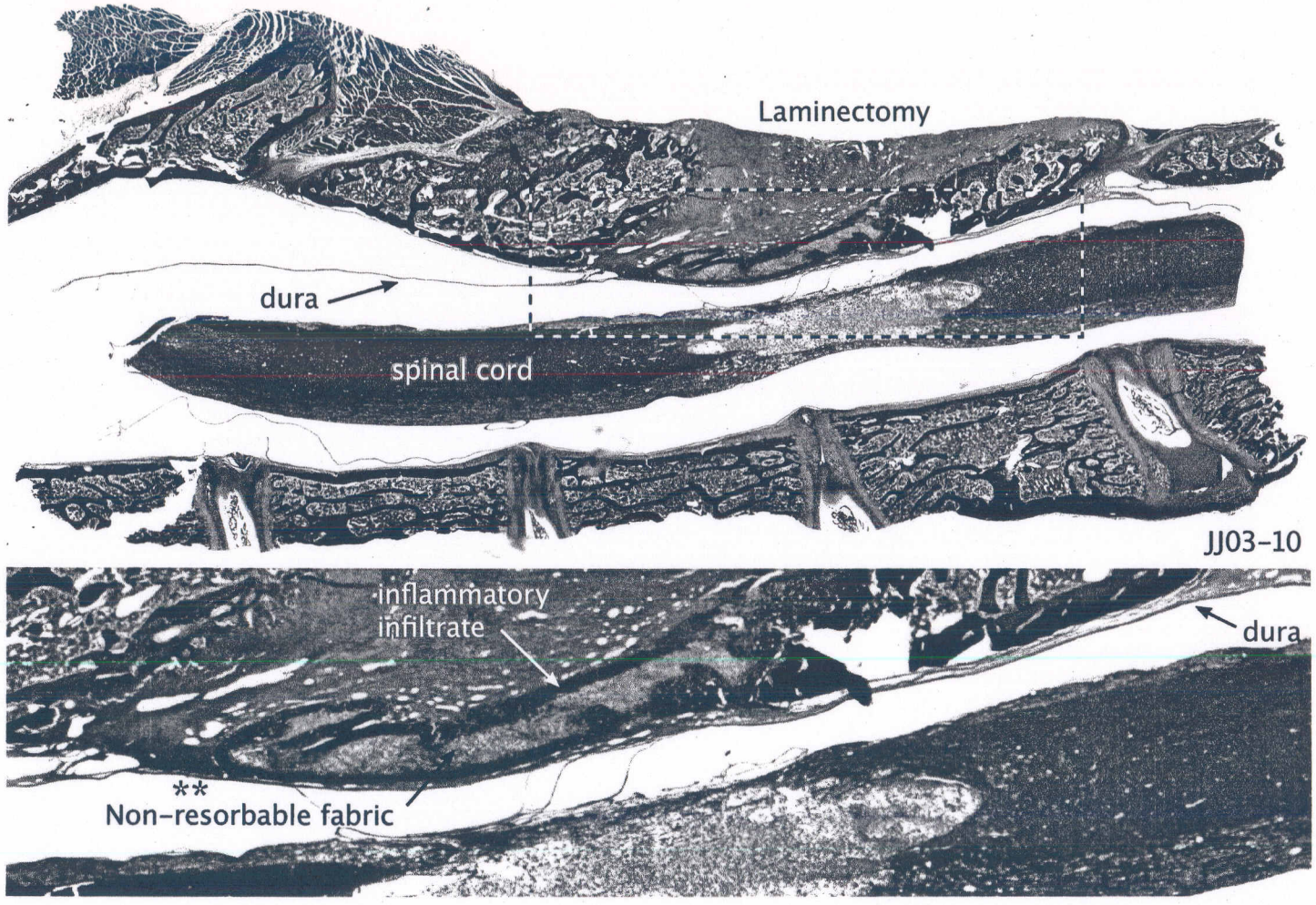


Figure 8. Non-resorbable fabric (NF) epidural barrier at 2 weeks after a 12.5 mm contusion. The NR membrane is partially invaginated and surrounded by cells (red color, probably inflammatory cells). The dura adhered to the ventral side of the epidural barrier (**). There was minimal subdural adhesion.

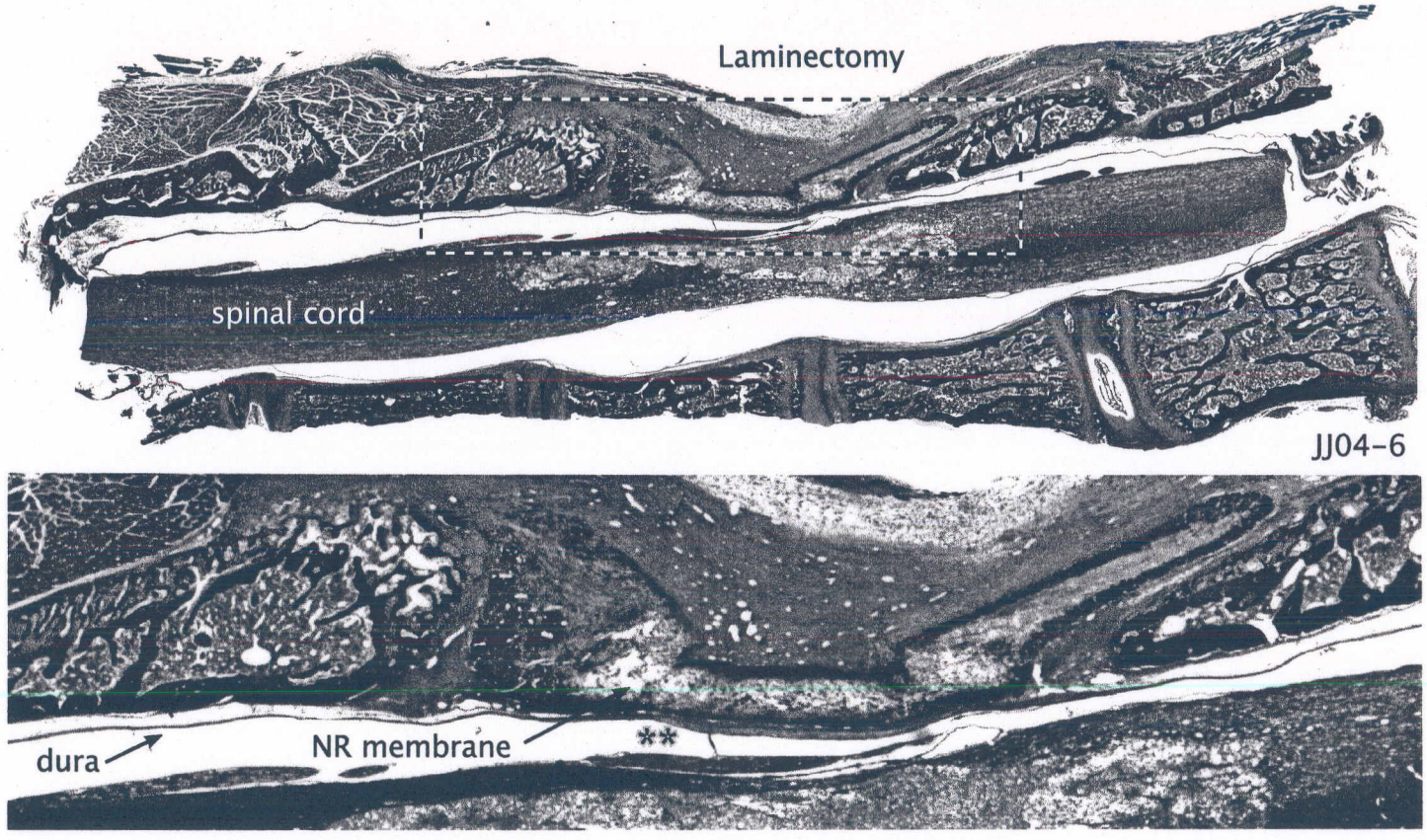


Figure 9. Non-resorbable fabric (NF) epidural barrier at 2 weeks after a 12.5 mm contusion. The barrier was surrounded by inflammatory cells (red). The dura clearly adhered (**) to the barrier over 50% of the laminectomy width. There were minimal subdural adhesions.

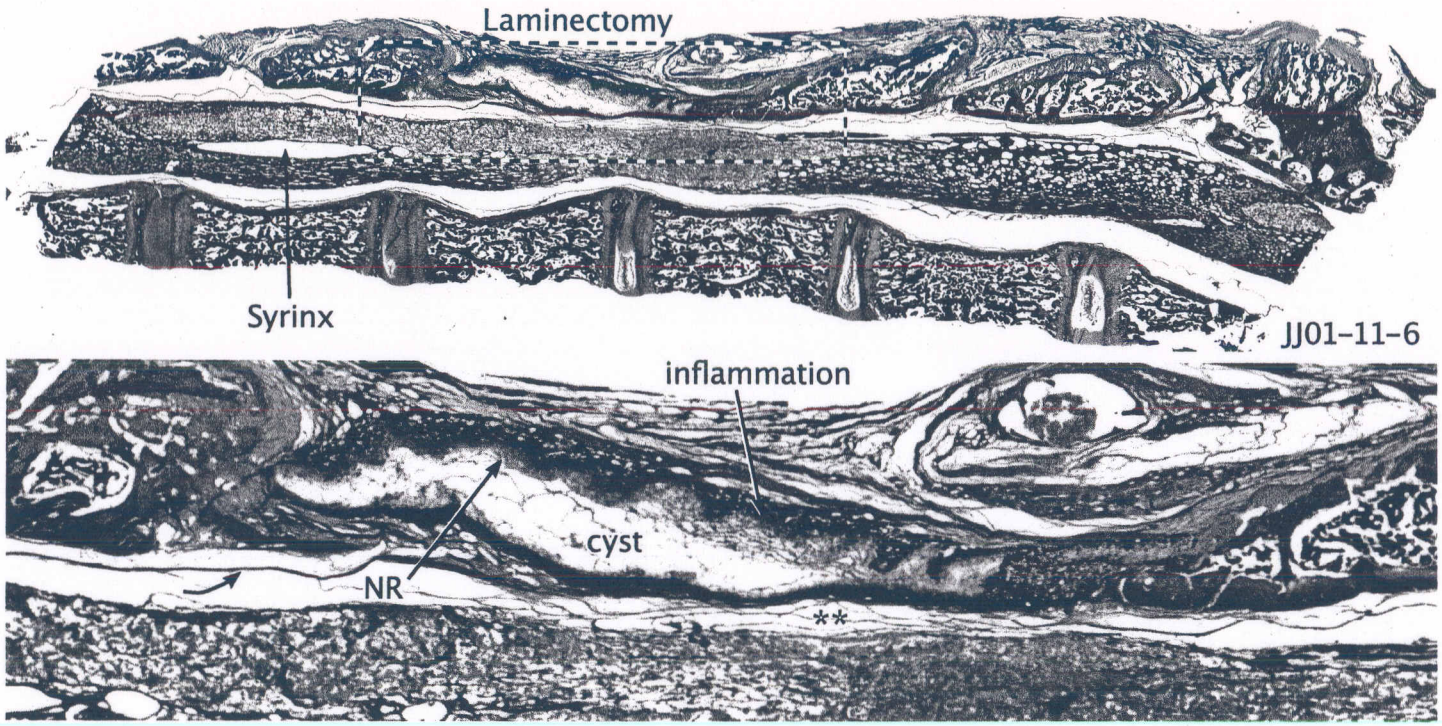


Figure 10. Non-resorbable fabric (NF) epidural barrier at 2 weeks after a 25-mm contusion. Note the presence of a syrinx (top panel). The spinal cord had widespread Wallerian degeneration reaching 3 segments above the contusion site and extensive microcystic changes below. The lower panel shows an enlargement of the laminectomy side. A red-stained layer of inflammatory cells lined the dorsal surface of the barrier. A cyst had developed on the ventral side of the barrier. The dura was clearly adherent to ventral surface of the cyst. Scar tissues had crept around the rostral corner of the laminectomy.

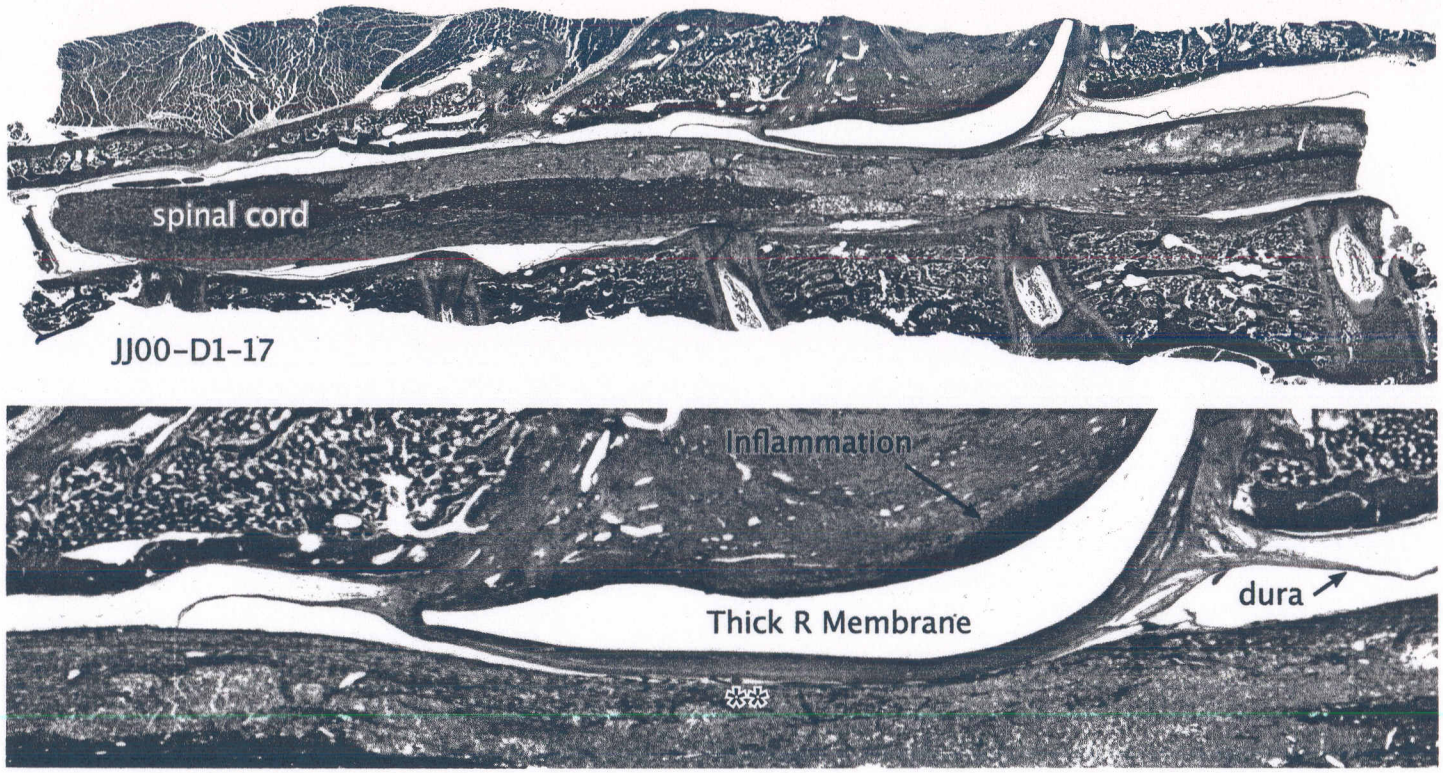


Figure 11. Thick resorbable epidural barrier (RB) at 2 weeks after a 12.5-mm contusion. The membrane was not stained. Fibrotic tissue had crept around the edges of the membrane and the dura was tightly adherent to the scar below the membrane. In addition, the stain shows a collection of inflammatory cells (red) on the dorsal surface of the barrier. The membrane was mechanically pressing on the cord.

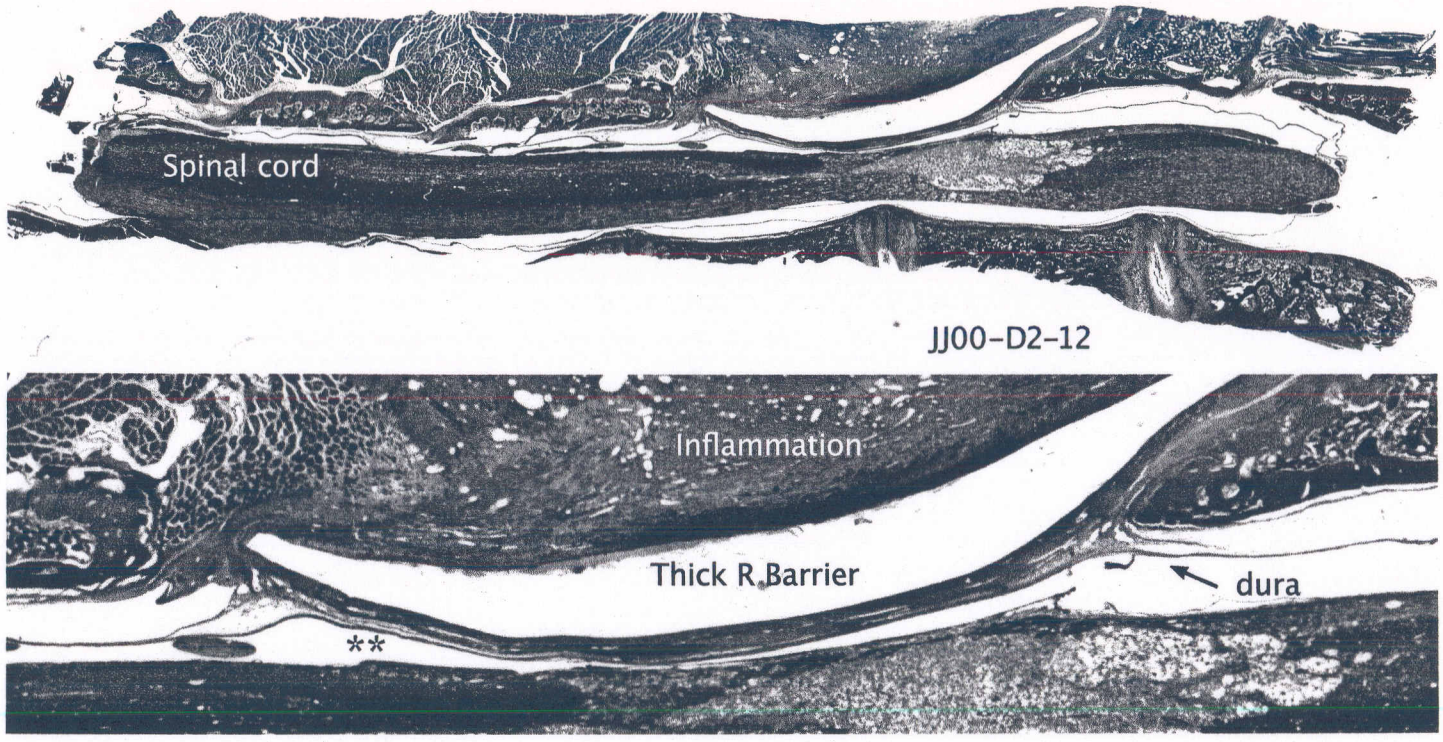


Figure 12. Thick resorbable epidural barrier (RB) at 2 weeks after a 12.5 mm contusion. The barrier was still present and not stained. Collagenous scar tissue had crept around the edges of the membrane barrier and enveloped the barrier. The dura is tightly adherent to the scar below the membrane (**). Note the collection of inflammatory cells (red) on the dorsal surface of the barrier. No subdural adhesions were present.

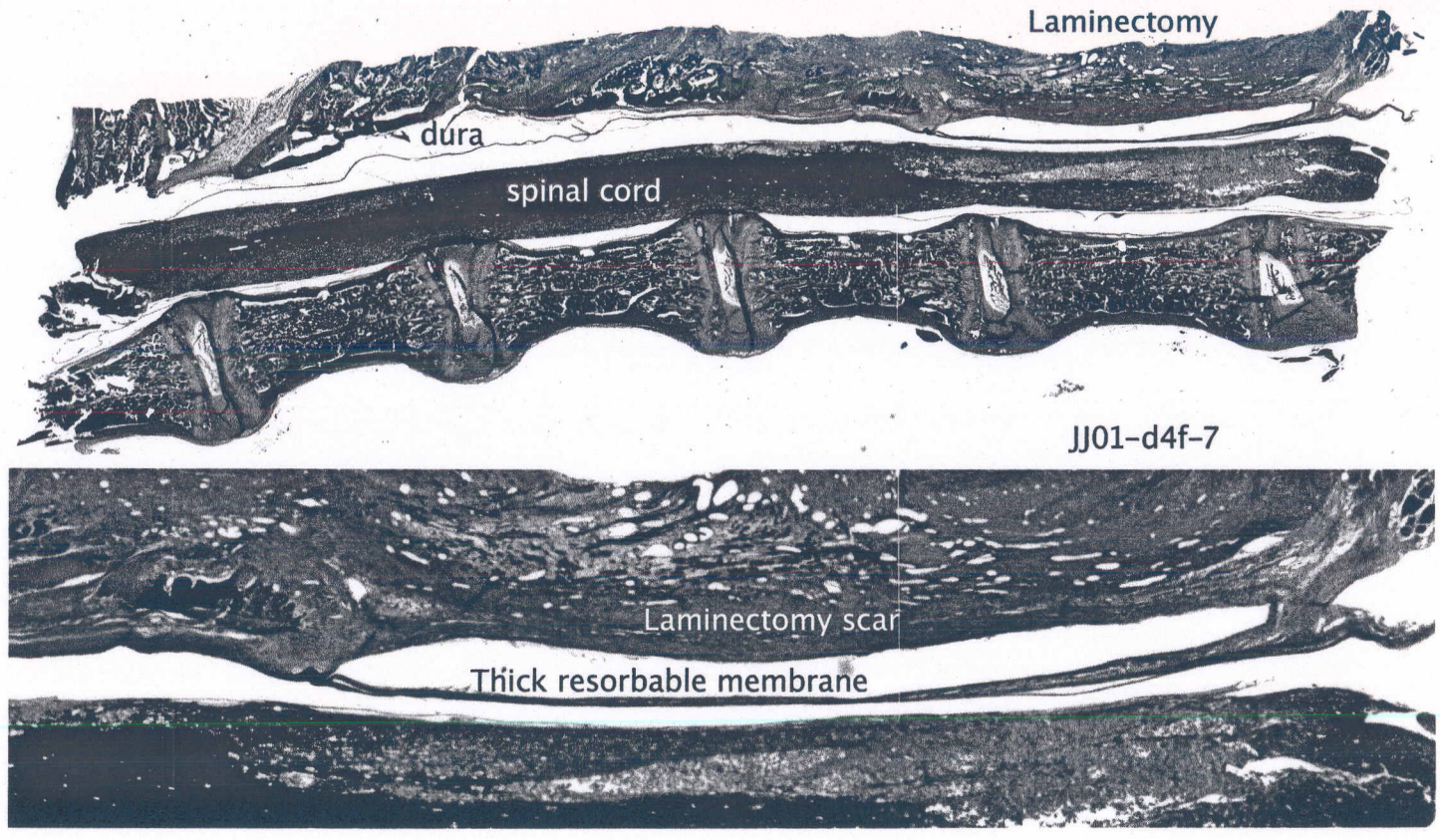


Figure 13. Thick resorbable epidural barrier (RB) at 2 weeks after a 12.5 mm contusion injury. A thin layer of collagenous tissue (blue) formed below the membrane. Little or no inflammation was present on the dorsal side of the barrier. Nevertheless, the dura adhered closely to the ventral surface of the barrier. Subdural adhesions were minimal.

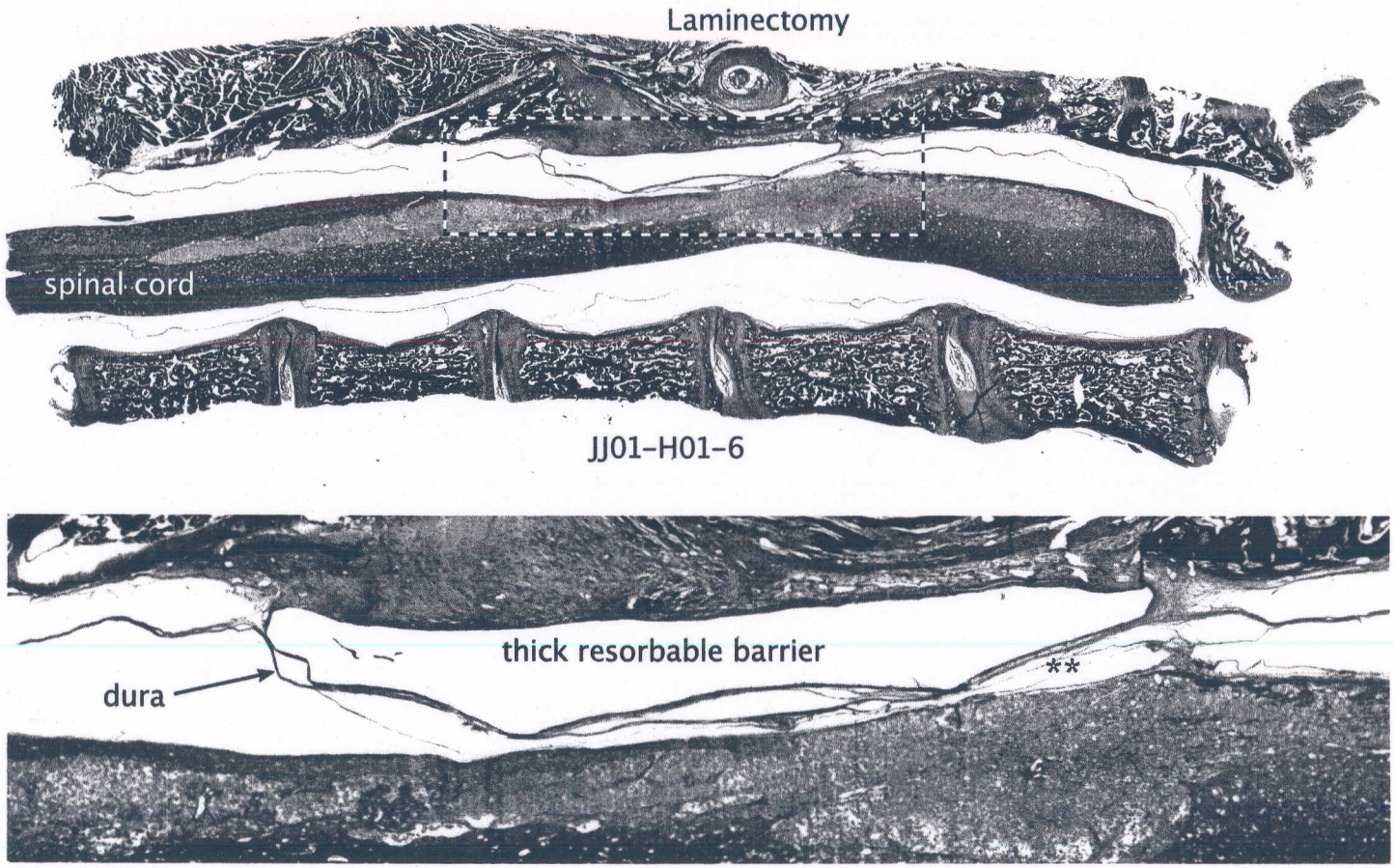


Figure 14. Thick resorbable epidural barrier at 2 weeks after a 25-mm weight drop contusion. The barrier does not seem to be compressing the spinal cord in this case due to an ample spinal canal. However, the dura is attached to the resorbable membrane in some areas (**) but does not appear to be free in other areas. The spinal cord is severely damaged, as expected from a 25-mm weight drop, with extensive Wallerian degeneration above and below the contusions site.

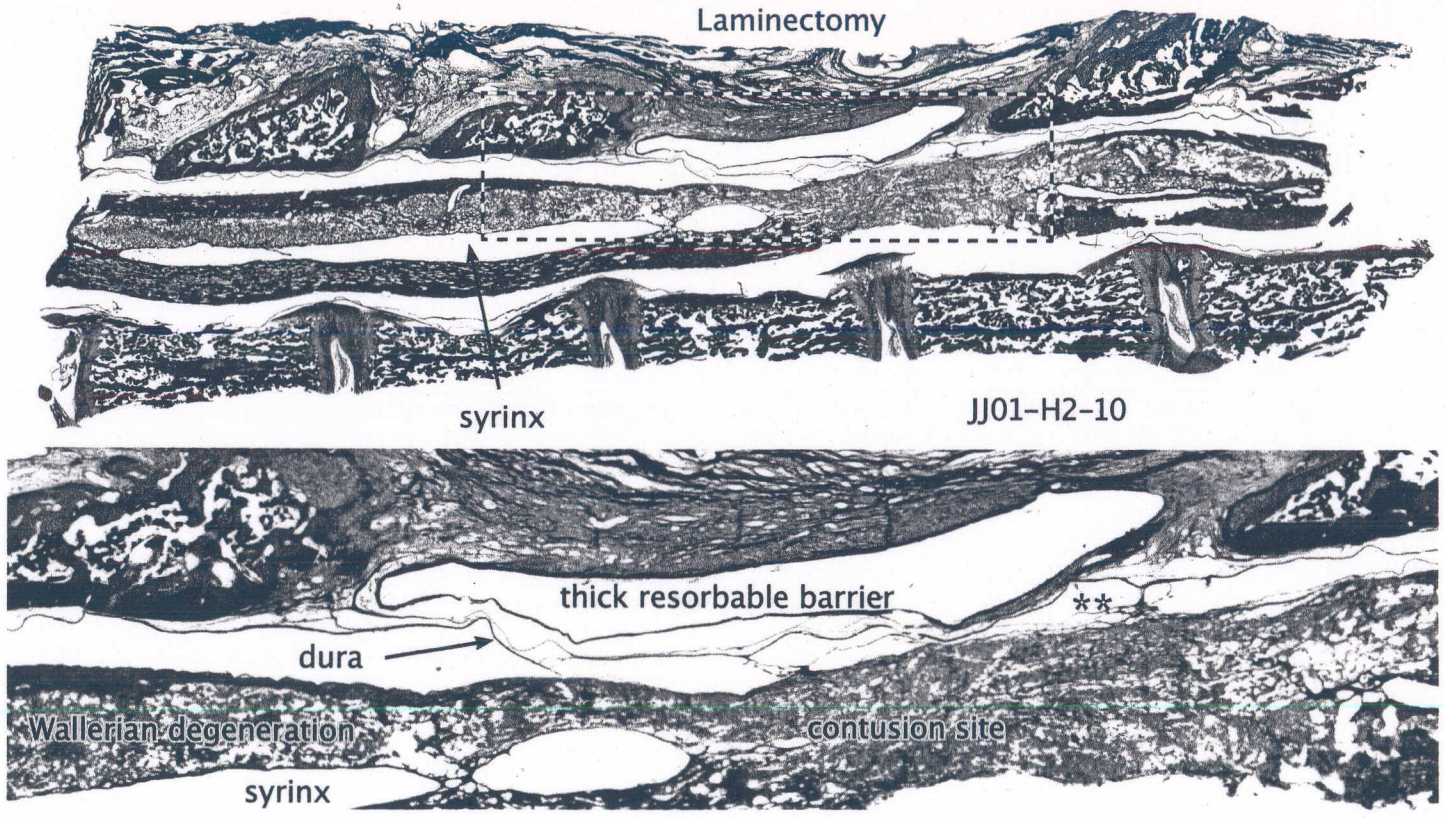


Figure 15. Thick resorbable epidural dural barrier at 2 weeks after 25-mm spinal cord contusion. Note the severe damage in the spinal cord, with complete loss of axons at the impact site, the presence of bilobulated syringomyelic cyst (expansion of the central canal), and Wallerian degeneration of the dorsal column.

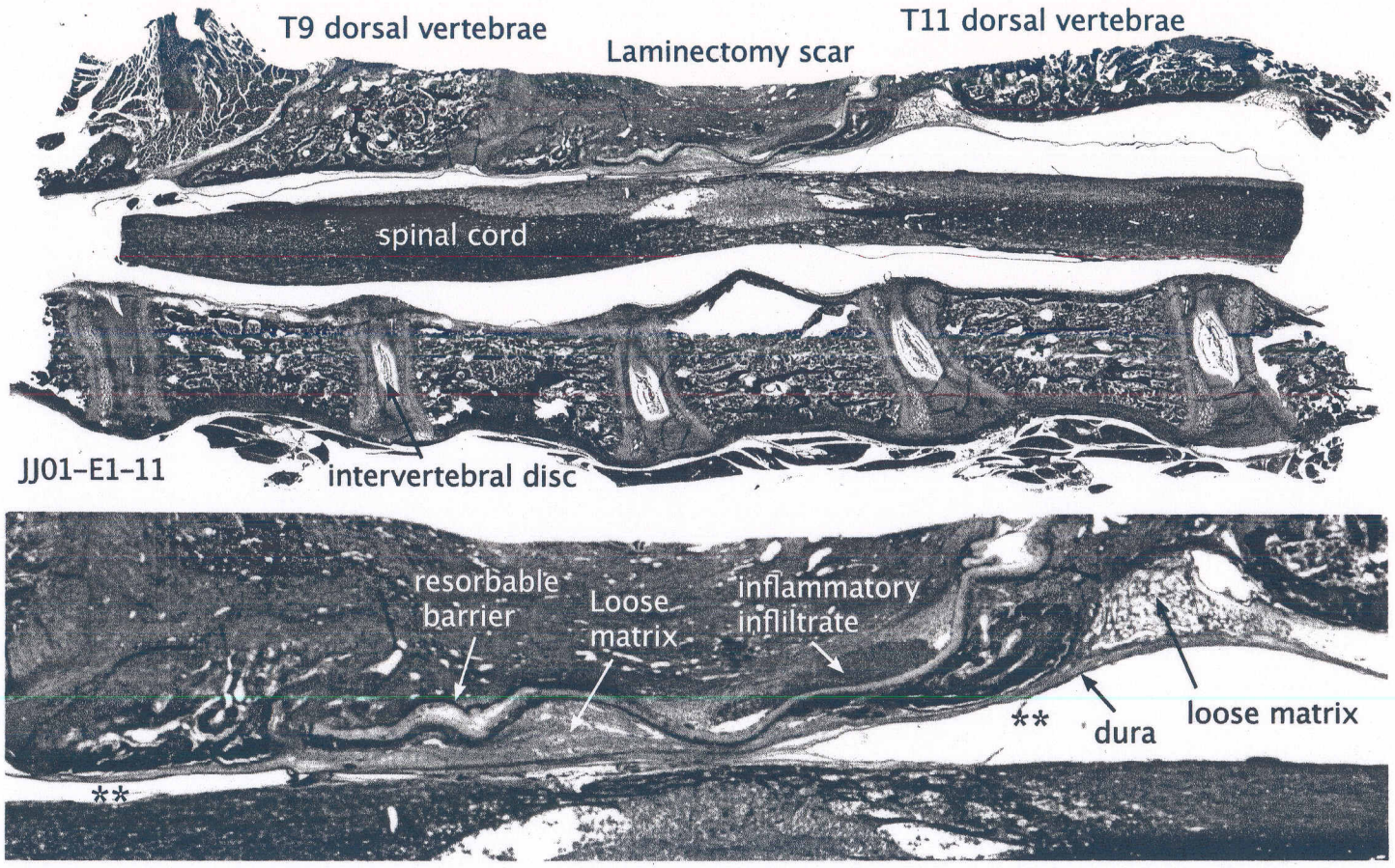


Figure 16. Thin resorbable epidural barrier at 2 weeks after a 12.5-mm contusion. A laminectomy scar (blue) has formed. The thin resorbable barrier can be seen as undulating membrane that stretched from the T9 to T10 dorsal vertebral processes. A pocket of inflammatory infiltrate is present on the dorsal surface of the barrier. While the dura was adherent (**) to bone at the edge of the laminectomy, a loose matrix of tissue separated the barrier from the dura in most places. Note the minimal damage in the spinal cord.

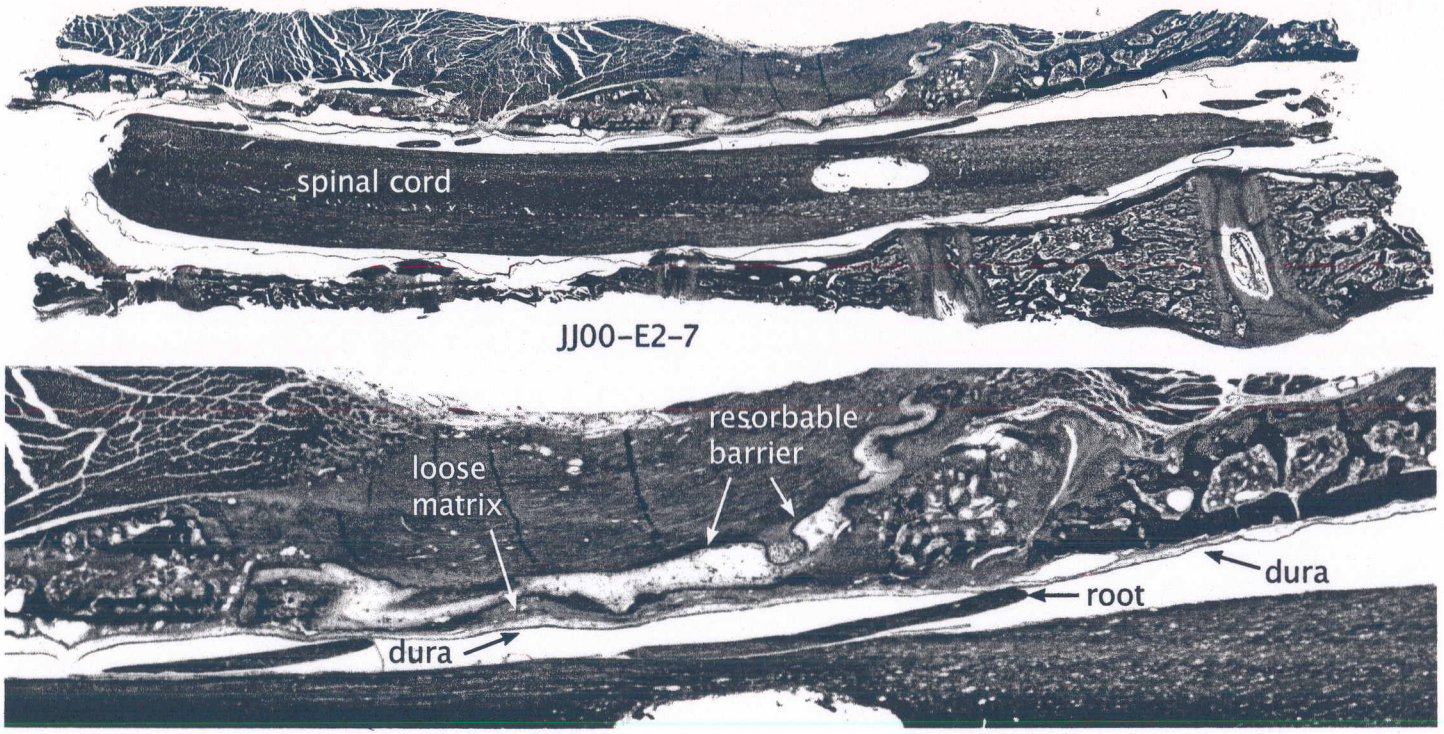


Figure 17. Thin epidural resorbable fiber (RF) barrier at 2 weeks after a 12.5-mm contusion. A laminectomy scar has formed on top of the barrier. Below the barrier, a loose matrix of tissue is present. The dura is not adherent or loosely adherent to this matrix. Note the minimal damage in the spinal cord at the contusion site; this is partly due to the particular section of the cord. Nevertheless, the surrounding cord appears to be relatively undamaged.

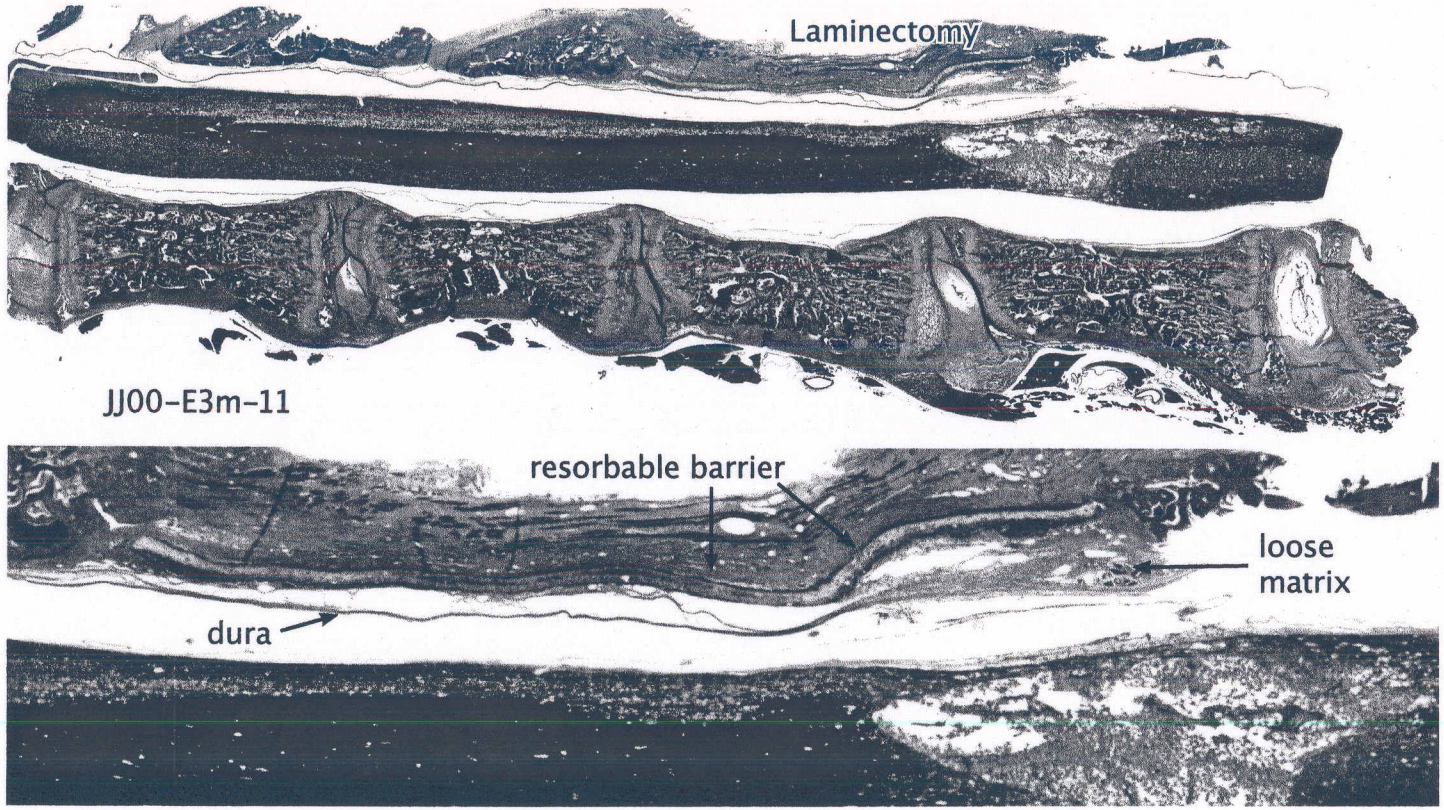


Figure 18. Thin epidural resorbable barrier (RB) at 2 weeks after a 12.5-mm spinal cord contusion. The barrier is clearly visible as an undulating membrane that stretched across the laminectomy site. The dura is clearly not attached to the membrane. A loose collagenous matrix is present between the membrane and the dura in at one end. Note the minimal damage in the spinal cord.

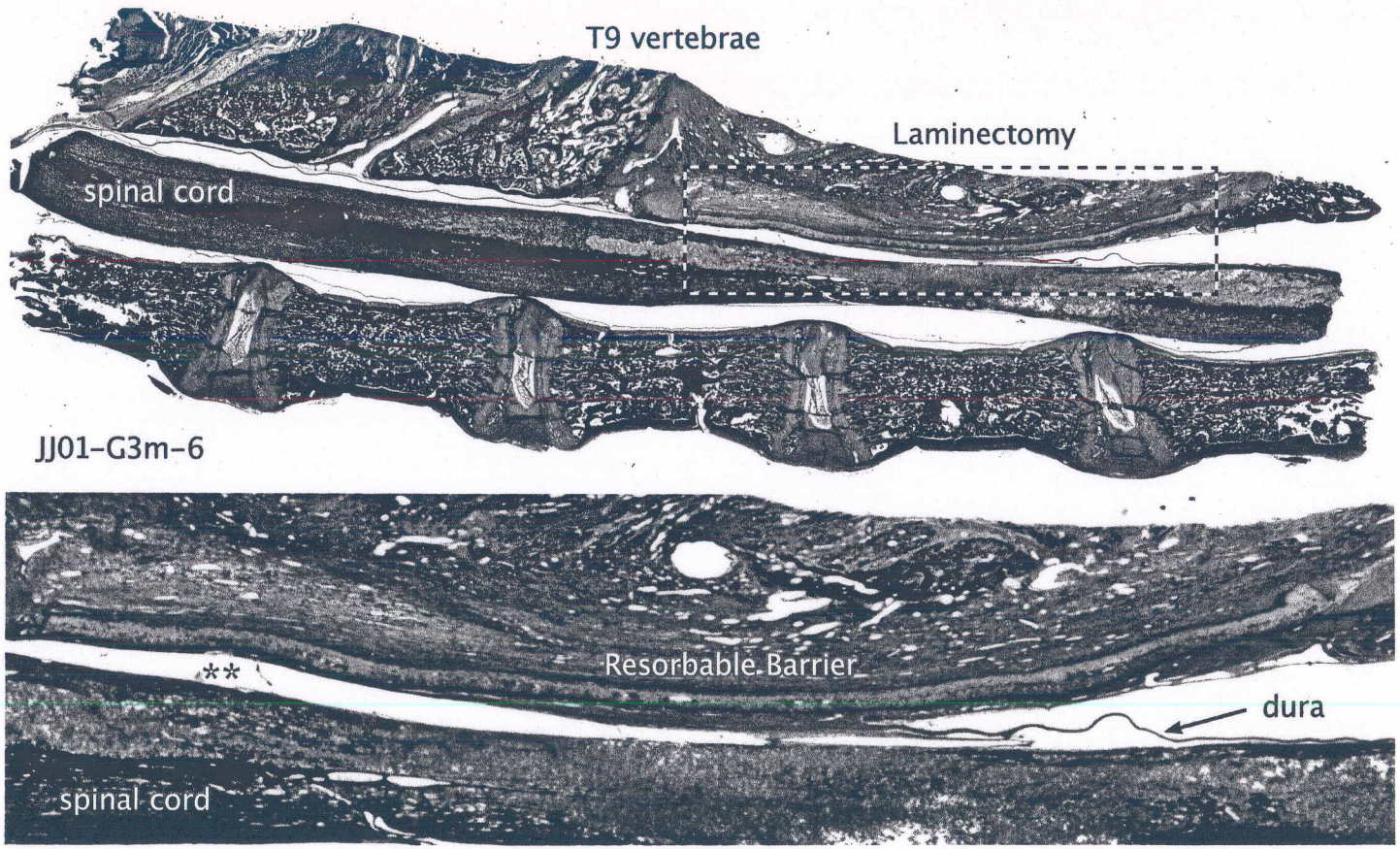


Figure 19. Thin epidural resorbable barrier at 2 weeks after a 25.0-mm contusion injury. A dense laminectomy scar has formed. The resorbable PLA/PVCA fabric is still present at 2 weeks. The dura adhered extensively to the fabric at certain locations (**). The spinal cord appeared severely injured with almost complete cell loss at the contusion site and Wallerian degeneration extending rostrally and caudally.

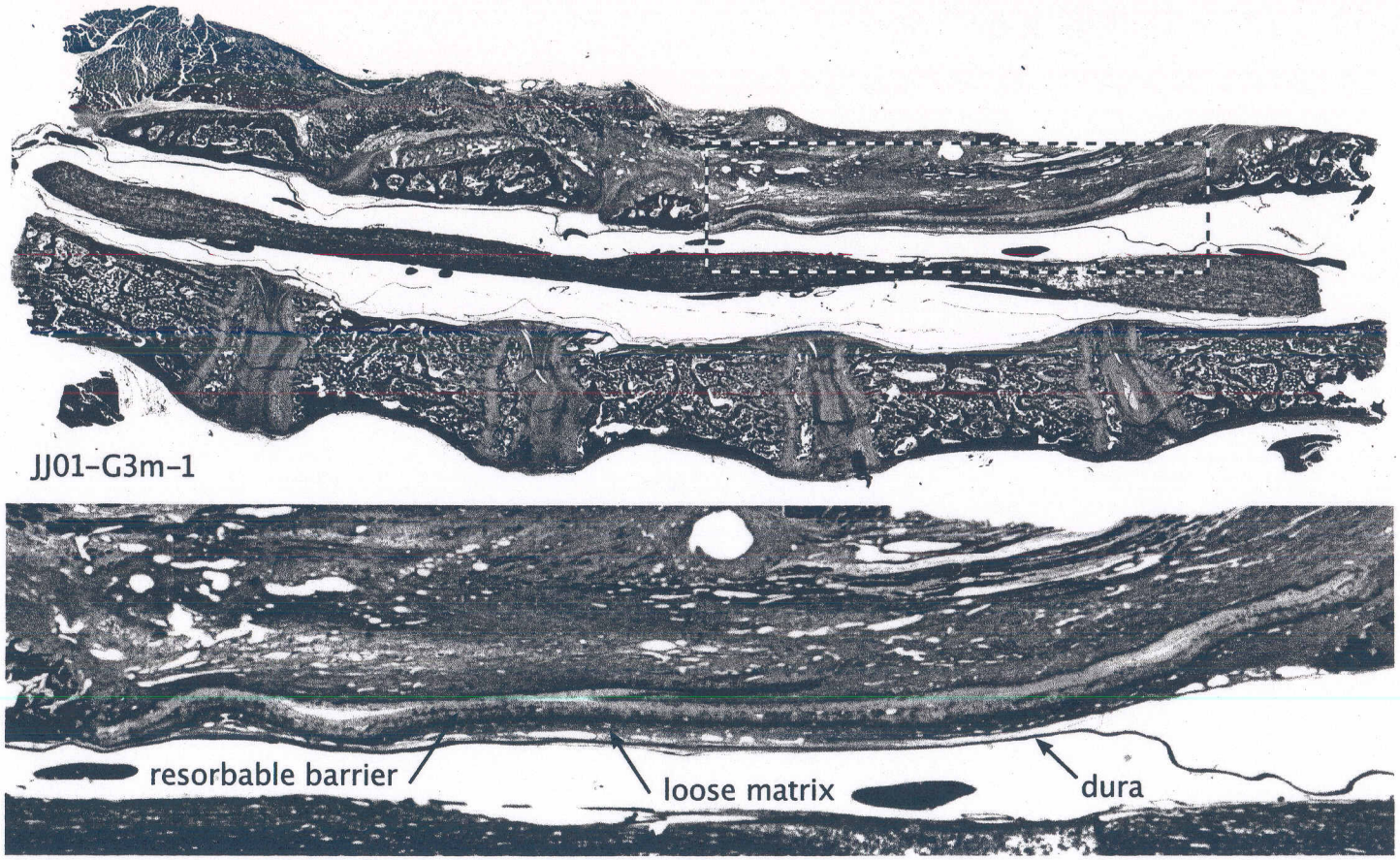


Figure 20. Thin epidural PLA/PCA resorbable fabric barrier at 2 weeks after a 25-mm contusion injury. This is a more lateral view of the spinal cord shown in figure 19. The barrier spanned the laminectomy site and not as much scar tissue had crept around the barrier edges. A loose tissue matrix is present between the dura and the barrier. Note the thin red-stained layer coating the barrier. There were no subdural adhesions.

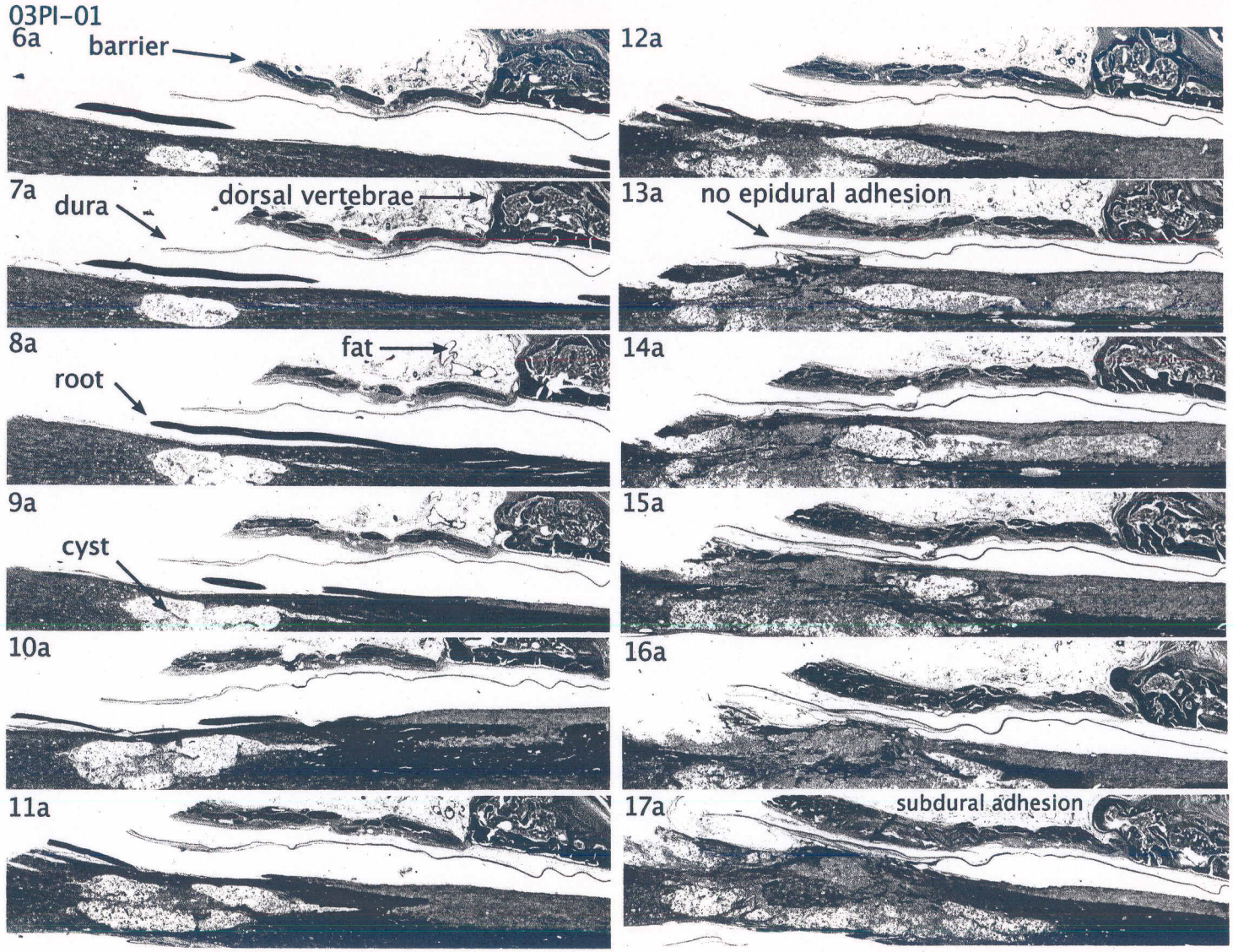


Figure 21. Thin epidural resorbable PCA/PLA fabric barrier at 6 weeks after a 12.5-mm contusion injury. No epidural adhesions were seen but occasional subdural adhesions were present.

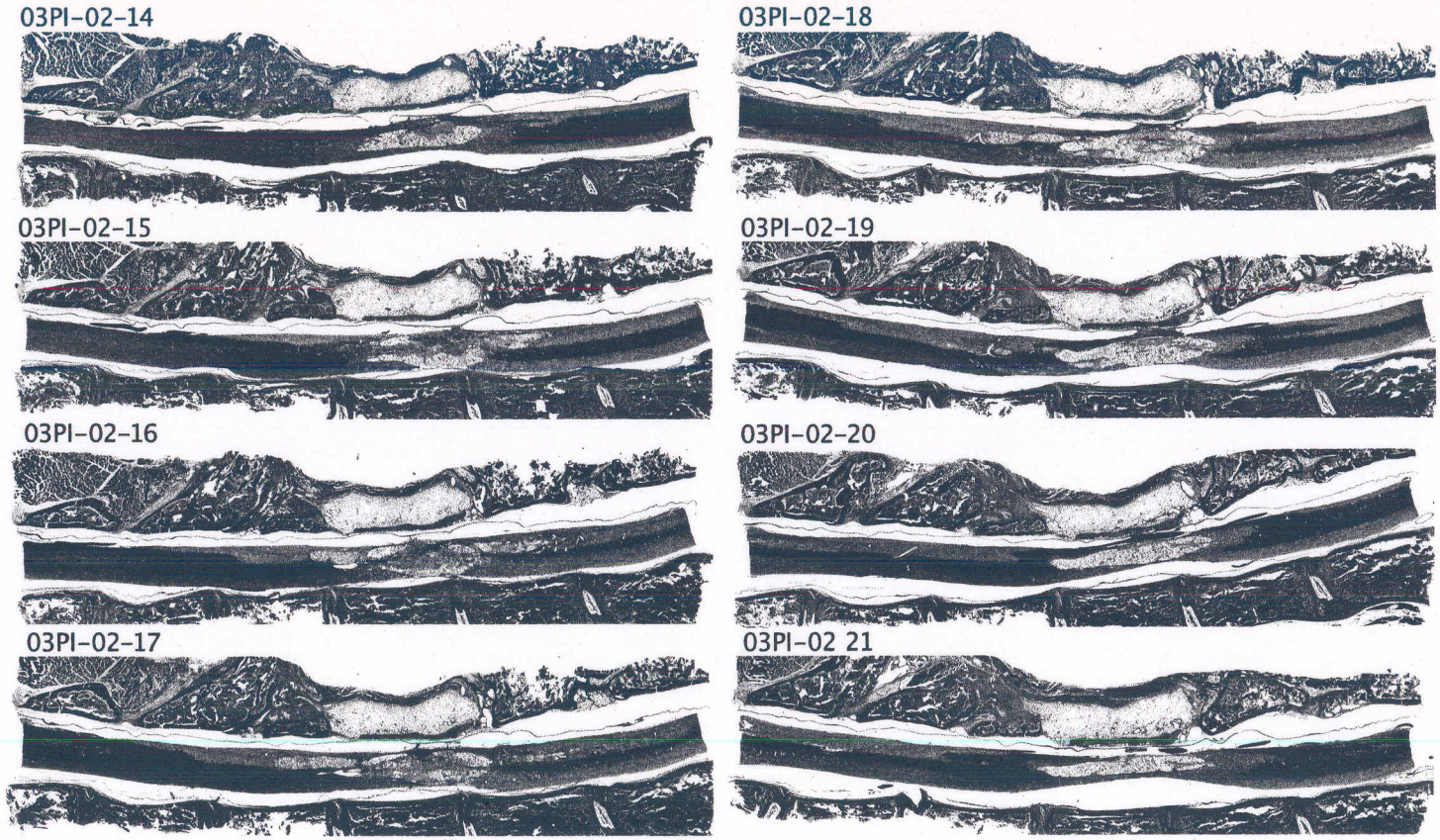


Figure 22. Thin epidural resorbable PLA/PCA fabric barrier at 6 weeks after a 12.5-mm contusion. The barrier is still present at 6 weeks although it is clearly beginning to break up. There was no epidural adhesion although occasional subdural adhesions were present (section 18-20).

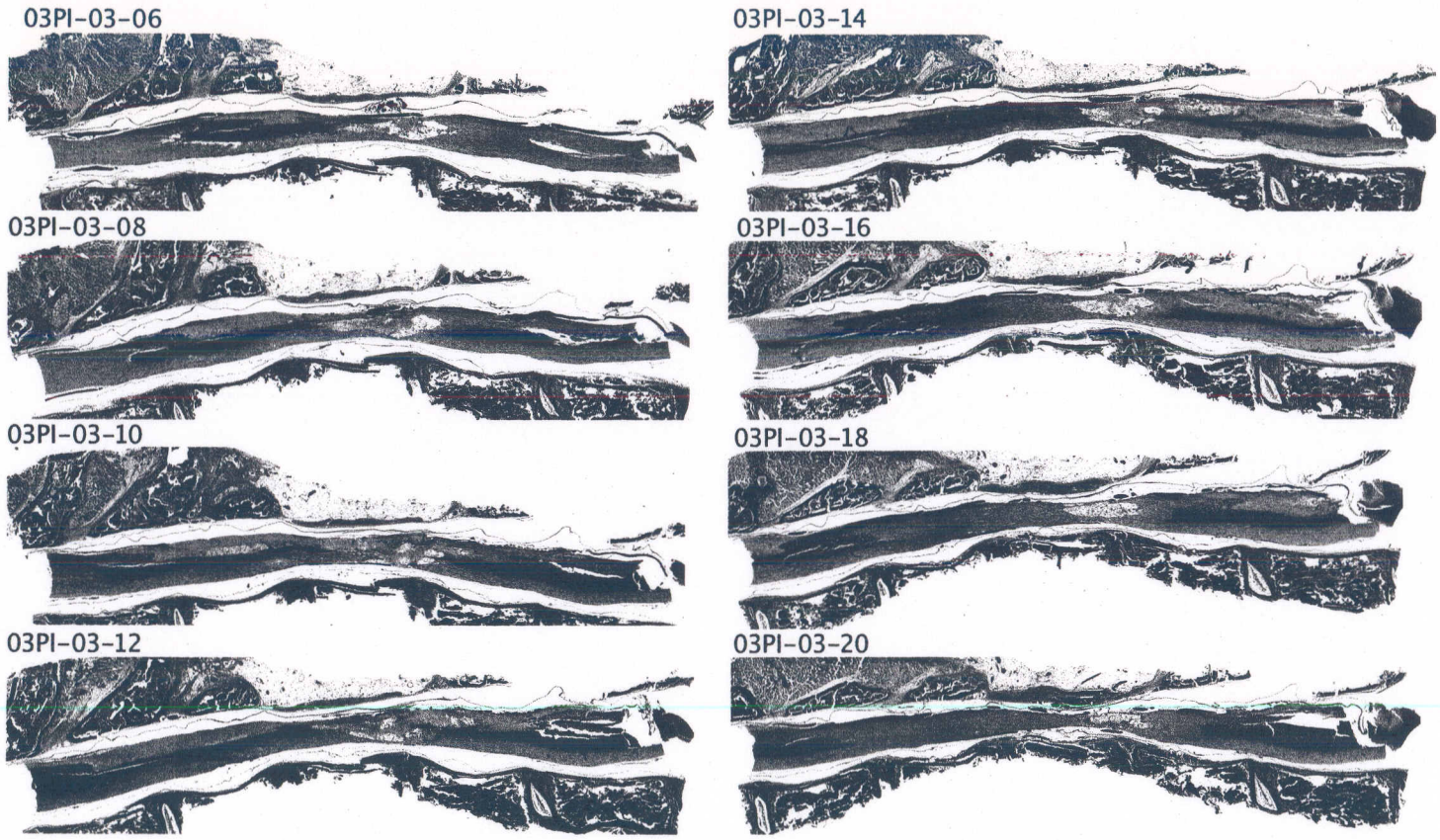


Figure 23. Thin epidural resorbable PLA/PCA fabric barrier at 6 weeks after 12.5-mm spinal cord contusion. There was no epidural adhesion although some subdural adhesions were present (section 18-20).

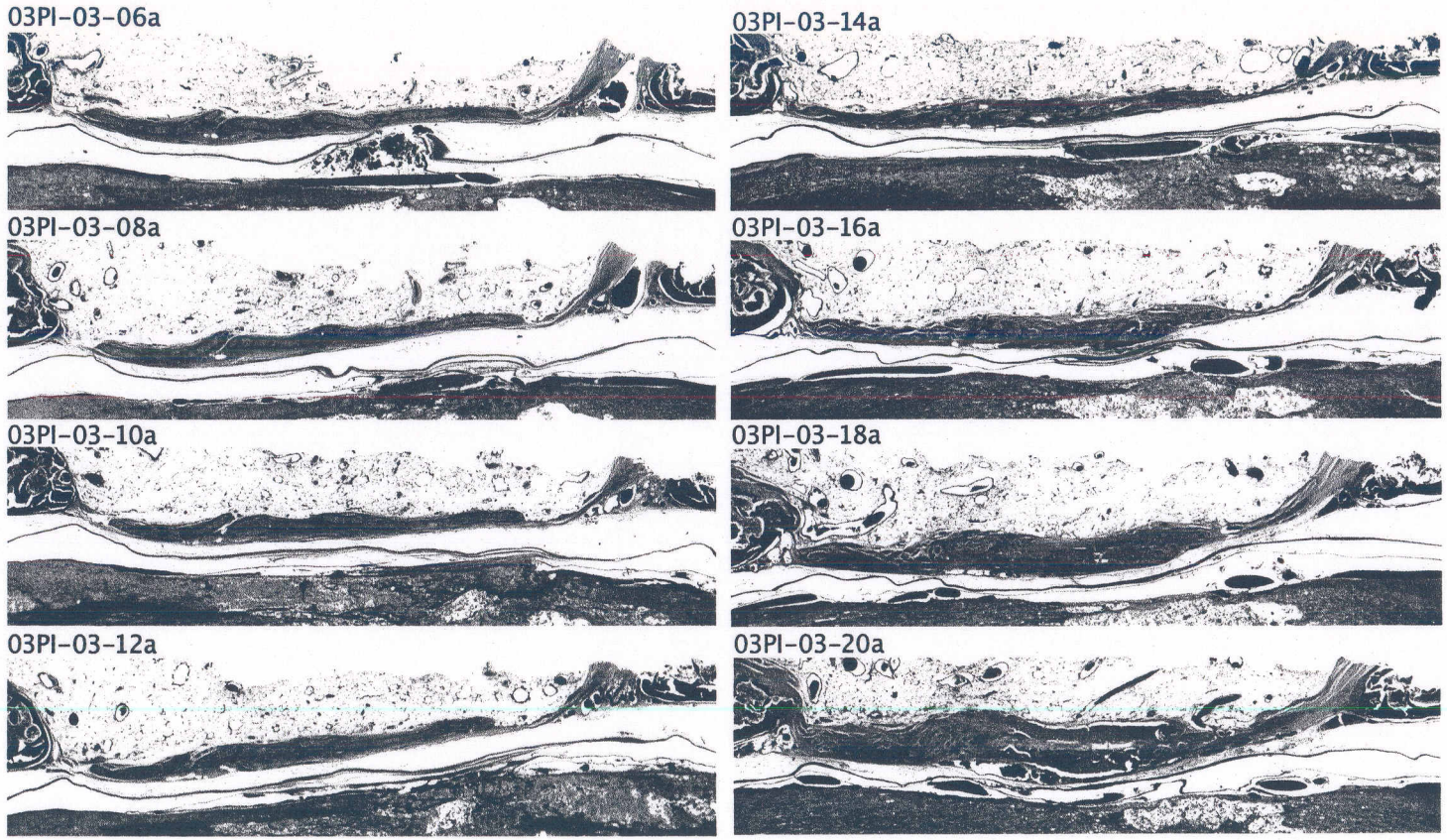


Figure 24. Thin epidural resorbable PLA/PCA fabric barrier at 6 weeks after a 12.5-mm spinal cord contusion. There was no epidural adhesion although some subdural adhesions were present (section 06).

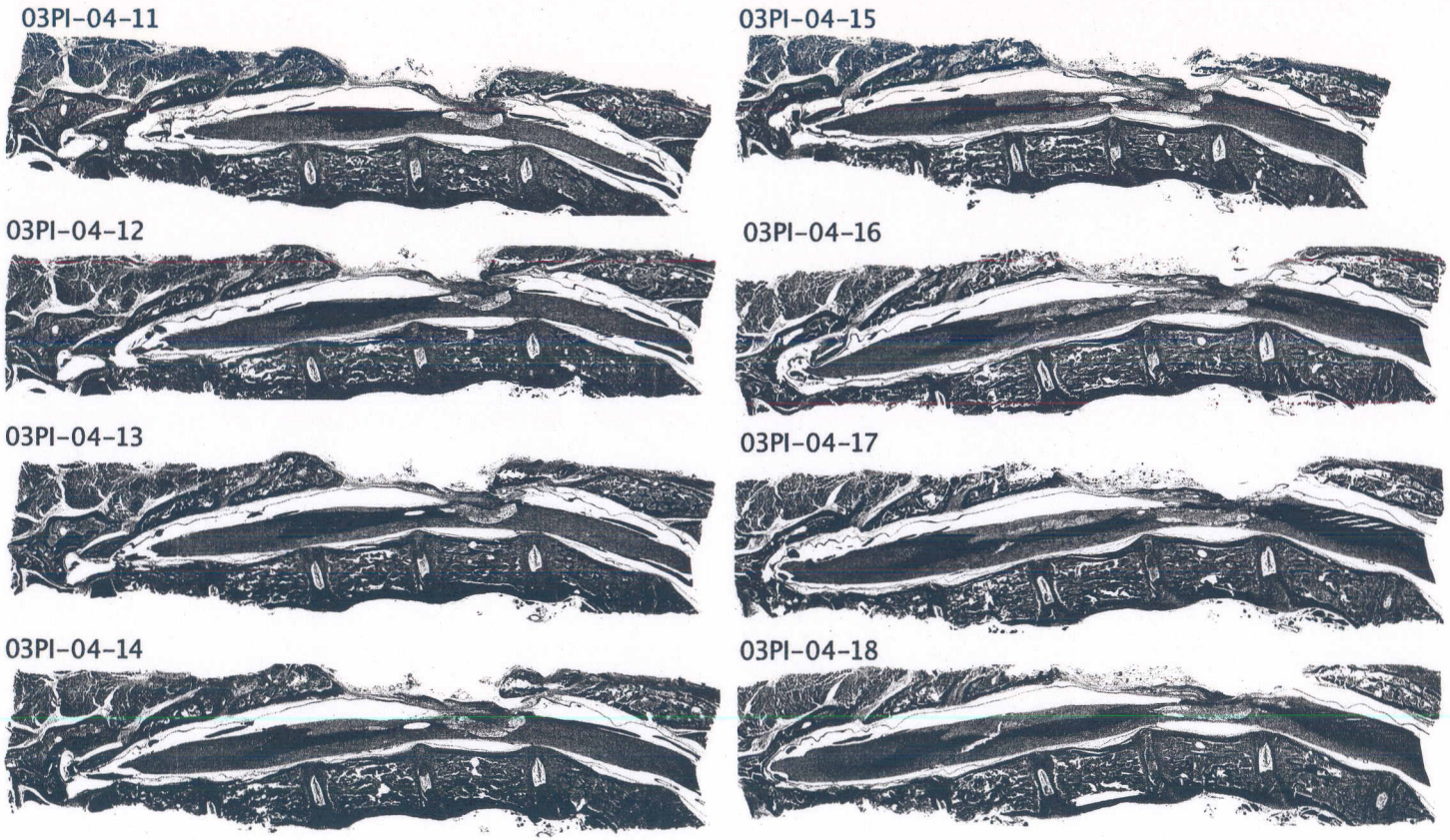


Figure 25. Thin epidural resorbable PLA/PCA fabric barrier at 6 weeks after 12.5-mm spinal cord contusion. Both epidural and subdural adhesions can be seen, particular at the caudal side (right) of the laminectomy site.

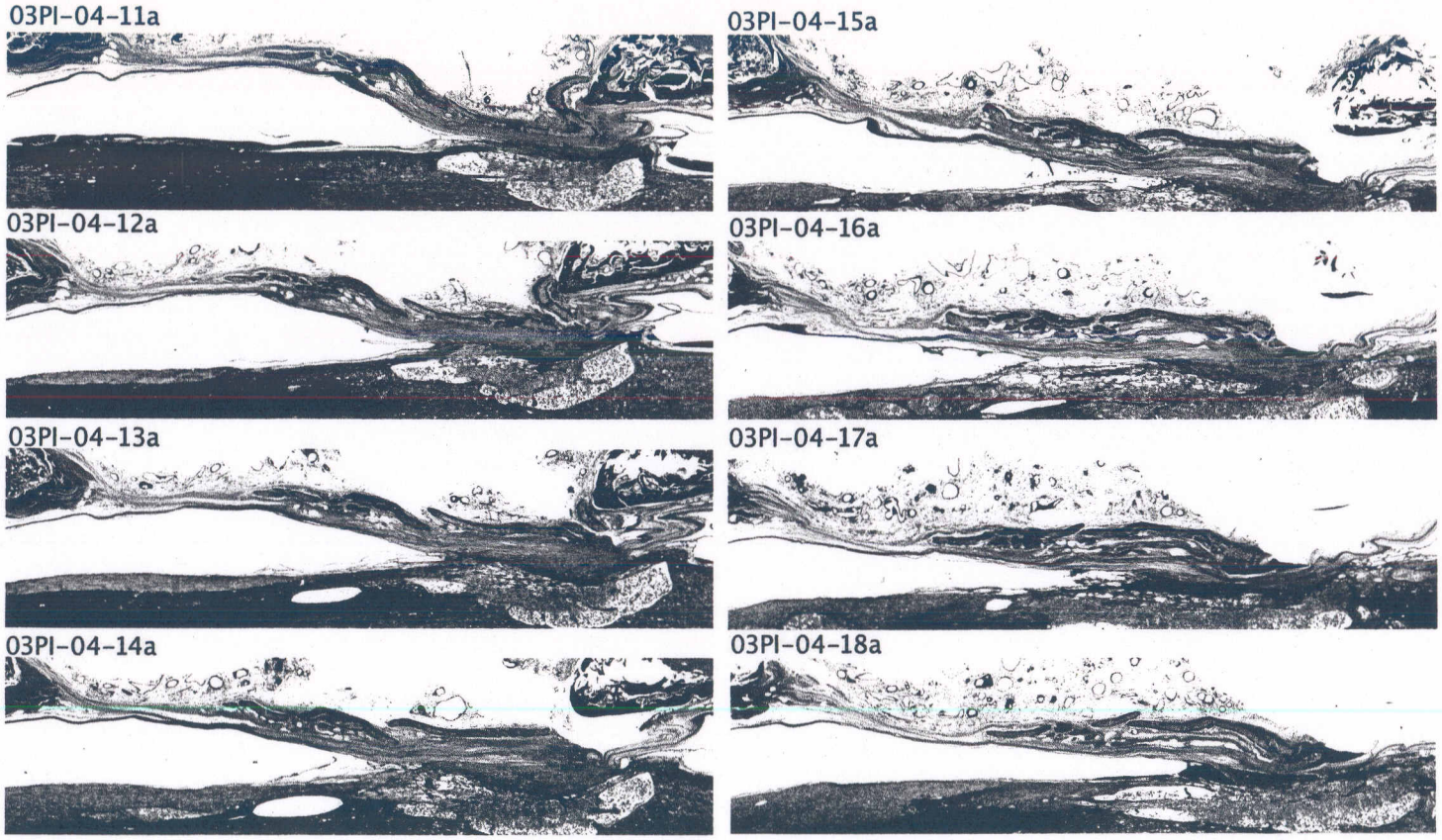


Figure 26. Thin epidural resorbable PLA/PCA fabric barrier at 6 weeks after a 12.5-mm spinal cord contusion. On the caudal side of the laminectomy site, an area of epidural and subdural adhesion had developed, associated with evidence of spinal cord damage.

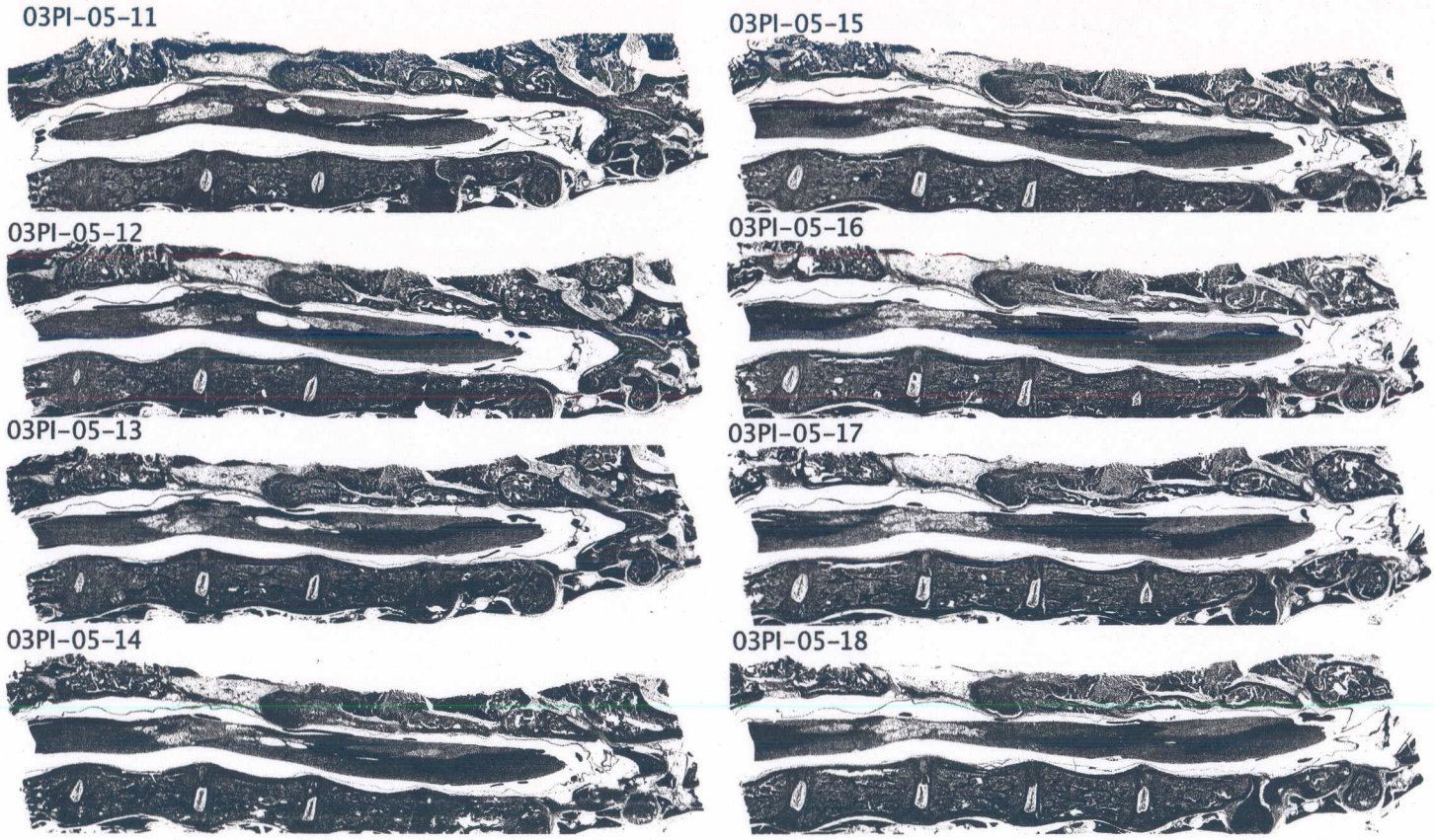


Figure 27. Thin epidural resorbable PLA/PCA fabric barrier at 6 weeks observed at 12.5-mm spinal cord contusion. Occasional epidural and subdural adhesions were present. See next figure for higher magnification views of the laminectomy site.

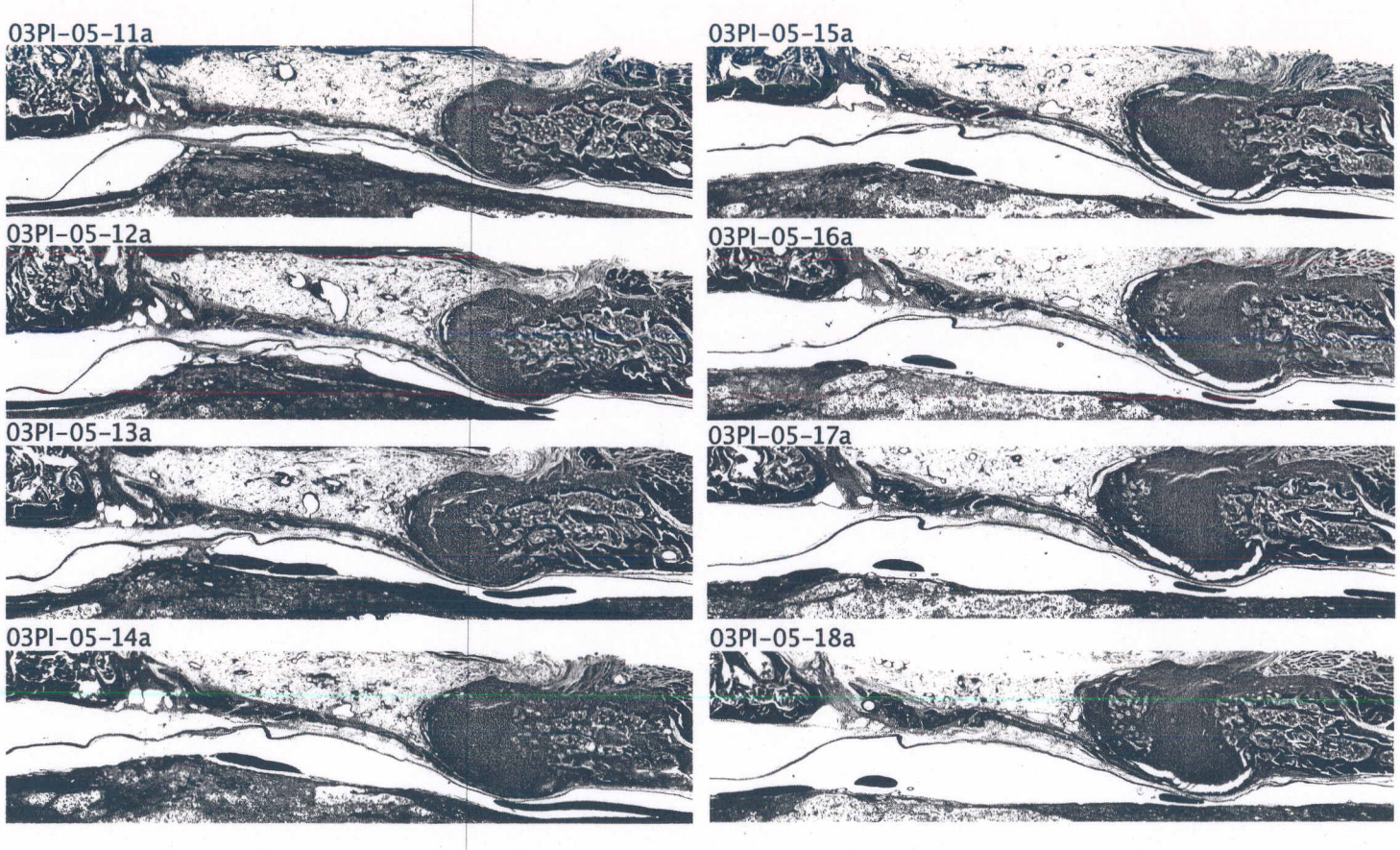


Figure 28. Thin epidural resorbable PLA/PCA fabric barrier at 6 weeks after a 12.5-mm spinal cord contusion. On the lower side of the laminectomy site, one area of epidural adhesion can be seen at the caudal laminectomy edge.

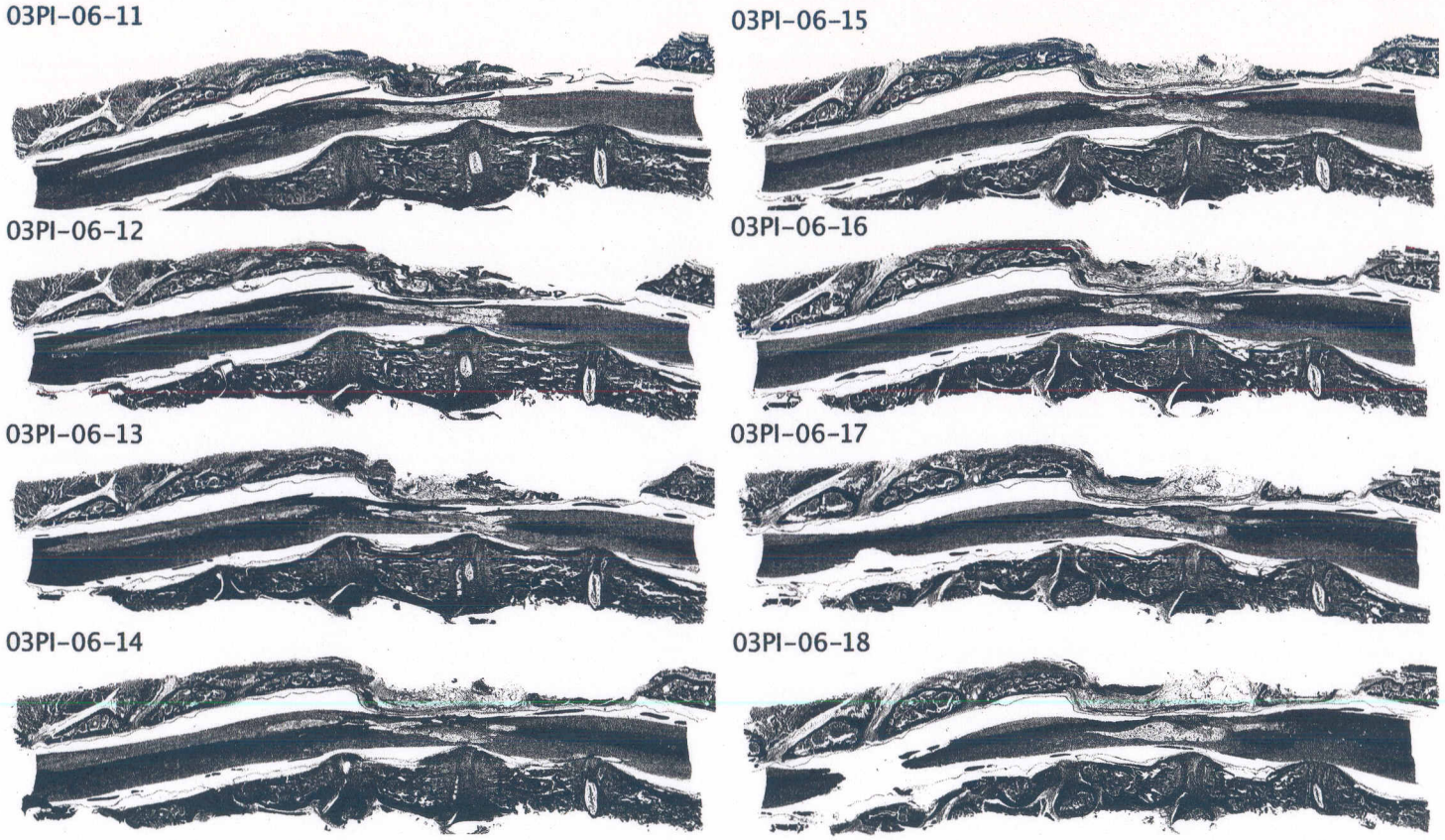


Figure 29. Thin epidural resorbable PLA/PCA fabric barrier at 6 weeks after a 12.5-mm spinal cord contusion. The spinal cord may be partially compressed at the laminectomy site. See next figure for higher magnification images of the laminectomy site.

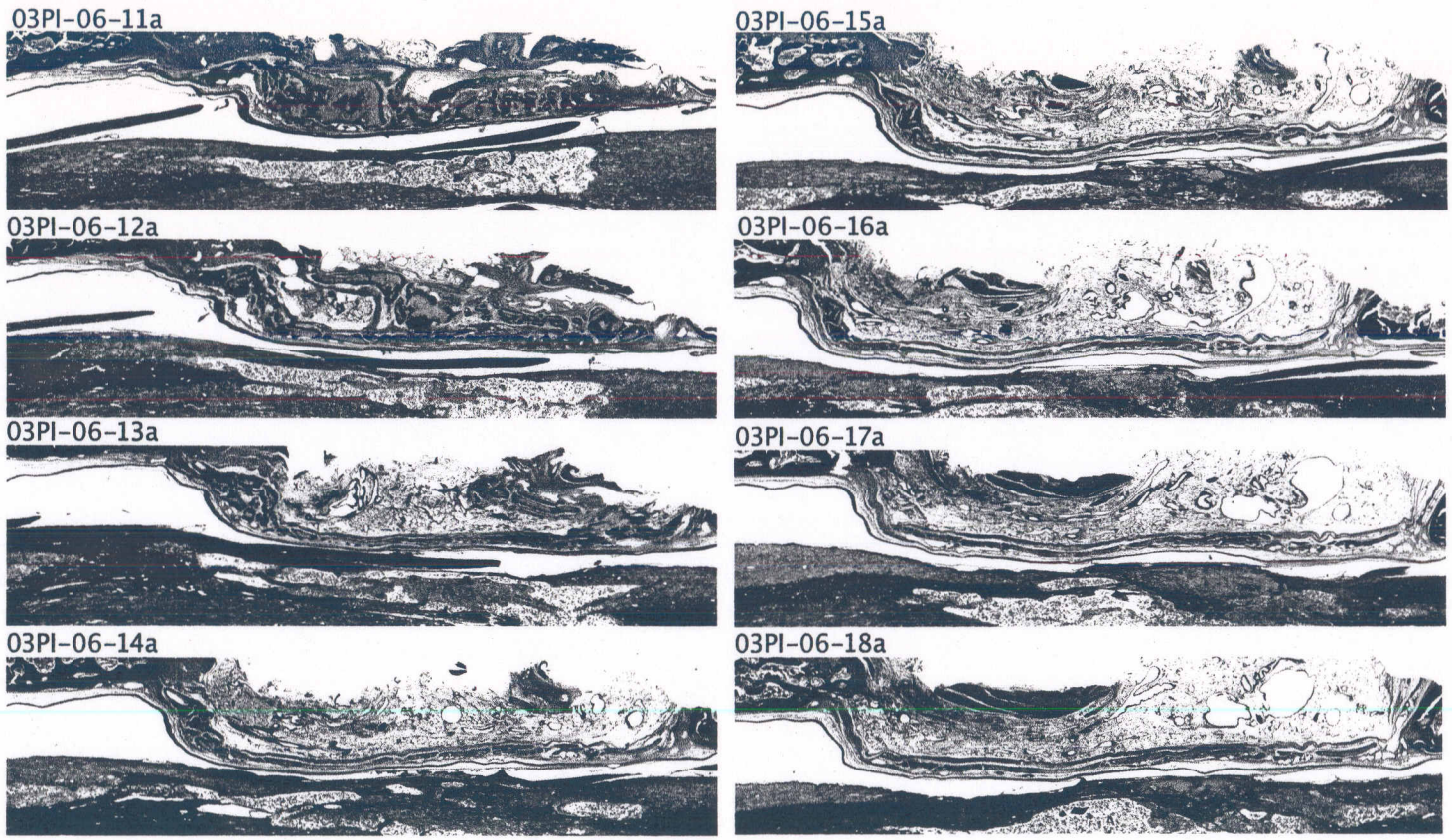


Figure 30. Thin epidural resorbable PLA/PCA fabric barrier at 6 weeks after a 12.5-mm spinal cord contusion. Although the dura appears to be attached to the barrier, closer inspection (right panels) indicate a space between the dura and the barrier. Only occasional epidural and subdural adhesions can be seen.

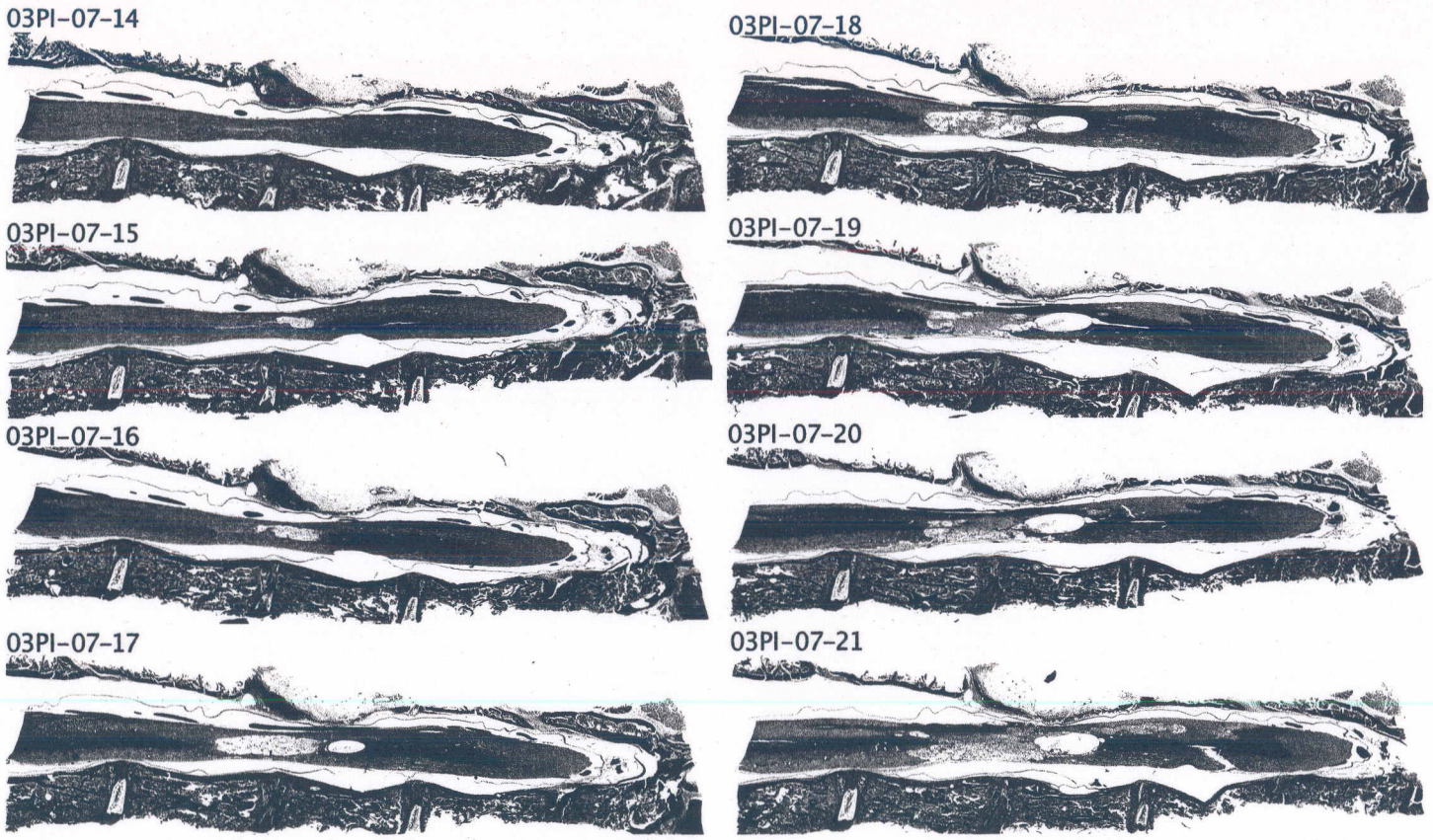


Figure 31. Thin epidural resorbable barrier at 6 weeks after a 12.5-mm spinal cord contusion. Some epidural and sudural adhesions can be seen (see next figure for higher magnification images) at the laminectomy site.

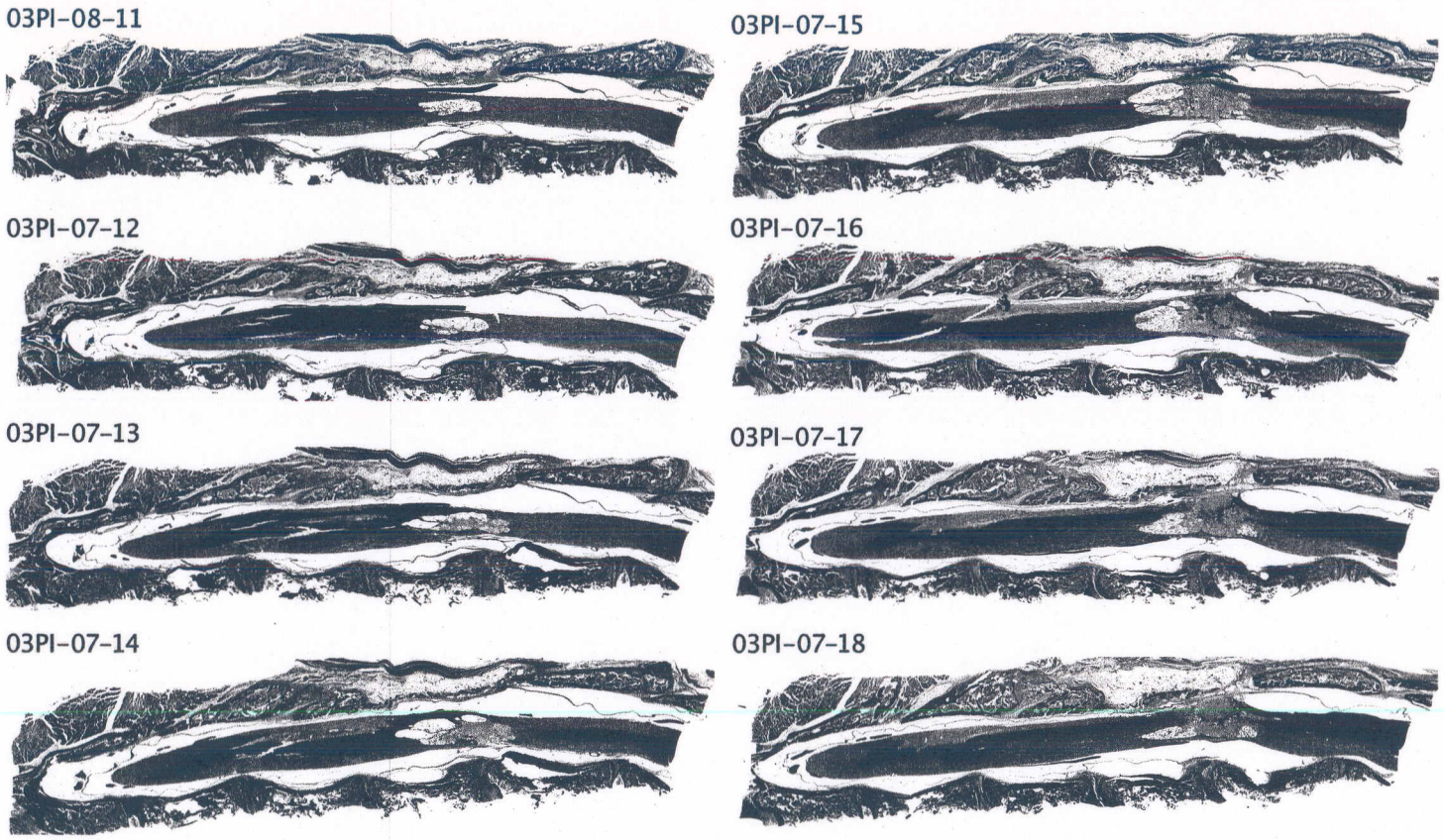


Figure 33. Thin epidural resorbable PLA/PCA fabric barrier at 6 weeks after a 12.5-mm spinal cord contusion. Some collagenous scar had infiltrated above and below the barrier particularly the caudal side of the contusion, resulting in both epidural and subdural adhesions. See next figure for higher magnification views of the laminectomy site.

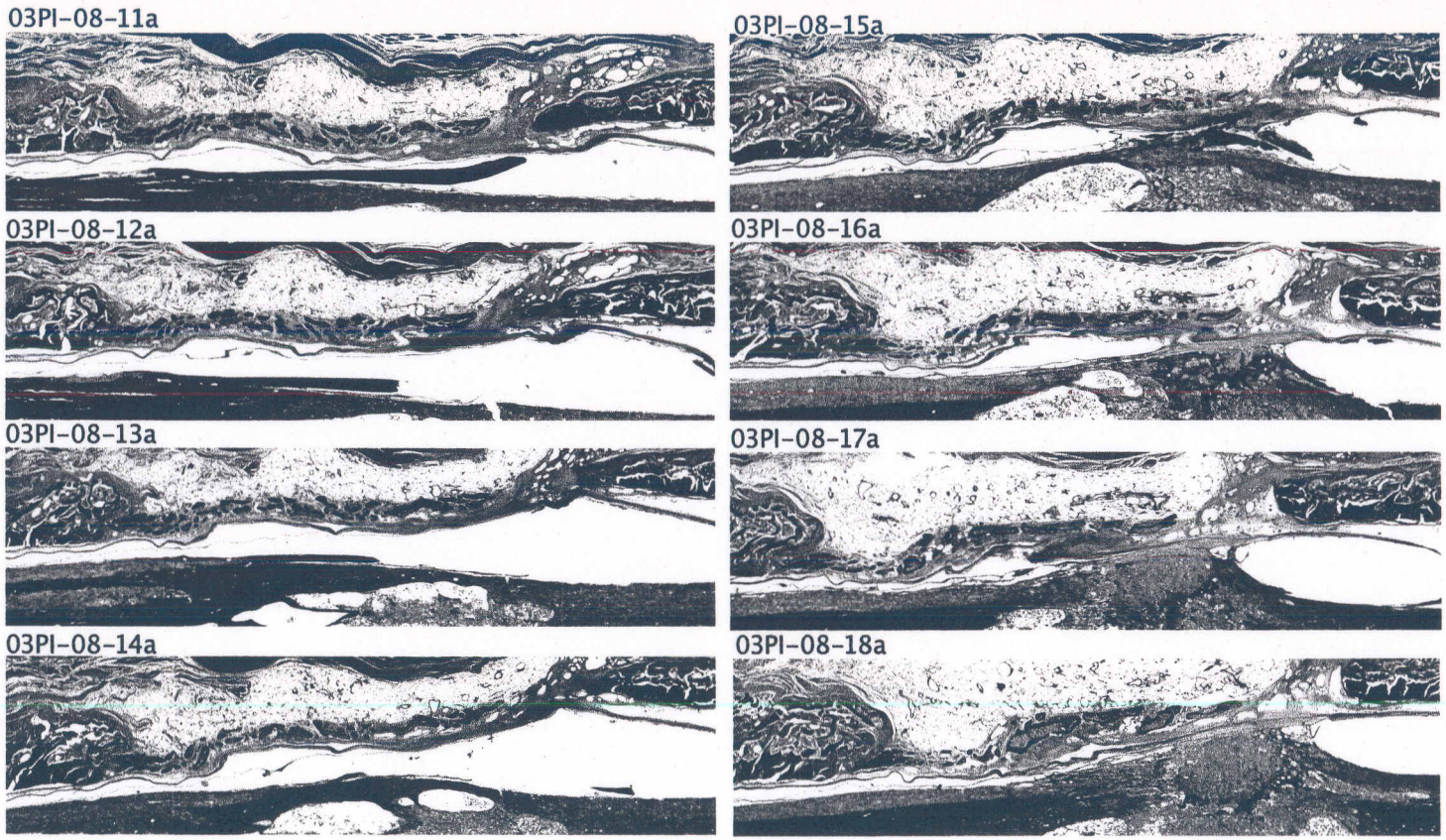


Figure 34. Thin epidural resorbable PLA/PCA fabric barrier at 6 weeks observed at 12.5-mm spinal cord contusion. On the caudal side of the laminectomy site, scar tissue appeared to have invaded into the ventral side of the barrier. In sections 15a-17a, an area of epidural and subdural adhesion can be see, along with changes in the underlying spinal cord.

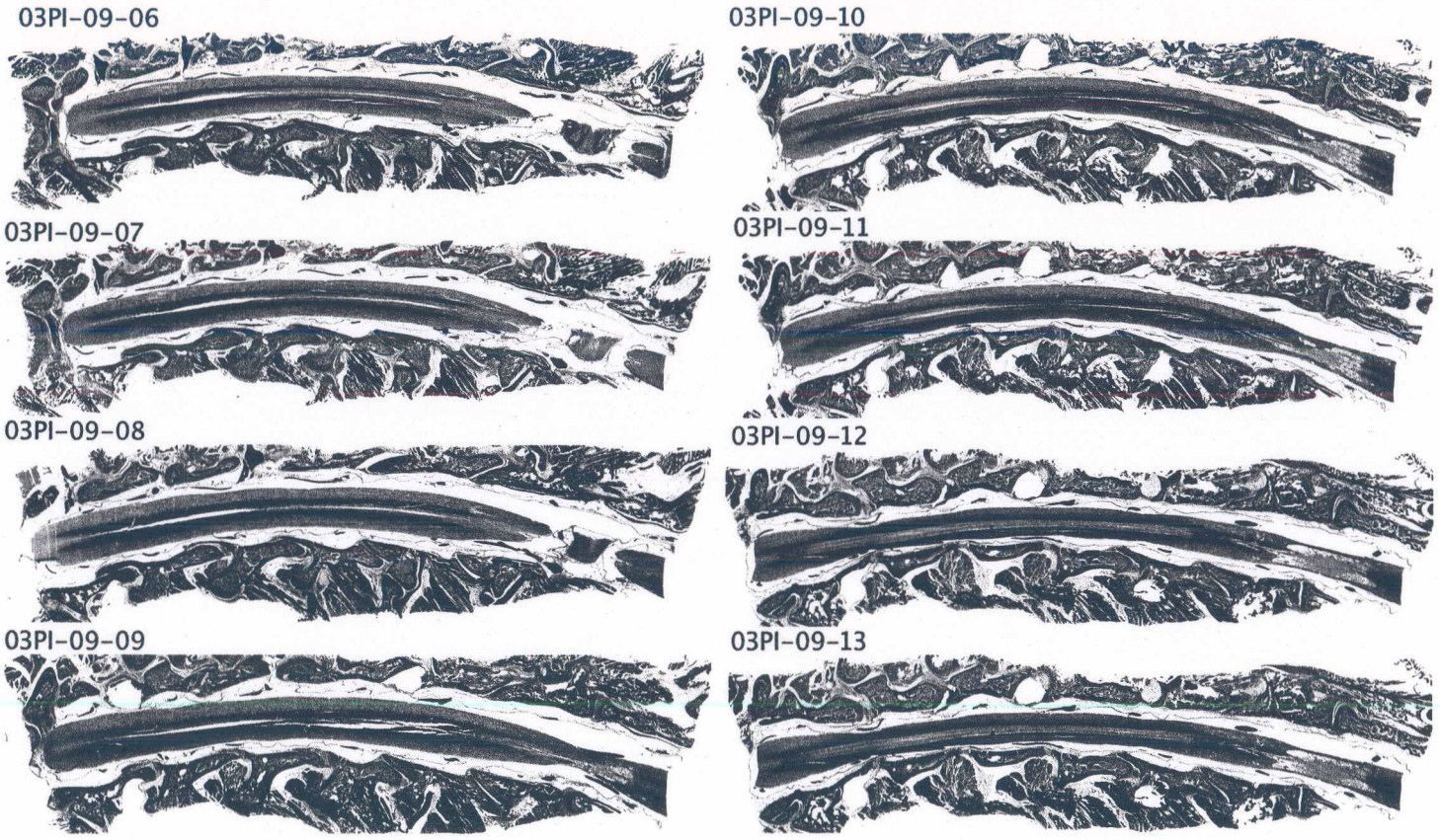


Figure 35. Thin epidural resorbable PLA/PCA fabric barrier at 6 weeks after a 12.5-mm spinal cord contusion. The spinal column had been horizontally sectioed with the injury site to the right. Sections 06 to 08 (top three left images on the right panel) show the laminectomy scar. The spinal cord is atrophic at the injury site and dips below the section plane. The horizontal sections show no adhesions of dura to the spinal cord or surrounding tissues.

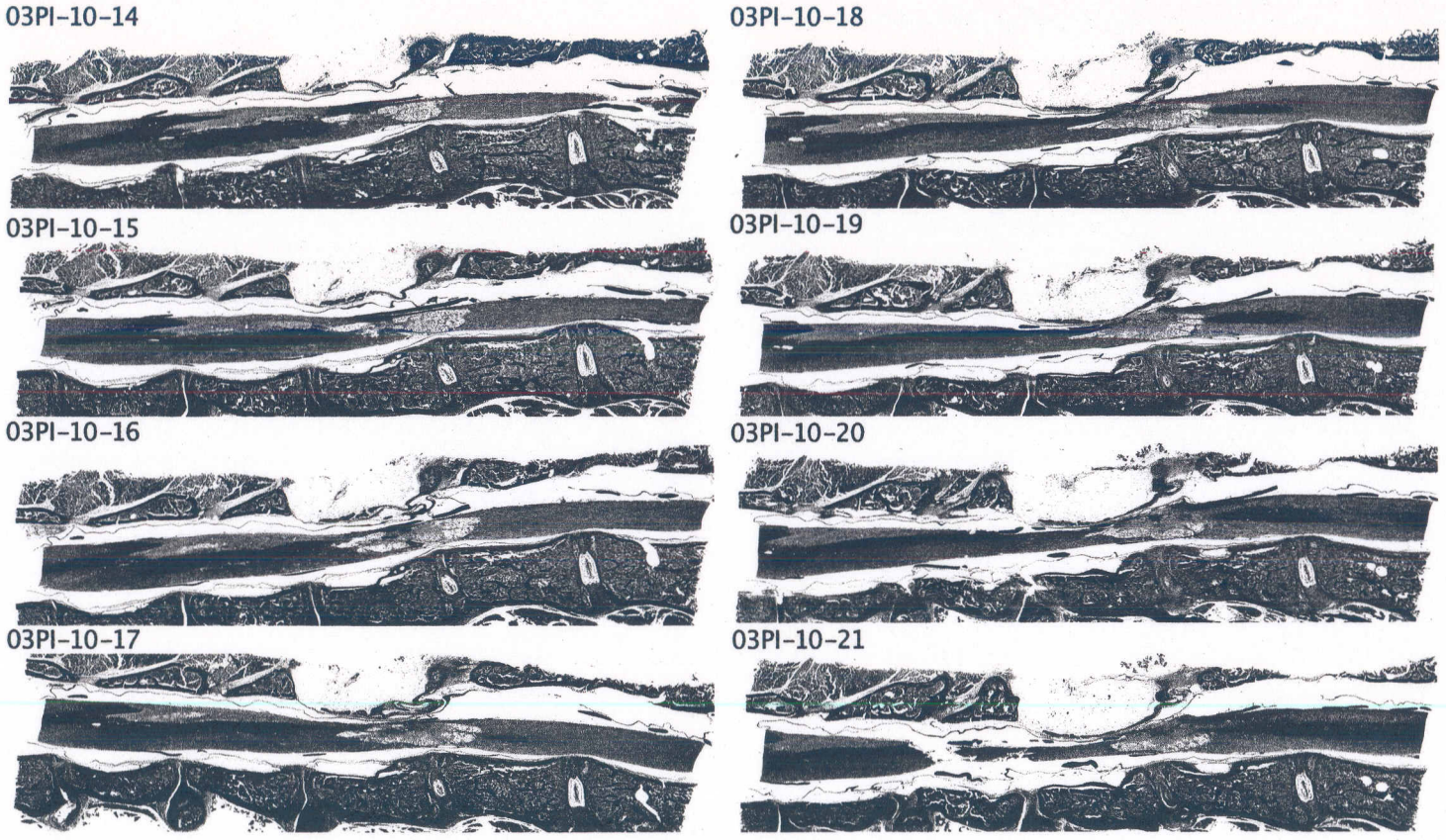


Figure 36. Thin epidural resorbable PLA/PCA fabric barrier at 6 weeks after 12.5-mm spinal cord contusion. Some epidural and subdural adhesions may be present on the caudal side of the laminectomy site (see next figure for higher magnification views).

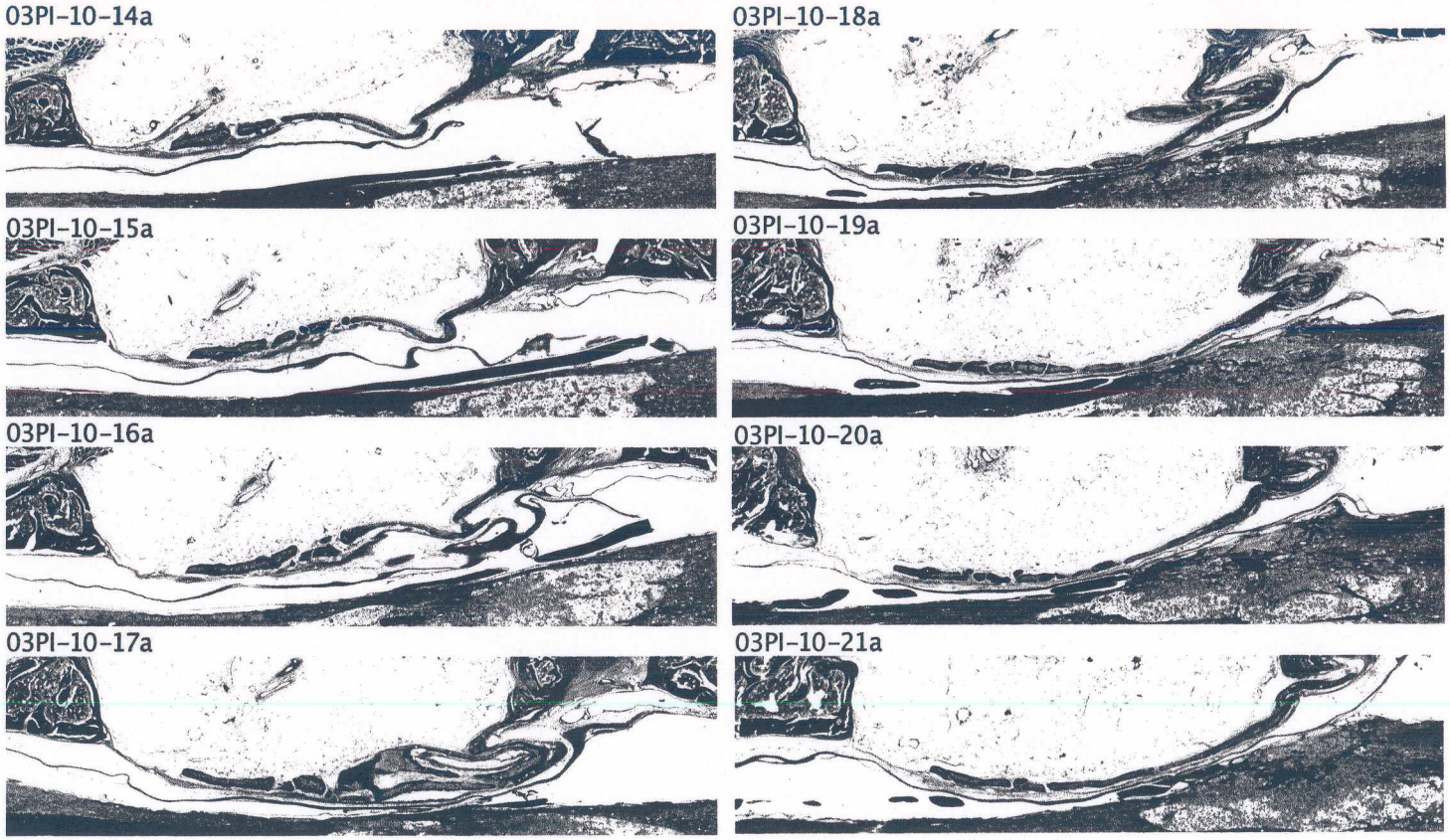
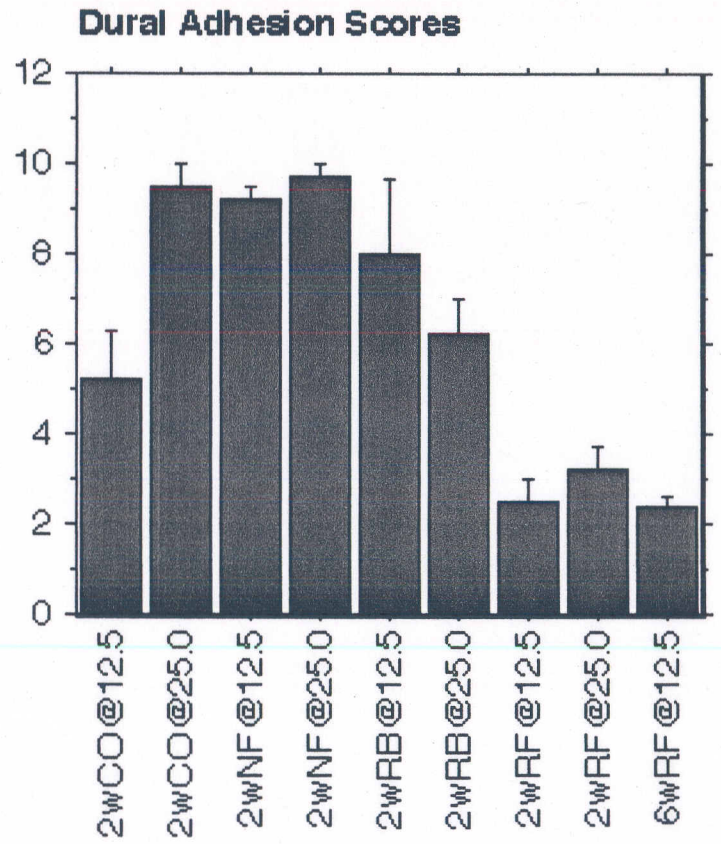


Figure 37. Thin epidural resorbable PLA/PCA fabric barrier at 6 weeks after a 12.5-mm spinal cord contusion. A area of epidural and subdural adhesion can be seen (section 18a-20a)



Bonferroni/Dunn Post-hoc Comparisons

X	Y	Δ(Y-X)	p-value
2wCO@12.5	2wCO@25.0	+4.25	=0.0002*
2wCO@12.5	2wNF@12.5	+4.00	=0.0004*
2wCO@12.5	2wRB@12.5	+2.75	=0.0107
2wCO@12.5	2wRF@12.5	-2.75	=0.0107
2wCO@25.0	2wNF@25.0	+0.25	=0.8072
2wCO@25.0	2wRB@25.0	-3.25	=0.0030*
2wCO@25.0	2wRF@25.0	-6.25	<0.0001*
2wCO@12.5	6wRF@12.5	-2.85	=0.0020*
2wRF@12.5	6wRF@12.5	-1.25	=0.2203
2wRF@12.5	2wRF@25.0	+0.50	=0.6259

ANOVA F=22.45, P<0.0001
* significant at p<0.005 vs. matched injury control

Figure 38. Dural Adhesion Scores (DAS) for contusion experiments. The mean DAS scores and standard errors of means are shown in the graph (left). Analysis of Variance (ANOVA) indicated significant differences among the treatment groups (F=22.45, p<0.0001). Post-hoc analyses compared the two untreated injury control groups (2wCO@12.5, 2wCO@25.0), each of the treatment groups (2w, 6w; CO=Control, NF=Nonresorbable Fabric, RB=Resorbable Barrier, RF=Resorbable Fabric) against matched injury controls, and NF treatment for 2 weeks versus 6 weeks The p-value for significance p=0.005. The table lists the treatment groups compared, mean differences, and p-values of comparisons.

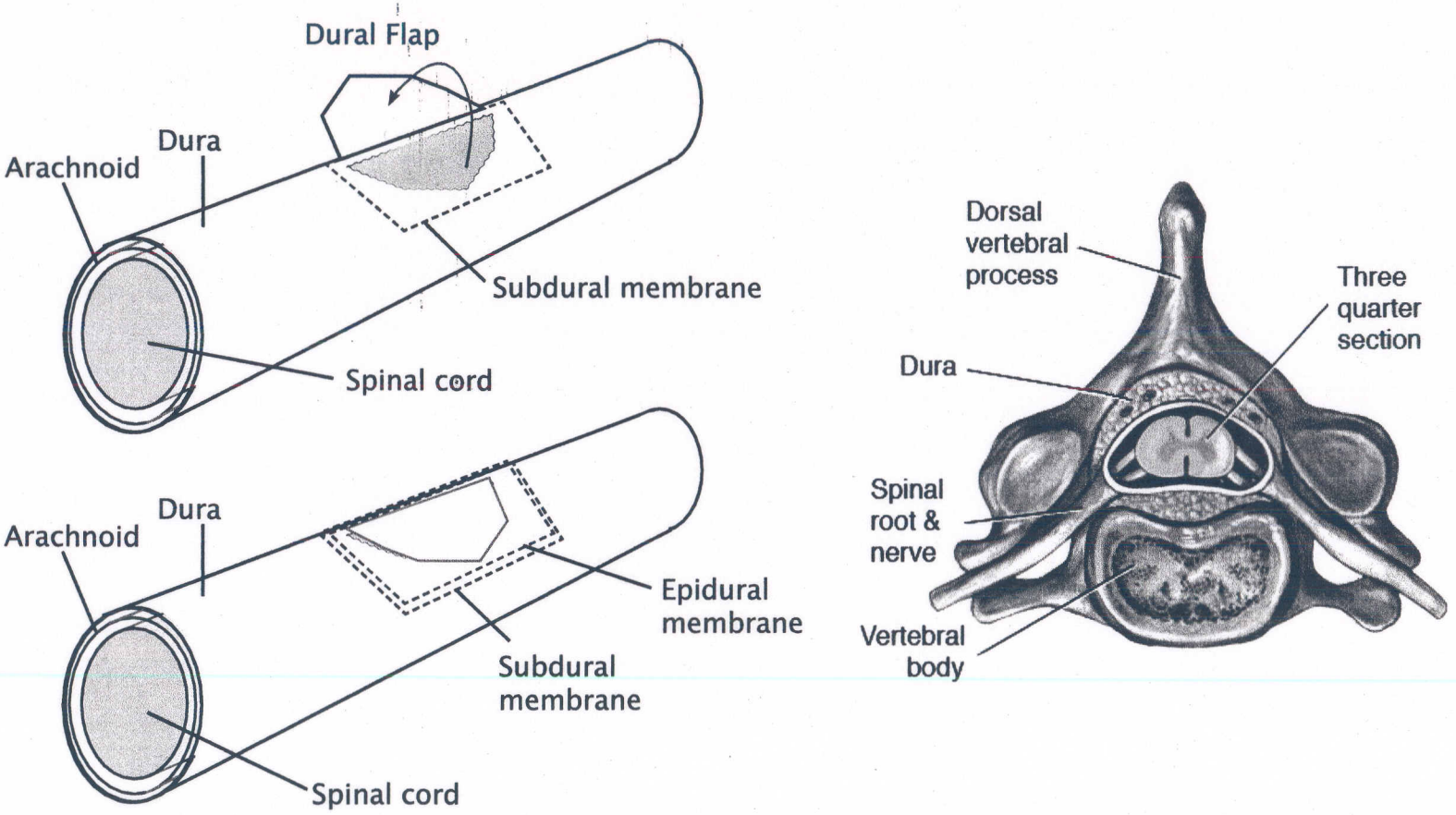


Figure 39. The sandwich dural repair method. A dural flap was cut, like an envelope. The spinal cord is cut with careful hemostasis. A thin absorbable membrane is placed beneath the dura (subdural), the dural flap is returned to its original closed position, and another thin absorbable membrane is placed on top (epidural).

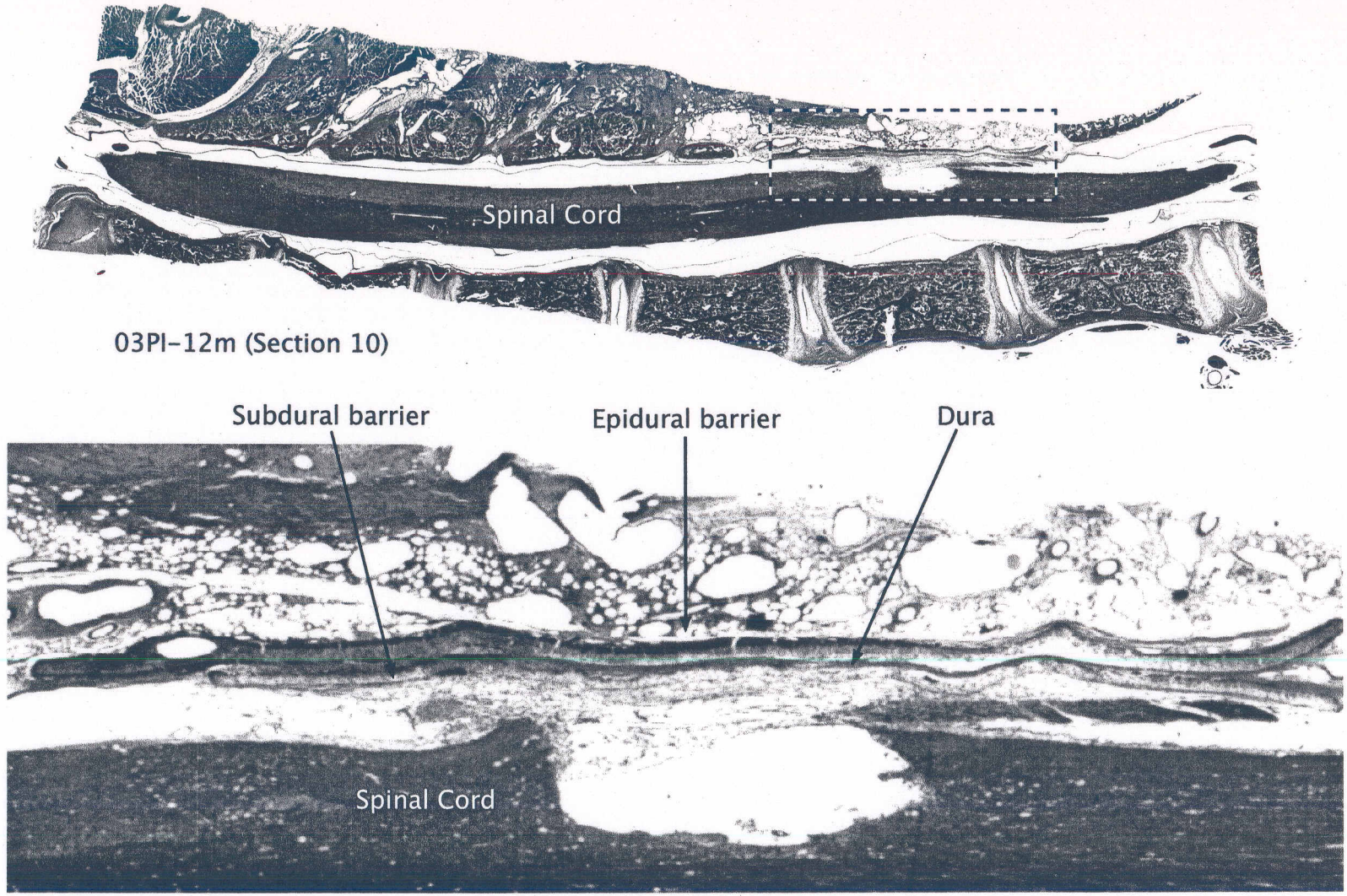


Figure 40. A "sandwich" dural repair at 6 weeks after a three-quarter section of the spinal cord. The dura can be seen between the epidural and subdural barriers. A piece of fat had been placed on top of the epidural membrane. The loose cystic fatty material replaced where the laminectomy scar usually would be. The subdural barrier was barely discernible. The dura can be seen within the "sandwich". A loose matrix was present between the spinal cord and the subdural layer. The area of spinal cord damage has a cavity but the cord surrounding the vacity shows remarkably little loss of cellular material (red color).

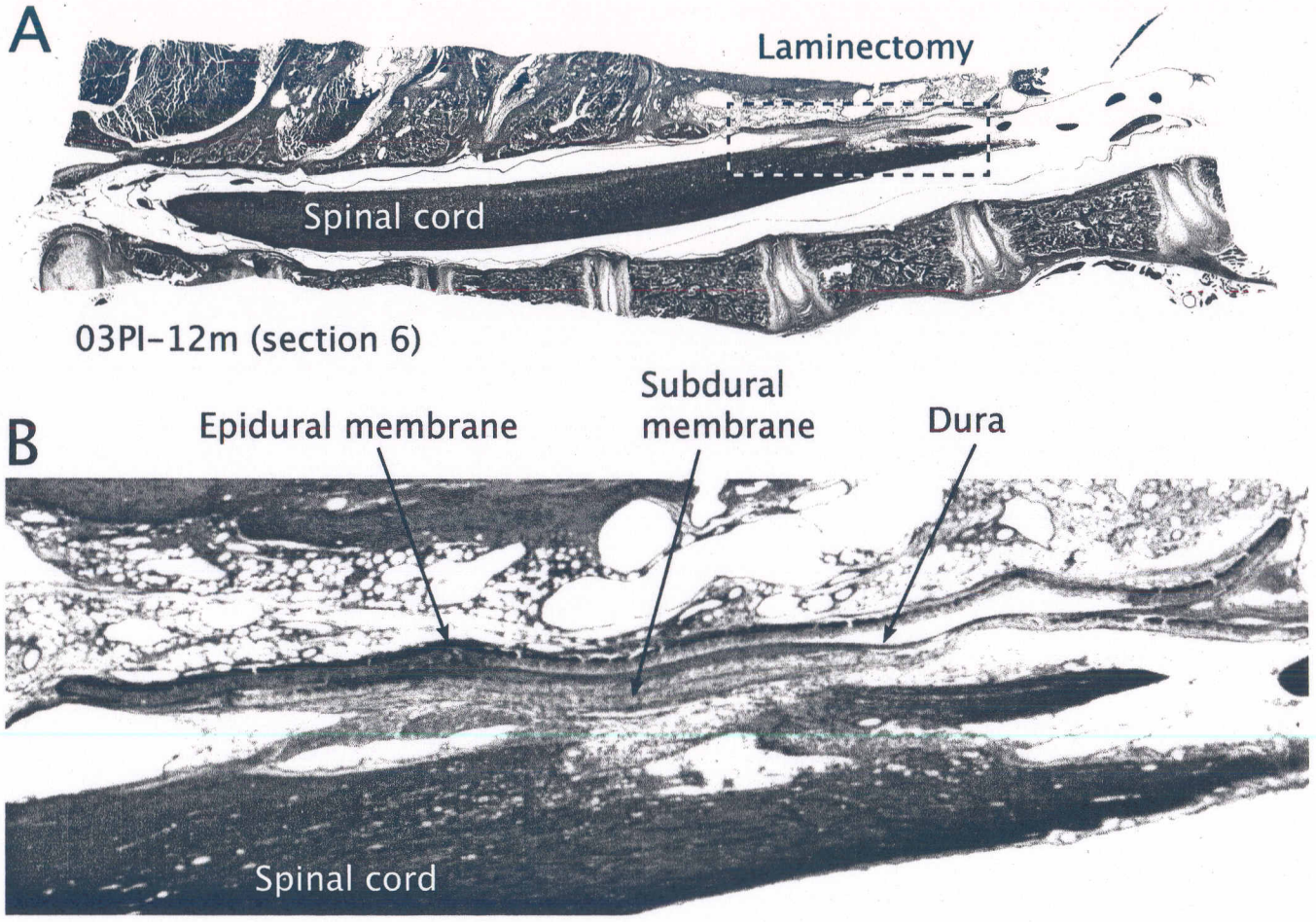


Figure 41. A "sandwich" dural repair at 6 weeks after a three-quarter section of the spinal cord. The subdural membrane was beginning to break up and a loose matrix was present between the spinal cord and subdural barrier.

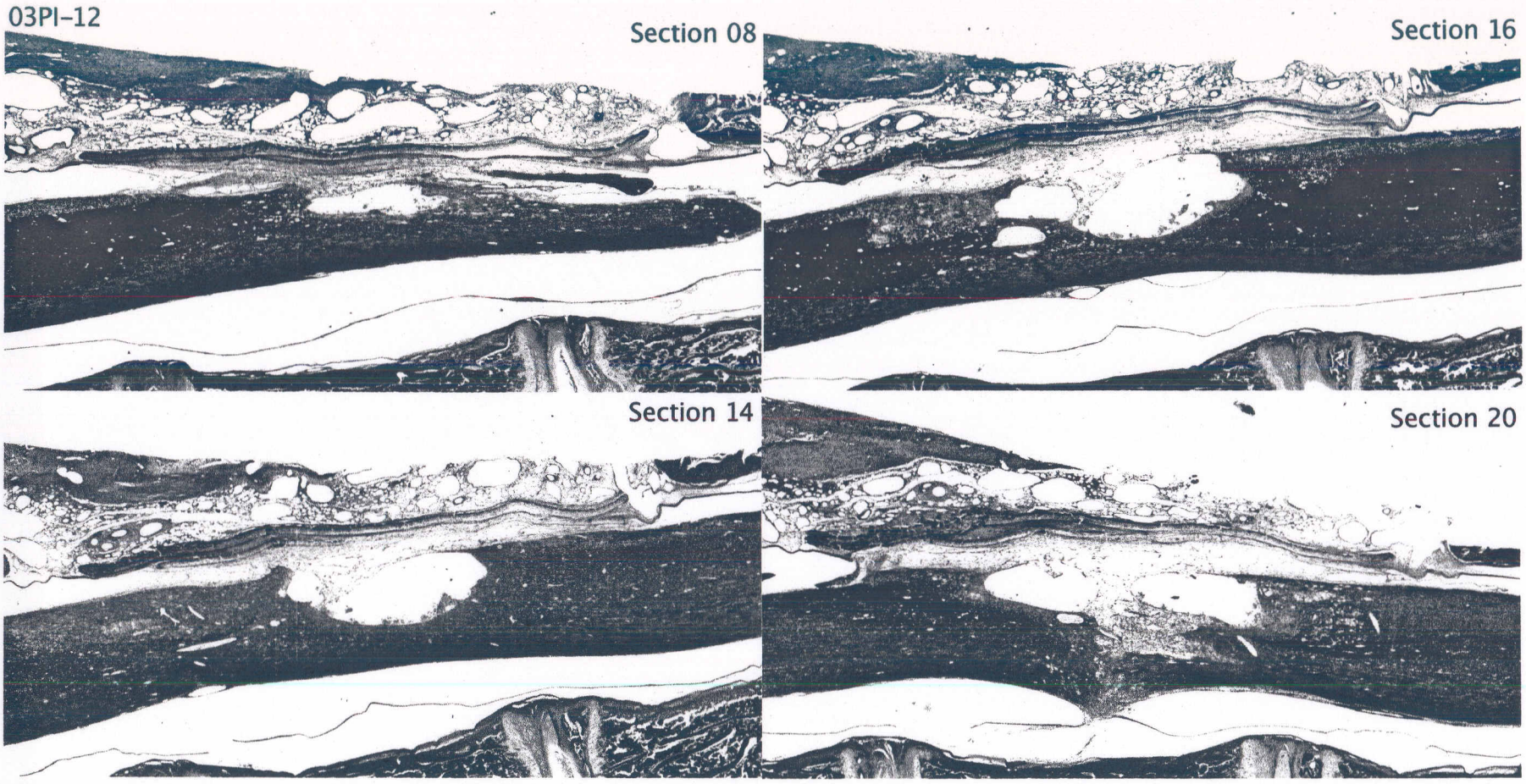


Figure 42. A "sandwich" dural repair at 6 weeks after a three-quarter section of the spinal cord. Four additional views of 03PI-12, 6 weeks after a three-quarter section of the T10 cord and "sandwich repair" of the dural opening with an epidural and a subdural barrier. A piece of fat had been placed above the epidural barrier. Most of the fat was cystic. No or few inflammatory infiltrate was present. The cystic cavity resulting from the cut of the spinal cord can be seen in all sections, as well as the epidural barrier. A loose tissue matrix with no collagen (blue) is present between the spinal cord and the barely discernible subdural barrier. Note that because this was a three-quarter section of the spinal cord, sparing the right ventral quarter of the spinal cord (section 08). The cut can be seen to extend across the entire thickness of the spinal cord in section 20.

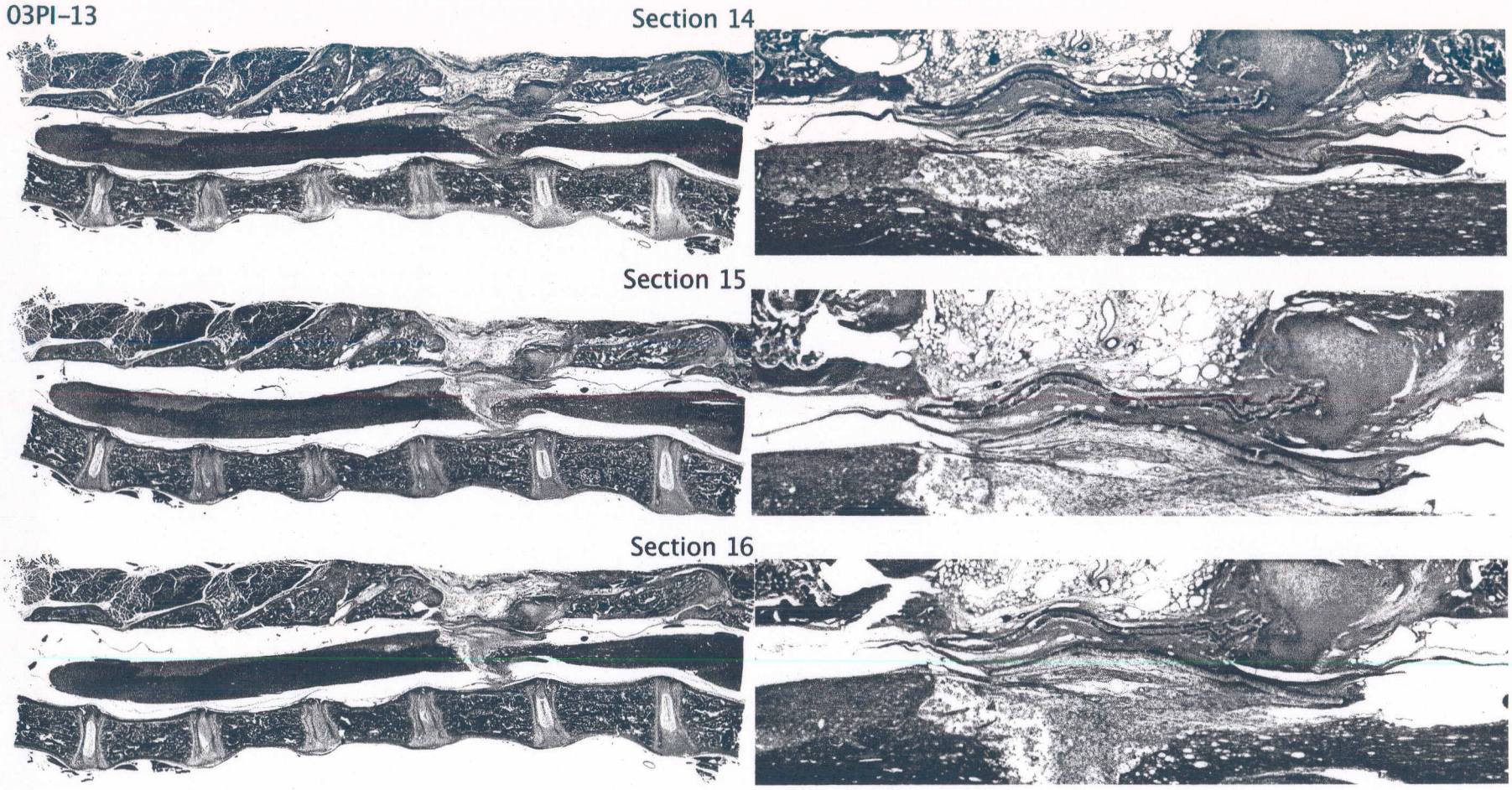


Figure 43. A "sandwich" dural repair at 6 weeks after a three-quarter section of the spinal cord. Three sagittal views of 03PI-13 at 6 weeks after a three-quarter section of the T10 cord and "sandwich repair" of the dural opening with an epidural and a subdural barrier. A piece of fat had been placed above the epidural barrier. The left panels show low-power views of the spinal cord while the right panels show enlargements of the laminectomy site. The epidural and subdural barriers can be clearly seen in section 14. However, fibroblasts have infiltrated into the space between the barrier. No or few inflammatory infiltrate was present. A non-collagenous cellular matrix has filled the cut and the space between the spinal cord and the subdural barrier. Although dura can be clearly seen to be entering the space between the two barriers, the dura merged with the scar tissue between the two barriers.

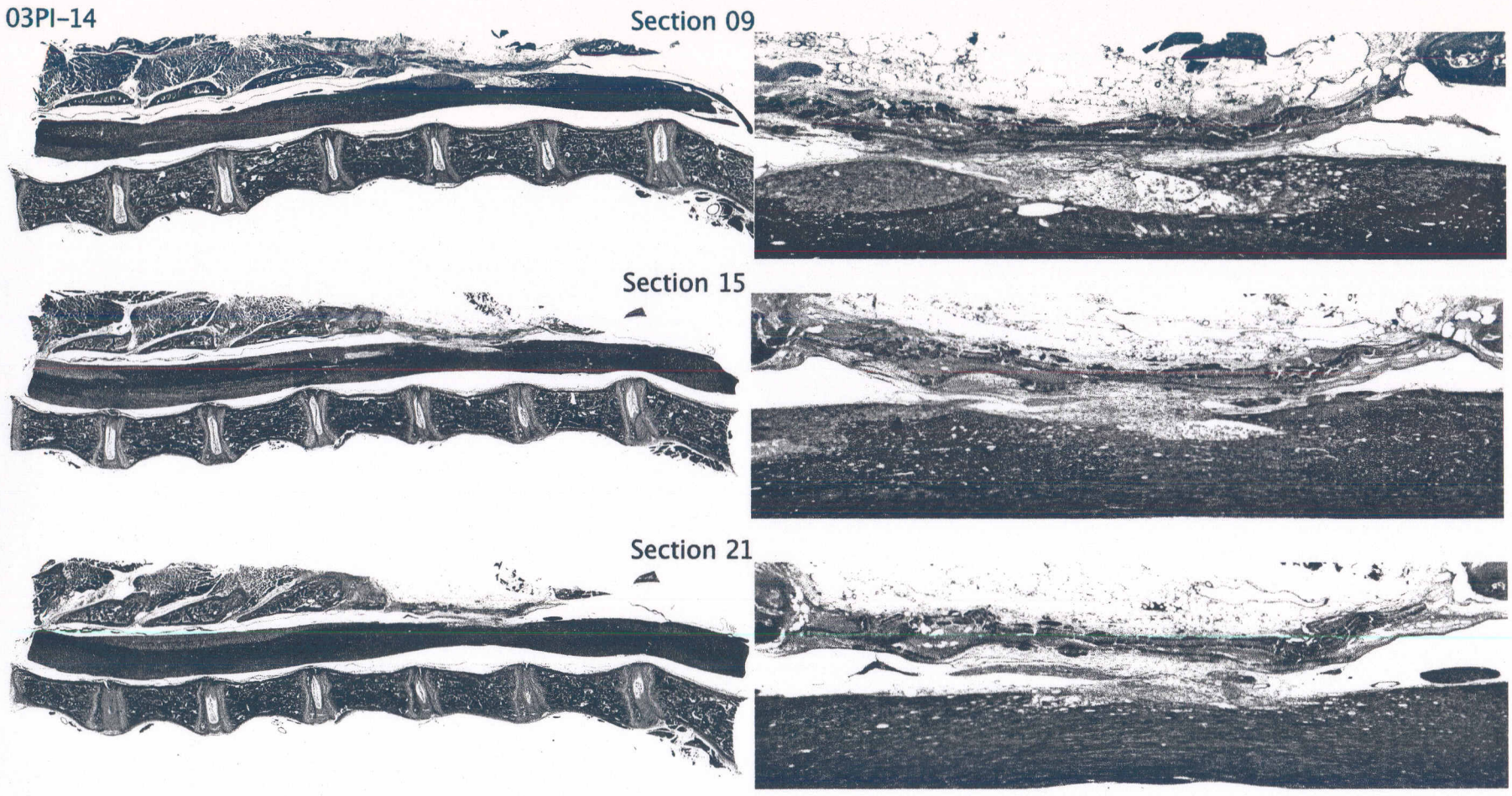


Figure 44. A "sandwich" dural repair at 6 weeks after a three-quarter section of the spinal cord. Three sagittal views of 03PI-14 at 6 weeks after a three-quarter section of the T10 cord and "sandwich repair" of the dural opening with an epidural and a subdural barrier. A piece of fat had been placed above the epidural barrier. The left panels show low-power views of the spinal cord while the right panels show enlargements of the laminectomy site. The epidural and subdural barriers are beginning to break up. Some collagenous material have infiltrated into the space between the barrier. Although dura can be clearly seen to be entering the space between the two barriers, the dura merged with the collagenous material between the two barriers. A non-collagenous cellular matrix has filled the cut site in the spinal cord.

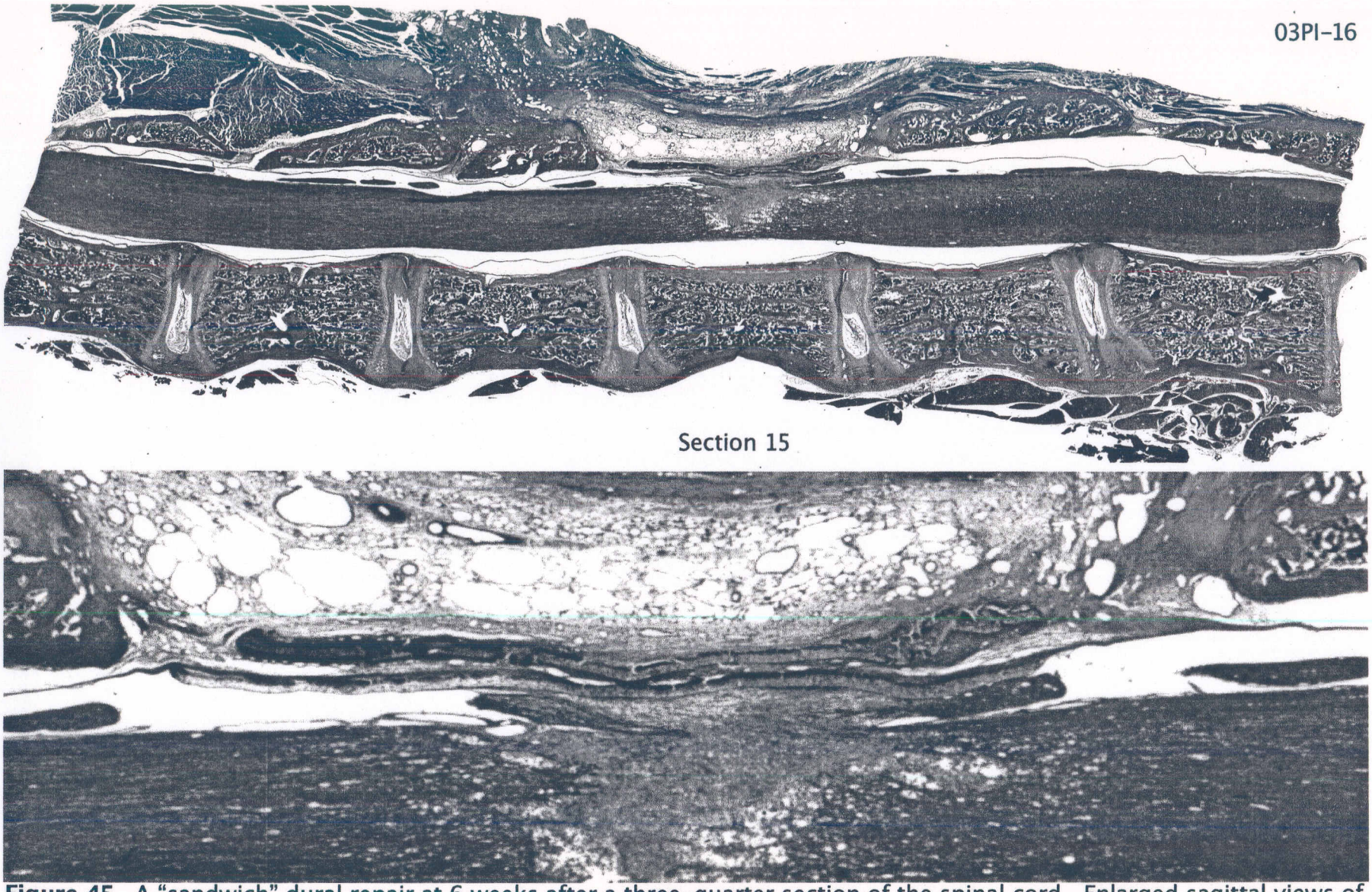


Figure 45. A "sandwich" dural repair at 6 weeks after a three-quarter section of the spinal cord. Enlarged sagittal views of 03PI-16 at 6 weeks after a three-quarter section of the T10 cord and "sandwich repair" of the dural opening with an epidural and a subdural barrier. A piece of fat had been placed above the epidural barrier. The top panel shows a lower power view of the spinal cord and bottom panel is an enlargement of the repair site. Both the top and bottom barriers can be clearly seen. However, subdural adhesions can be seen.

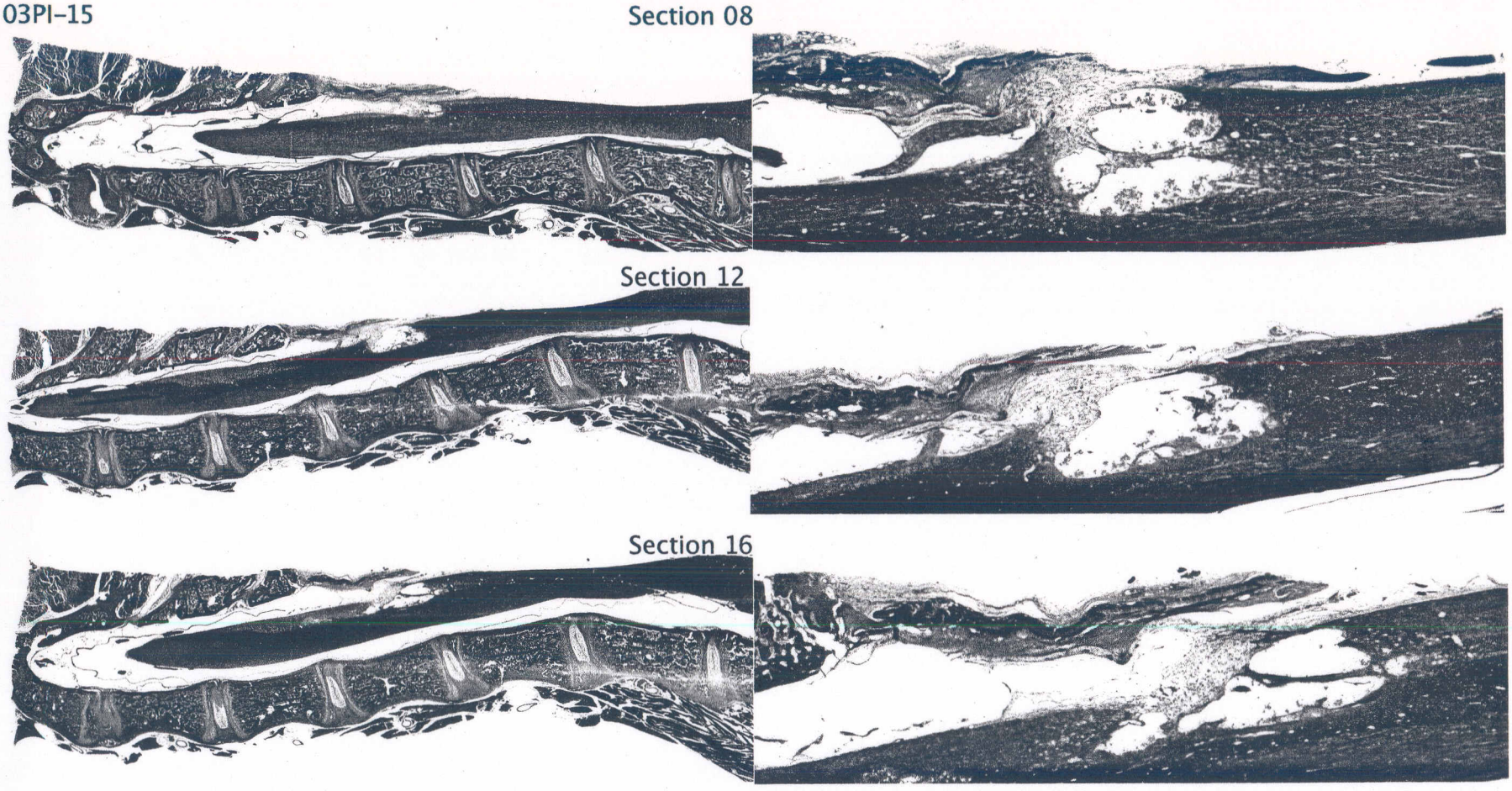


Figure 46. A "sandwich" dural repair at 6 weeks after a three-quarter section of the spinal cord. Three sagittal views of 03PI-15 at 6 weeks after a three-quarter section of the T10 cord and "sandwich repair" of the dural opening with an epidural and a subdural barrier. A piece of fat had been placed above the epidural barrier. The left panels show low-power views of the spinal cord while the right panels show enlargements of the laminectomy site. The epidural and subdural barriers are beginning to break up. Unfortunately, part of the site was lost in histological processing. However, some collagenous material have infiltrated into the space between the barriers. A non-collagenous cellular matrix was present between the spinal cord and the subdural barrier..

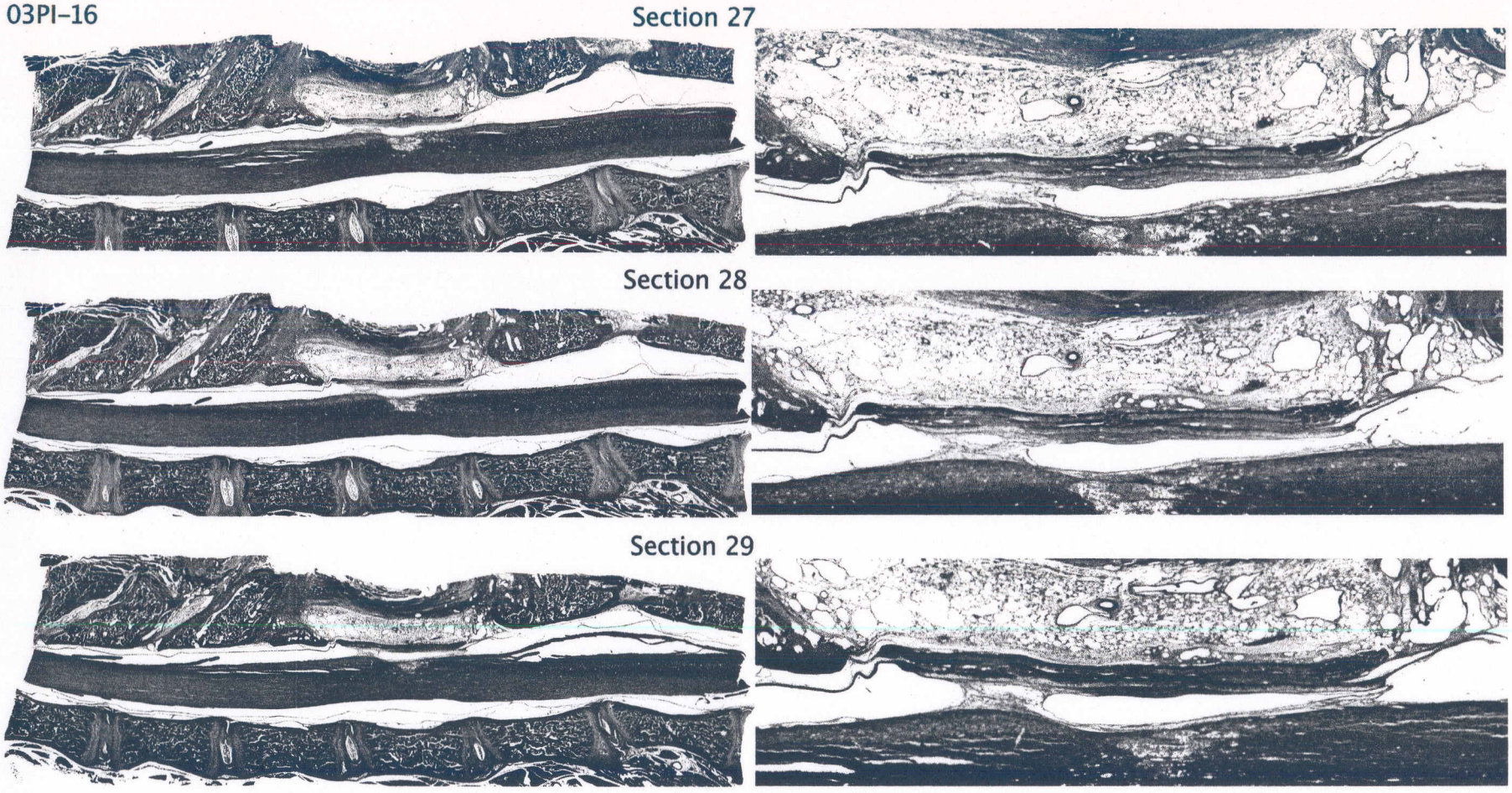


Figure 47 A "sandwich" dural repair at 6 weeks after a three-quarter section of the spinal cord. Three sagittal views of 03PI-16 at 6 weeks after a three-quarter section of the T10 cord and "sandwich repair" of the dural opening with an epidural and a subdural barrier. A piece of fat had been placed above the epidural barrier. The left panels show low-power views of the spinal cord while the right panels show enlargements of the laminectomy site. Some collagenous material have infiltrated into the space between the barriers but the barriers clearly repaired the dural opening with minimal epidural or subdural adhesions.

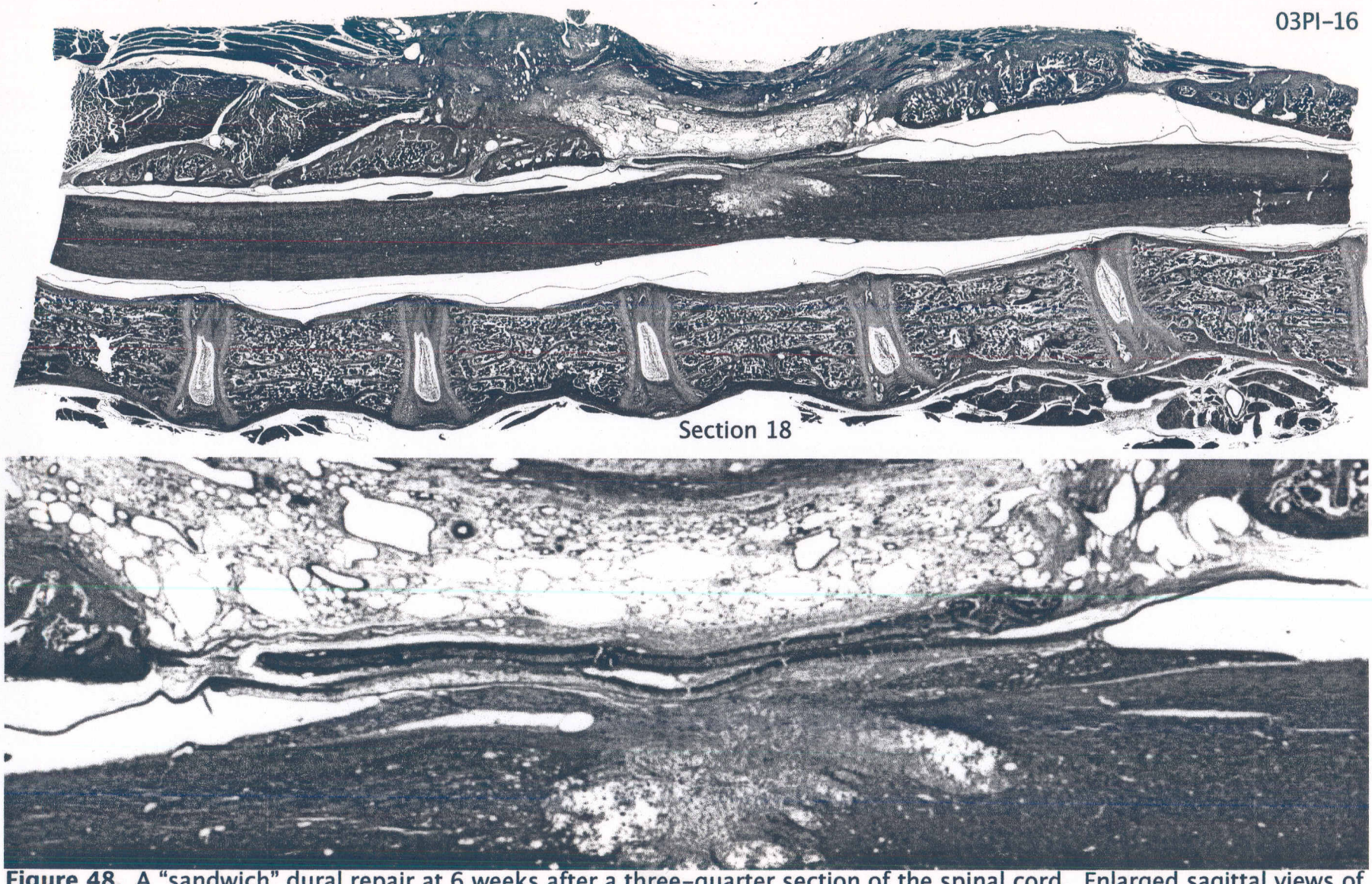


Figure 48. A "sandwich" dural repair at 6 weeks after a three-quarter section of the spinal cord. Enlarged sagittal views of 03PI-16 at 6 weeks after a three-quarter section of the T10 cord and "sandwich repair" of the dural opening with an epidural and a subdural barrier. A piece of fat had been placed above the epidural barrier. The top panel shows a lower power view of the spinal cord and bottom panel is an enlargement of the repair site. Both the top and bottom barriers can be clearly seen. However, subdural adhesions can be seen.

Figure 49. A "sandwich" dural repair at 6 weeks after a three-quarter section of the spinal cord. Enlarged sagittal views of 03PI-17 at 6 weeks after a three-quarter section of the T10 cord and "sandwich repair" of the dural opening with an epidural and a subdural barrier. A piece of fat had been placed above the epidural barrier. This is an example of a failed repair. Note the invasion of scar into the cut spinal cord.

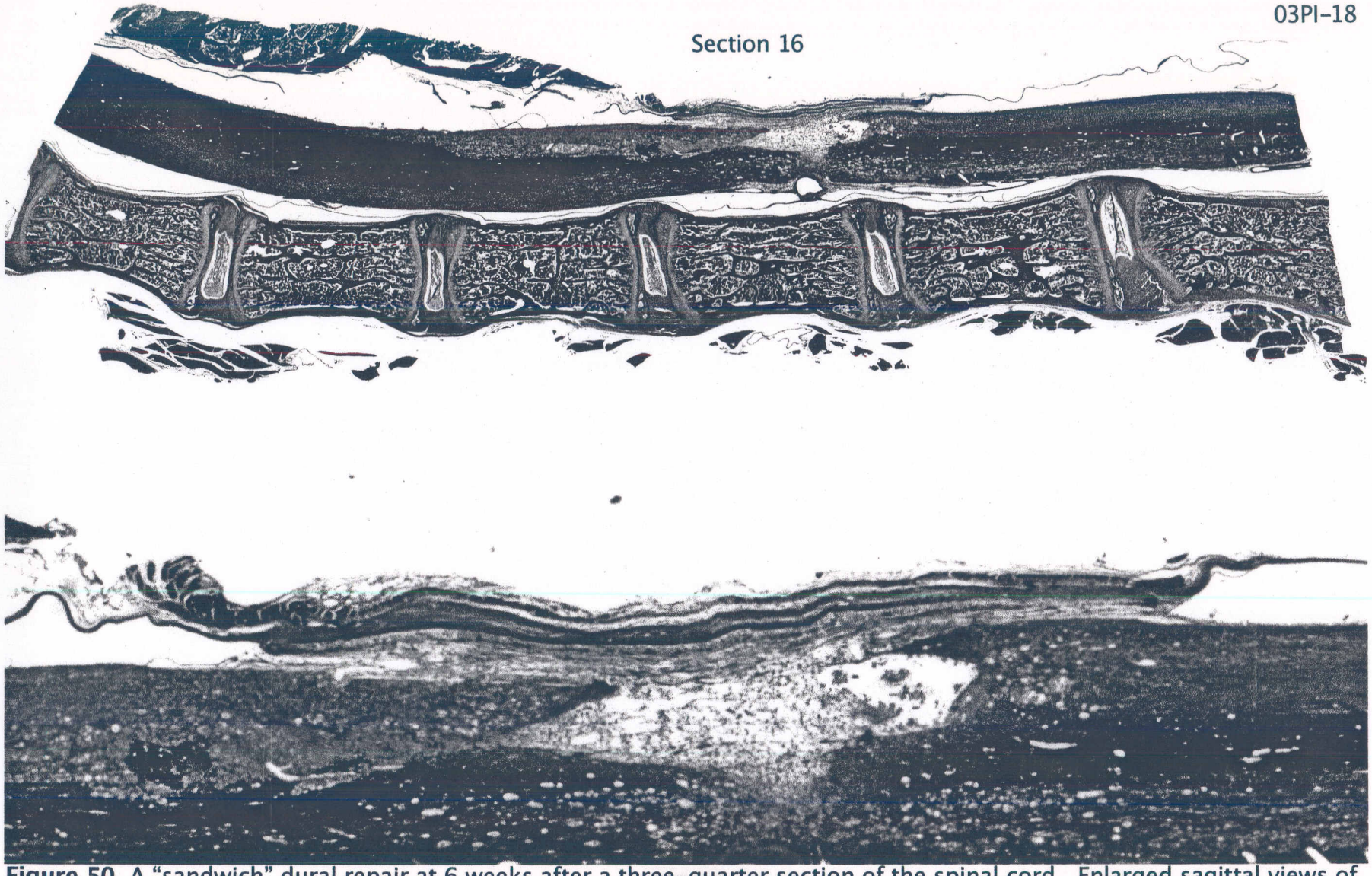


Figure 50 A "sandwich" dural repair at 6 weeks after a three-quarter section of the spinal cord. Enlarged sagittal views of 03PI-18 at 6 weeks after a three-quarter section of the T10 cord and "sandwich repair" of the dural opening with an epidural and a subdural barrier. A piece of fat had been placed above the epidural barrier.

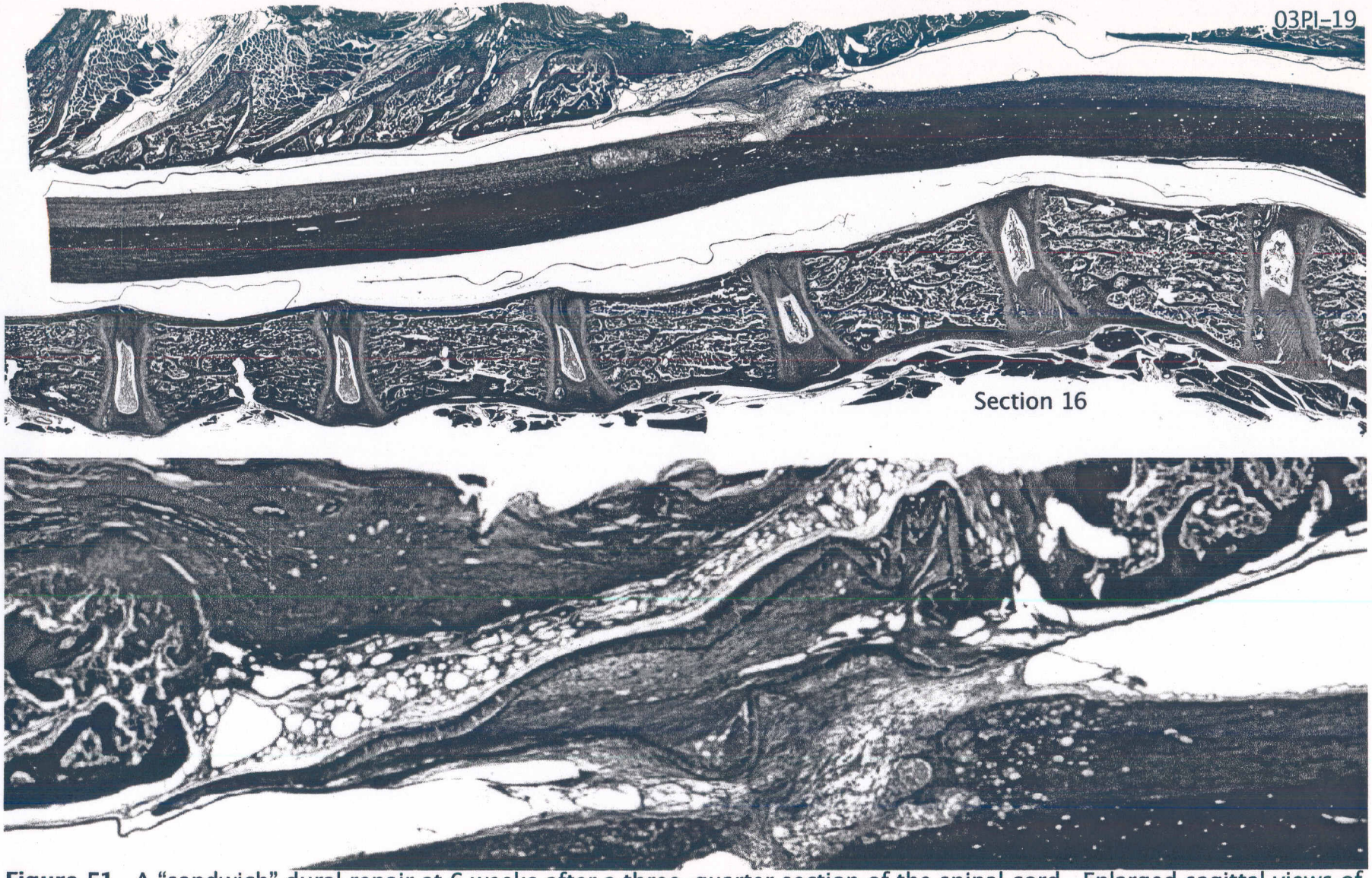


Figure 51. A "sandwich" dural repair at 6 weeks after a three-quarter section of the spinal cord. Enlarged sagittal views of 03PI-19 at 6 weeks after a three-quarter section of the T10 cord and "sandwich repair" of the dural opening with an epidural and a subdural barrier. A piece of fat had been placed above the epidural barrier. Note that both the epidural and sudural barriers barrier had buckled. Some collagenous scar tissue had entered into the sandwich area. A loose matrix of tissue was present between the subdural barrier and the spinal cord.

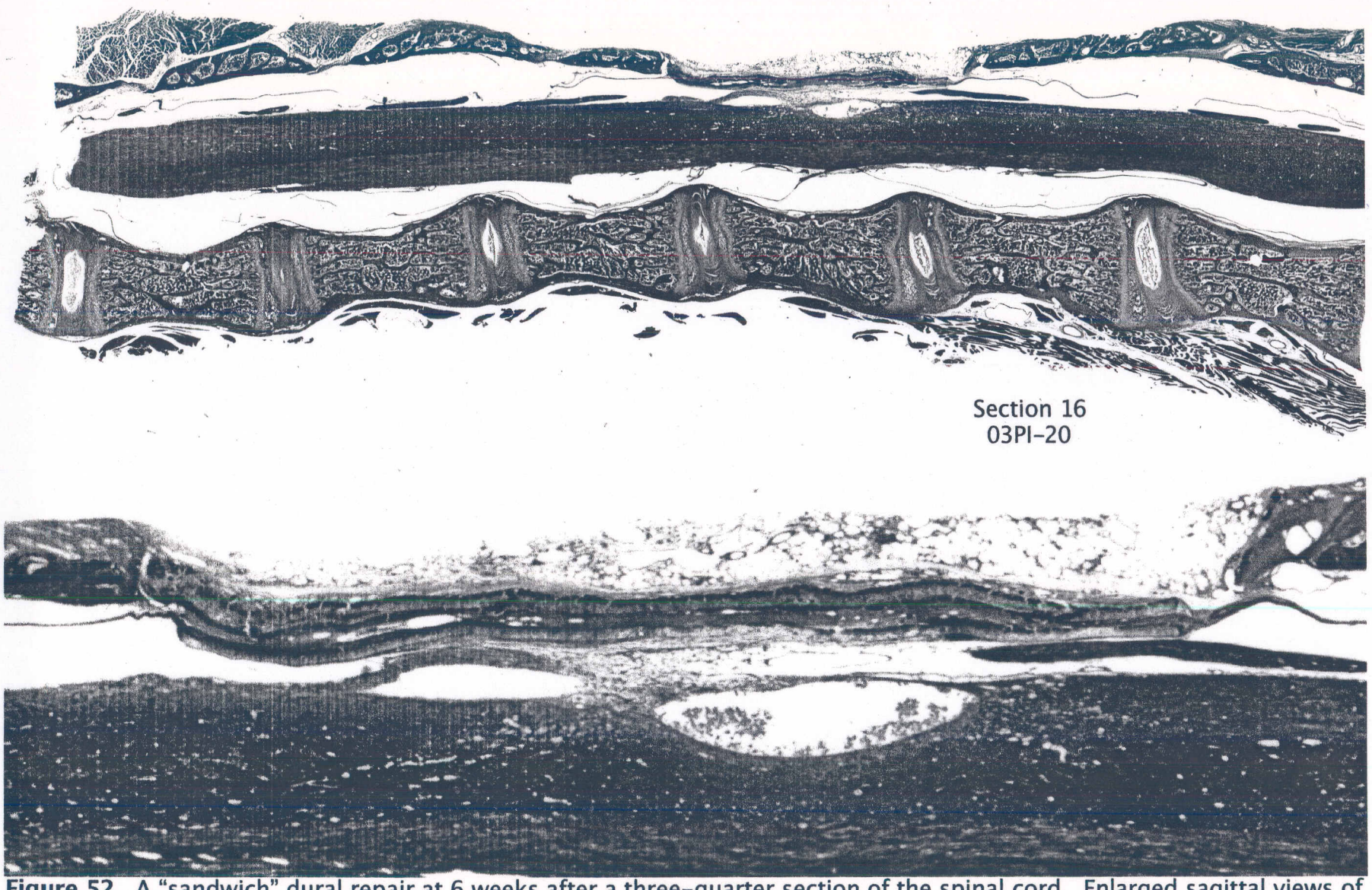


Figure 52. A "sandwich" dural repair at 6 weeks after a three-quarter section of the spinal cord. Enlarged sagittal views of 03PI-20 at 6 weeks after a three-quarter section of the T10 cord and "sandwich repair" of the dural opening with an epidural and a subdural barrier. A piece of fat had been placed above the epidural barrier. Note the presence of a spinal root on right (caudal) side of the laminectomy with no evidence of tethering.