Novel intrathecal delivery system for treatment of spinal cord injury

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Abstract

A novel, localized method for potential delivery of therapeutic agents to the injured spinal cord was investigated. The strategy consists of a polymeric drug solution that gels after injection into the subarachnoid space (SAS). By dispersing therapeutic agents in the polymeric solution, a method is provided for localized delivery to the spinal cord. To determine whether intrathecal injection of this drug delivery system (DDS) would affect cerebrospinal fluid (CSF) flow, a spinal canal model was built using dimensional analysis. Blocking up to 52% of the modeled subarachnoid space of the spinal canal caused minimal pressure differences (9.22 ± 1.45 Pa), suggesting that implantation of a DDS would not subject the spinal cord to increased pressure. The safety of the DDS was also assessed in vivo by injecting collagen into the SAS of Sprague Dawley rats. Controls received injections of artificial CSF (aCSF). Collagen or aCSF was injected at the T2-T3 spinal level of both uninjured rats and rats injured with a 20g compression clip. The injected collagen persisted in the SAS for at least 8 weeks post-implantation and did not elicit an inflammatory reaction in either uninjured or injured animals. Long-term functional behavior was evaluated with the Basso, Beattie, and Bresnahan (BBB) scale weekly for 8 weeks. Functional behavior was similar in the collagen and aCSF groups, also indicating that the DDS was safe. This minimally invasive DDS may provide an alternative, safe method to deliver therapeutic agents intrathecally.
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Introduction

Intrathecal delivery is an effective way to distribute drugs for spinal or cerebral conditions, such as chronic pain and spinal cord injury (SCI). Infusion pumps can be used to deliver agents continuously into the subarachnoid space (SAS). Intrathecal infusion is currently used clinically to deliver analgesics for chronic pain and antispasticity drugs for severe spasticity (Burchiel and Hsu, 2001; Kumar et al., 2001; Zuniga et al., 2002). Continuous intrathecal delivery has also been shown to be beneficial in experimental SCI (Ankeny et al., 2001; Jakeman et al., 1998; Kojima and Tator, 2000, 2002; Lee et al., 1999; Namiki et al., 2000;

Rabehevsky et al., 2000; Ramer et al., 2000; Shibayama et al., 1998). For example, delivery of brain derived neurotrophic factor (BDNF) to the injured spinal cord has been shown to enhance Schwann cell proliferation and myelination, the sprouting of cholinergic fibers, and provide neuroprotection and functional recovery after traumatic SCI (Jakeman et al., 1998; Namiki et al., 2000). Delivery of basic fibroblast growth factor (FGF-2) also provided neuroprotection (Lee et al., 1999) and enhanced functional recovery in rats after SCI (Rabchevsky et al., 2000). Intrathecal infusion of both epidermal growth factor (EGF) and FGF-2, two mitogens used extensively in neural stem cell studies, stimulated the proliferation and migration of precursor cells from the adult rat spinal cord ependymal layer in normal (Kojima and Tator, 2000) and injured (Kojima and Tator, 2002) rats. These findings suggest that sustained

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delivery of the appropriate agents can improve outcome after SCI.

Although intrathecal delivery with an infusion pump provides reliable, continuous administration of agents, catheterization of the SAS is an invasive technique, which may require a hemilaminectomy for exposure of the vertebral interspace. Also, implantation of the catheter can cause adverse reactions, such as chronic inflammation and dural fibrosis, or result in spinal compression or infection, as has been shown in rodents and dogs (Jones and Tuszynski, 2001; Yaksh, 1999). Another limitation is that a second intervention may be required to remove the catheter and pump (Storkson et al., 1996; Thorne and Frey, 2001; Yaksh, 1999). Furthermore, the distribution of the infusate in the SAS is not localized, and the agents may be redistributed to the systemic and cranial circulation without reaching the intended target. Therefore, a higher concentration of infusate may be necessary to achieve the desired therapeutic effect.

Another intrathecal delivery strategy is encapsulated cell therapy (Aebischer et al., 1996). In this technique, primary cells or cells that are genetically engineered to produce a specific signaling molecule, such as a growth factor, are encapsulated in a polymeric membrane and implanted in the SAS. Although this strategy is promising, maintaining long-term cell function and viability of encapsulated cells is difficult to achieve. Furthermore, implantation of this drug delivery system (DDS) is highly invasive and the implant site may be distant from the intended target site. Therefore, a less invasive intrathecal DDS that provides localized delivery of therapeutic agents would be desirable. Such a system is described below.

The new DDS described herein consists of a biocompatible matrix capable of containing dispersed therapeutic agents such as growth factors. The DDS begins as a solution that, upon injection, gels quickly under physiological conditions and traps the dispersed agents. Due to its injectability, the DDS can be implanted in the SAS at the site of injury, thereby providing localized delivery of the confined agents. Moreover, implantation is minimally invasive, and its removal unnecessary since the matrix is biodegradable. Another advantage of this technique is its versatility since various types of therapeutic strategies, such as gene therapy (Shoichet et al., 1995), can be confined within the matrix singly or in combination providing diversified delivery strategies. Using this injectable DDS, localized distribution of therapeutic agents or strategies at the spinal cord injury site can be achieved in a minimally invasive manner optimizing their therapeutic benefits.

Our initial goal, described in detail here, was to determine the safety of this DDS. First, we assessed the effect of an injectable DDS using an in vitro model to ensure that cerebrospinal fluid (CSF) flow would not be adversely affected by its implantation. Second, the DDS was tested in uninjured and spinal cord injured rats in terms of behavior, histology, and immunohistochemistry. We chose to test
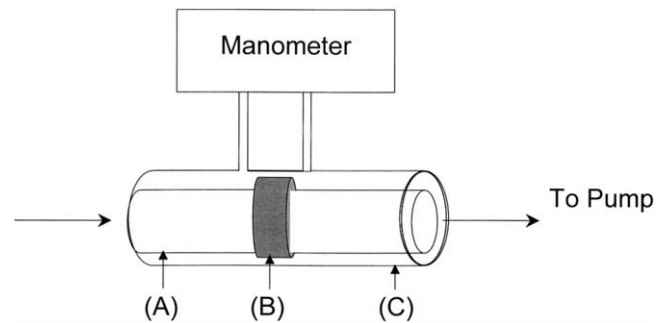

Fig. 1. In the spinal canal model, the solid rod (A) represents the spinal cord and pia mater, which are surrounded by the outer tube (C), representing the outer meninges. The gelled injectable matrix in the SAS is represented by (B). A manometer was attached in the middle of the model to measure pressure differences in the spinal canal after implantation of a matrix around the cord.

collagen as our DDS biomaterial because it is biocompatible, biodegradable, injectable, and fast-gelling.

Materials and methods

In vitro modeling of the subarachnoid space

A spinal canal model was developed using dimensional analysis to determine whether blocking the SAS with an implanted matrix would affect CSF flow. Disruption of CSF flow may create damaging clinical conditions that could negate the usefulness of the DDS; therefore the model helped test this possibility. The spinal canal was modeled as a concentric annulus where an inner, solid rod represented the spinal cord and pia mater, and an outer, hollow cylinder represented the dural sheath and arachnoid (Fig. 1). The model's outer layer was made of clear acrylic plastic and the inner rod was Tygon. The geometric dimensions of the model (spinal cord O.D. 1.27 cm; dural sheath I.D. 1.905 cm) were based on measurements of the human spinal canal (Beardsley et al., 1995; Holman et al., 1997; Robinson et al., 1994). The length of the model was 66 cm, allowing for entrance length effects to be dissipated after the first 6 cm and permitting fully developed laminar flow in the remainder of the model (White, 1991). Phosphate-buffered saline solution (PBS; Sigma–Aldrich Co., Oakville, ON) was continuously circulated throughout the model with a peristaltic pump (VWR Canlab, Ultra-low Flow Pump, Mississauga, ON) using silicone tubing (VWR Canlab). The tubing was connected to the outer acrylic tube using female polyvinylchloride connectors (I.D. 0.635 cm) attached to brass male fittings (National Pipe Thread size 0.635 cm). The model was easily filled with PBS using a four-way crossover Swagelok valve (Whitney 40″ series ball valves, Avon Valve and Fitting Company, Ltd., Scarborough, ON). A manometer (Capsuhelic Series 4000, Baker Instruments, Markham, ON) was attached to the outer annulus, which
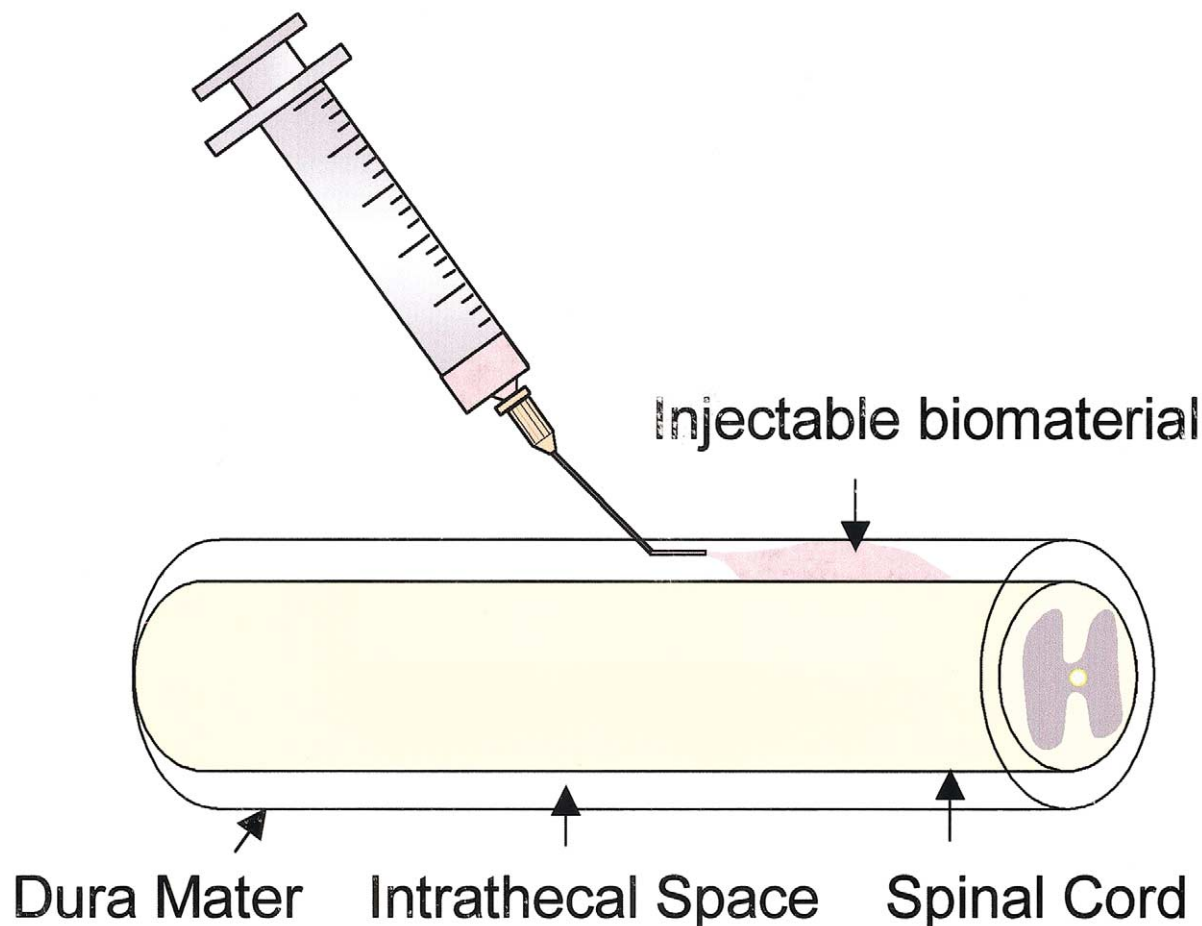
Fig. 2. Schematic drawing of injection of the collagen solution into the SAS using the nucleus hydrodissector. The collagen solution gels on the dorsal surface of the spinal cord and can provide localized delivery of entrapped agents at the injury site.

represented the outer meninges, in order to measure pressure differences across the model. The attachments of the manometer to the outer tube were made from acrylic plastic and were fitted with Tygon tubing (VWR Canlab). Flow was impeded by implanting matrices (i.e., Tygon tubing or Parafilm) of increasing wall thickness (ranging from 0.02 to 0.18 cm) around the inner rod simulating the implantation of a DDS in the SAS. The implanted matrices blocked up to 51.8% of the spinal canal. The flow dynamics of the in vitro model were matched to the CSF flow dynamics of the human spinal canal using the dimensionless Reynolds number,

$$Re_{human} = Re_{model}$$

or

$$[\rho VD/\mu]_{human} = [\rho VD/\mu]_{model},$$

where ρ is density, V is CSF velocity, D is the hydraulic diameter or $2\times$ (outer radius − inner radius), and μ is viscosity. The density ($\rho = 998.2$ kg/m^3) and viscosity ($\mu = 1.002 \times 10^{-3}$ Pa · s) of the PBS solution and CSF were assumed to be similar (Levin et al., 1981). Using the rate of

CSF formation of 0.35 ml/min (Mokri et al., 1998), the flow rate of the pump used to circulate PBS in the model was matched to that of the human canal.

Implantation studies

All animal procedures were performed in accordance with the Guide to the Care and Use of Experimental Animals (Canadian Council on Animal Care), and protocols were approved by the Animal Care Committee of the Research Institute of the University Health Network. The DDS matrix was injected into the SAS of 10 normal female Sprague Dawley rats (250–300 g; Charles River, Montreal, Que.) in order to establish a reproducible implantation method and to evaluate the safety of intrathecal implantation.

The matrix consisted of highly concentrated collagen solutions, 24–28 mg/ml (gifts from Dr. Dale DeVore and Mr. Peter Ciarametaro, Xium L.L.C., Westerly, RI), made from pepsin-solubilized bovine dermal collagen. These solutions gel quickly (within 1 min) when injected into physiological, aqueous environments (pH 7.4, T = 37°C) such

Table 1
Differential pressures measured in the spinal canal model

Percentage of blockage	Differential pressure (Pa) over time		
	2 h	4 h	24 h
0	0	0	0
5.2	0	0	0
44.2	4.23 ± 7.22	4.23 ± 7.22	4.23 ± 7.22
51.8	8.22 ± 1.45	9.22 ± 1.45	9.22 ± 1.45

Note. Increasing the blockage by implantation of a material in the model caused minimal pressure increase. Each measurement was made in triplicate, with the mean and standard deviation reported.

as CSF. The animals were anesthetized by inhalation of halothane and a mixture of nitrous oxide and oxygen (1:2). A laminectomy was performed at the T2 or T3 spinal level with the aid of an operating microscope to expose the dura. Twenty microliters of collagen solution were injected intrathecally. After injection, the overlying muscles and fascia were closed with 3.0 Vicryl sutures (Ethicon, Peterborough, ON), and the skin was closed with Michel clips. Rats were ventilated with pure oxygen for 2–3 min and were then placed under a heating lamp for recovery. Sham controls were injected with 20 μl of artificial cerebrospinal fluid (aCSF) (148 mM NaCl, 3 mM KCl, 0.8 mM $MgCl_2$, 1.4mM $CaCl_2$, 1.5 mM Na_2HPO_4, 0.2 mM NaH_2PO_4, 0.1 mg/ml of bovine serum albumin).

Injection of collagen in the intrathecal space was performed with a Visitec nucleus hydrodissector (Becton Dickinson Biosciences, Mississauga, ON) commonly used in cataract surgery. The hydrodissector is a 30 G needle, 22 mm long with a 45° angle blunt tip. The bent tip, 7 mm long, is inserted into the SAS for injection of the DDS. The dura mater overlying the left dorsal rootlets was lifted with the forceps (Dumont biologic tip, No. 5, FST, Vancouver, B.C.) and punctured with a sharp 30 G needle at the T3 level. The nucleus hydrodissector was then inserted through the dural opening. Twenty microliters of the collagen solution were injected over 20-30 s through a 1 ml syringe (Fig. 2). The hydrodissector was maintained intrathecally for an additional minute. The volume of the injected solution was chosen to be less than 10% of the total CSF volume (250 μl) in the rat (Yaksh, 1999) in order to attain widespread distribution of the DDS. It has been suggested that the volume of solutions injected in the intrathecal space should be 10% of the total CSF volume in order to get optimal distribution of the injectate (Rieselbach et al., 1962). For this reason, we began our investigation using this volume as a starting point.

Determination of safety in vivo by behavioral analysis

Twenty female Sprague Dawley rats were used to determine the neurobehavioral, histological, and immunohistochemical responses to intrathecal collagen injection. Six animals received collagen, and 4 aCSF (control group)

using the nucleus hydrodissector. Half the collagen group ($n = 3$) and half the aCSF group ($n = 2$) were sacrificed at 14 days, and the remainder at 56 days. In another 10 animals, 6 were injected intrathecally with collagen, and 4 with aCSF after moderate SCI produced by clip compression at the T2-T3 spinal level (Rivlin and Tator, 1977, 1978). In this injury model, the spinal cord was compressed for 1 min using a modified aneurysm clip calibrated to have a closing force of 20*g* (moderate injury). The collagen was then injected at the site of injury immediately after clip compression. In the injured group, half the animals ($n = 5$) were sacrificed at 14 days and the remainder at 56 days postsurgery. Postoperative urinary tract infections in the injured animals were treated with subcutaneous ampicillin (50 mg/ml) and/or gentamicin (8 mg/kg). The bladders of the SCI animals were manually expressed three times per day until sacrifice. Animals were housed in separate cages in a temperature-controlled room (27°C) with a 12-h light/dark cycle. Functional evaluation was performed weekly using the Basso, Beattie, and Bresnahan (BBB) scoring scale (Basso et al., 1996). Each leg was observed separately, and a score was given. Statistical analysis of the BBB results was performed by pooling legs for each group at the different time points. At the end of the experiment, the animals were deeply anesthetized by intraperitoneal injection of sodium pentobarbital. After an intracardiac injection of 1 ml of 1000 U/ml heparin, they were perfused with 10% neutral buffered formalin. The spinal cords were harvested and placed in 10% neutral buffered formalin for histological processing.

Histology

Formalin-preserved spinal cord samples of 0.5 to 1.0 cm in each direction rostral and caudal to the site of injection were harvested for histological processing. In the injured
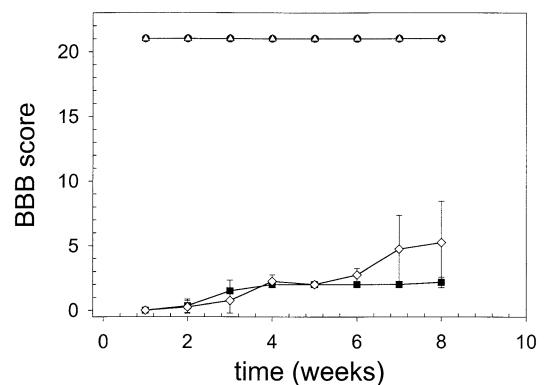

Fig. 3. BBB scores after injection of 20 μl of collagen or aCSF. Uninjured animals (●) injected with collagen had scores of 21 ± 0 for the duration of the experiment. The average score of uninjured animals injected with aCSF (△) was also 21 ± 0. Injured animals (■) injected with collagen had BBB scores of 2.2 ± 0.4 at the end of the experiment. Control animals (◇) had a score of 5.3 ± 3.2. There was no significant difference between animals injected with either collagen or aCSF ($p > 0.05$).
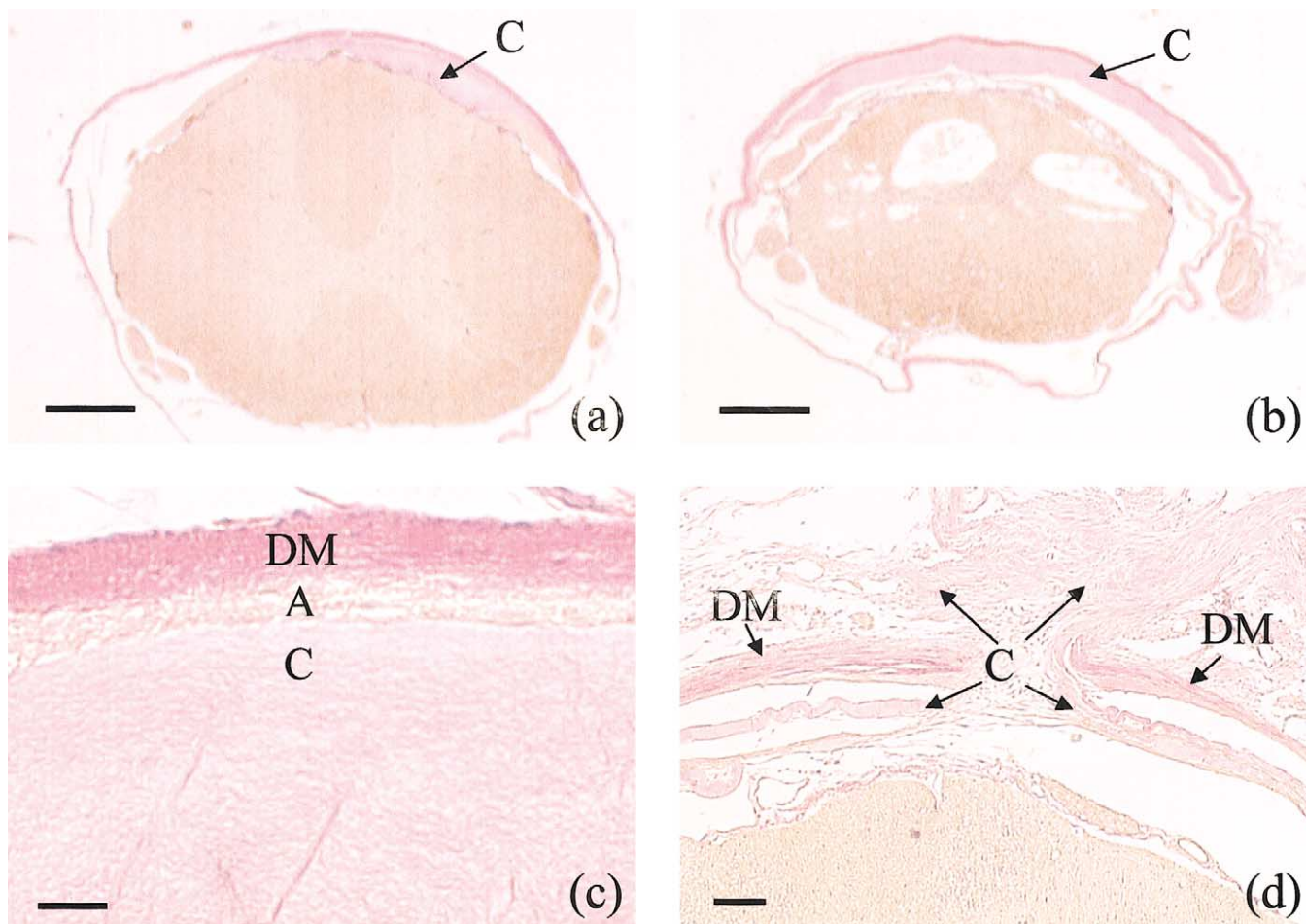
Fig. 4. (a) Cross-section of an uninjured spinal cord 56 days post-injection of the collagen solution (C) stained with Van Gieson's (scale bar = 500 μm). (b) Cross-section of a spinal cord after a 20g clip compression injury 56 days post-injection of the collagen solution (C) stained with Van Gieson's (scale bar = 500 μm). (c) Higher magnification view of (b) showing the dura mater (DM), arachnoid (A), and injected collagen (C) in the SAS stained with Van Gieson's (scale bar = 25 μm). (d) Injection site of the collagen solution (C) in a 14-day survival animal. In the same animal, some collagen is present outside the dura mater (DM) and in the dural opening which would effectively prevent CSF leakage (Van Gieson's stain, scale bar = 100 μm).

rats, the spinal cord segments encompassing the injection and injury sites were embedded in paraffin and 8-μm-thick serial, transverse sections were cut. Every tenth section was stained with nonacidic hematoxylin and eosin (H&E) for general morphology, and every 11th section with Van Gieson's, a collagen-specific stain (Luna, 1968). In the initial studies, the injected collagen was difficult to detect in the SAS. Since many histological stains, such as Masson's trichrome, contain acetic acid, dissolution of the injected acid-soluble collagen occurred during staining. Therefore, a modified H&E stain, composed of acid-free eosin, was used (Luna, 1968).

Immunohistochemistry

The remaining sections were processed for immunohistochemistry with glial fibrillary acidic protein (GFAP) and ED-1 to assess the astrocytic and inflammatory responses, respectively, in the spinal cords (Kojima and Tator, 2002).

Monoclonal mouse anti-GFAP and monoclonal mouse anti-ED-1 were purchased from Chemicon International, Inc. (Temecula, CA). In the GFAP protocol, the tissue was treated with 0.009% hydrogen peroxide in methanol for 30 min. The sections were washed with PBS and then placed in 10% heat-inactivated goat serum with 0.03% Triton-X for 1 h. The tissue was incubated with mouse anti-GFAP (1:200) overnight at 4°C. After washing the sections with PBS for 30 min, biotinylated goat anti-mouse IgG (1:500; Vector Laboratories, Burlington, ON) was used with an avidin–biotin peroxidase complex (ABC kit; Vector Laboratories) and VIP (Vector Laboratories) as the chromogen. Other sections were reacted for ED-1 to examine the extent of microglial and macrophage infiltration (Kojima and Tator, 2002). After incubation in Bouin's for 1 h at 60°C, the thoroughly washed sections were treated with 0.009% hydrogen peroxide in methanol. For antigen retrieval, the slides were placed in a microwave (1.4 kW) for 35 min in a pressure cooker filled with a sodium citrate (3 g/liter, pH
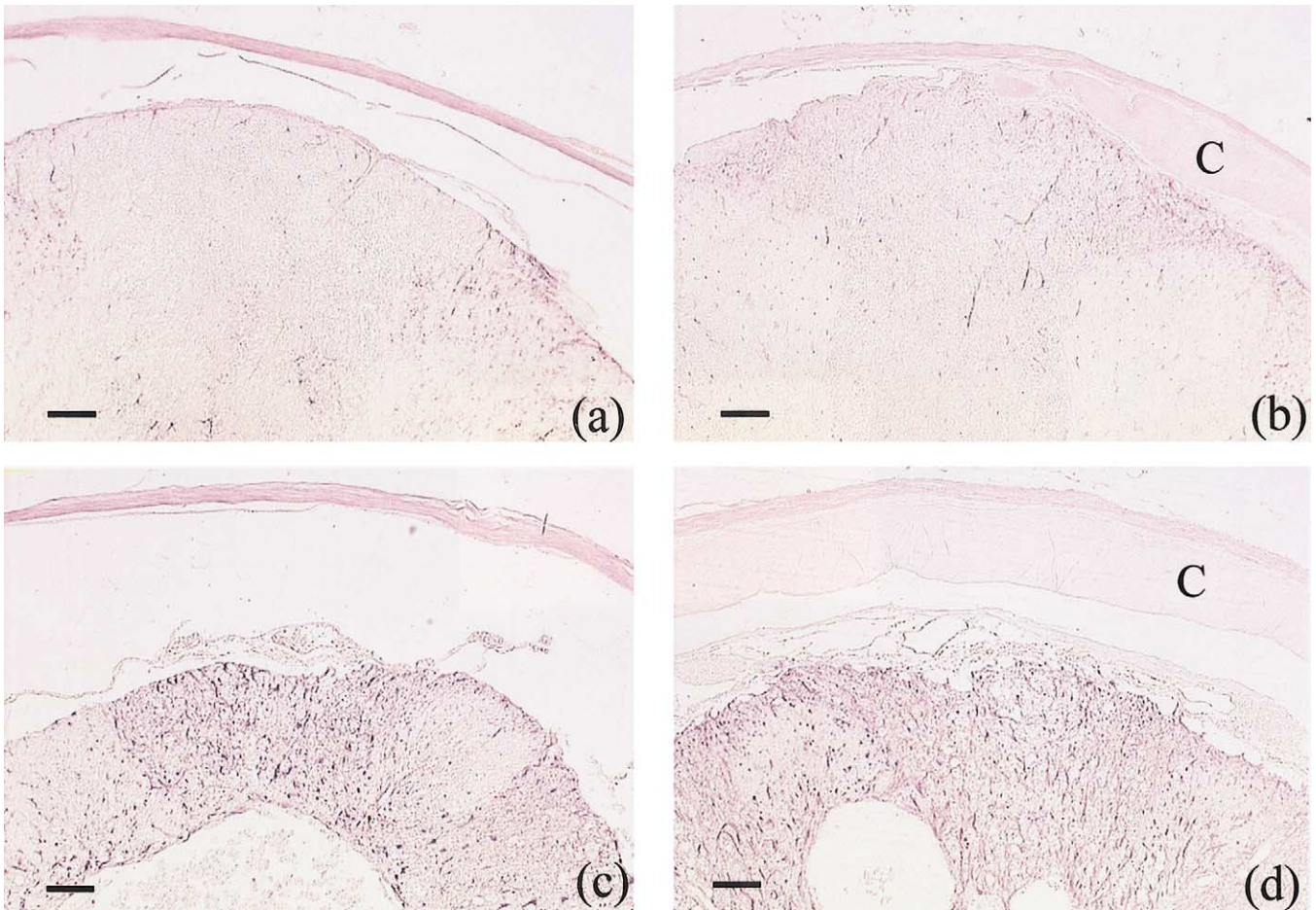
Fig. 5. Slight GFAP reactivity in the dorsal aspect of an uninjured rat injected with aCSF 56 days post-implantation (scale bar = 100 μm). (b) Similar GFAP reactivity as in (a) in the dorsal aspect of an uninjured cord injected with collagen (C) after 56 days (scale bar = 100 μm). (c) Increased GFAP reactivity in the gray and white matter of an injured spinal cord injected with aCSF after 56 days (scale bar = 100 μm). (d) Similar GFAP reactivity as in (c) in the gray and white matter of an injured spinal cord injected with collagen (C) after 56 days (scale bar = 100 μm).

6) solution. The sections were blocked with 4% normal goat serum in 0.1% Triton-X prior to overnight incubation with mouse anti-rat ED-1 (1:50) at 4°C. The slides were incubated with biotinylated goat anti-mouse IgG (1:500) for 1 h followed by an avidin–biotin peroxidase complex (ABC kit) with VIP as the chromogen. Images of the sections were captured using the Bioquant image analysis program (R&M Biometrics, Inc., Nashville, TN) interfaced with a Nikon CCD camera mounted on a Nikon Eclipse TE 300 microscope (Tokyo, Japan).

Results

In vitro spinal canal model

Using a spinal canal model, the effect of implanting an intrathecal DDS on CSF flow was studied. At 24 h, a maximal differential pressure increase of only 9.96 Pa (with a mean and standard deviation of 9.22 ± 1.45 Pa, $n = 3$) was recorded after blocking 51.8% of the simulated SAS

(Table 1). In our model, fully developed laminar flow was established between 2 and 24 h, and stability in the flow was noted in the pressure measurements, which did not change over time. The results suggest that implantation of a matrix in the SAS would not affect CSF flow, since in the human spinal canal there is normally a wide range of pressures, between 637.4 and 1912 Pa (Mokri et al., 1998). Based on these results, the safety of intrathecal implantation of the DDS was tested in Sprague Dawley rats.

Behavioral analysis

Functional evaluation of uninjured and injured animals injected with either collagen or aCSF was assessed weekly with the BBB scale by scoring each leg separately. Uninjured animals injected with either collagen (pooled legs, $n = 6$) or aCSF ($n = 4$) had BBB scores of 21 ± 0 during the 8 weeks of the experiment (Fig. 3). Injured animals injected with either collagen or aCSF had similar BBB scores at 56 days. Injured animals injected with collagen (pooled legs, $n = 6$) had a final BBB score of 2.2 ± 0.4. Injured animals
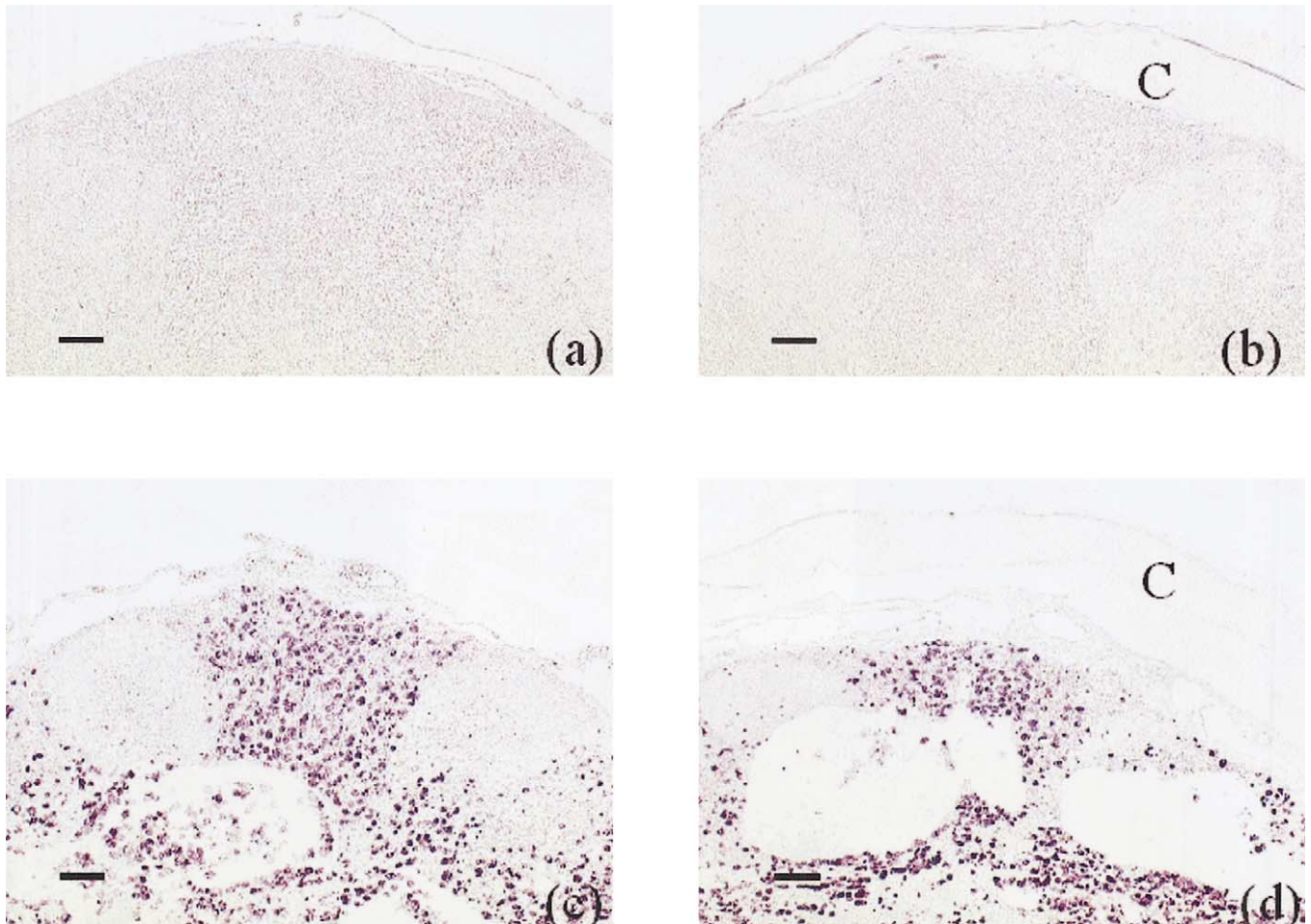
Fig. 6. (a) No ED-1 expression was seen in the uninjured spinal cord injected with aCSF after 56 days (scale bar = 100 μm). (b) As in (a), there was no ED-1 expression in the uninjured cord injected with collagen (C) after 56 days (scale bar = 100 μm). (c) Extensive ED-1 expression in the gray and white matter of an injured spinal cord injected with aCSF after 56 days (scale bar = 100 μm). (d) Similar extensive ED-1 expression as in (c) in the gray and white matter of an injured spinal cord injected with collagen (C) after 56 days (scale bar = 100 μm).

injected with aCSF had a final BBB score of 5.3 ± 3.2 (pooled legs, $n = 4$). There was no statistically significant difference between these groups, as evaluated with a t test (95% confidence interval) followed by post-hoc analysis with a Mann–Whitney rank sum test ($P = 0.114$). The larger variation in the control group was due to one animal with a final BBB score of 8, while the other animal had a final score of 3 in the best leg. Histologic analysis of the animal with the highest BBB score in the control group revealed that the injury was less severe and resulted in much less cavitation than in the other control animal. Thus, the injection of collagen in the SAS was not detrimental to the animal's functional behavior.

Histology

Histologic analysis of the uninjured and injured cords at both 14 and 56 days showed that the collagen persisted in the SAS. It was located over the dorsal aspect of the spinal cord, only partially filling the SAS (Figs. 4a and b), with none of the collagen in the ventral SAS. The collagen adhered to the dura mater and was easily distinguished from it with Van Gieson's stain (Fig. 4c). It spread a total distance of 5.1 ± 1.2 mm (mean ± standard deviation) rostro-caudally along the dorsal aspect of the cord. There was no difference in the spread of the collagen solution between the 14- and 56-day survival groups, demonstrating its stability in vivo. There were some differences in the axial distribution, probably due to the presence of anatomical structures such as the nerve rootlets. During injection, some collagen leaked from the puncture site into the epidural space, but due to its fast gelling properties, the dural opening became occluded, confining most of the collagen in the SAS (Fig. 4d). Blockage of the puncture site by collagen is probably beneficial, since it prevents CSF leakage, which could lead to other complications (Kumar et al., 2001). Arachnoiditis or syringomyelia was not seen in either the collagen or the aCSF groups. Thus, there were no histological differences between animals injected with either collagen or aCSF in the uninjured or injured groups.
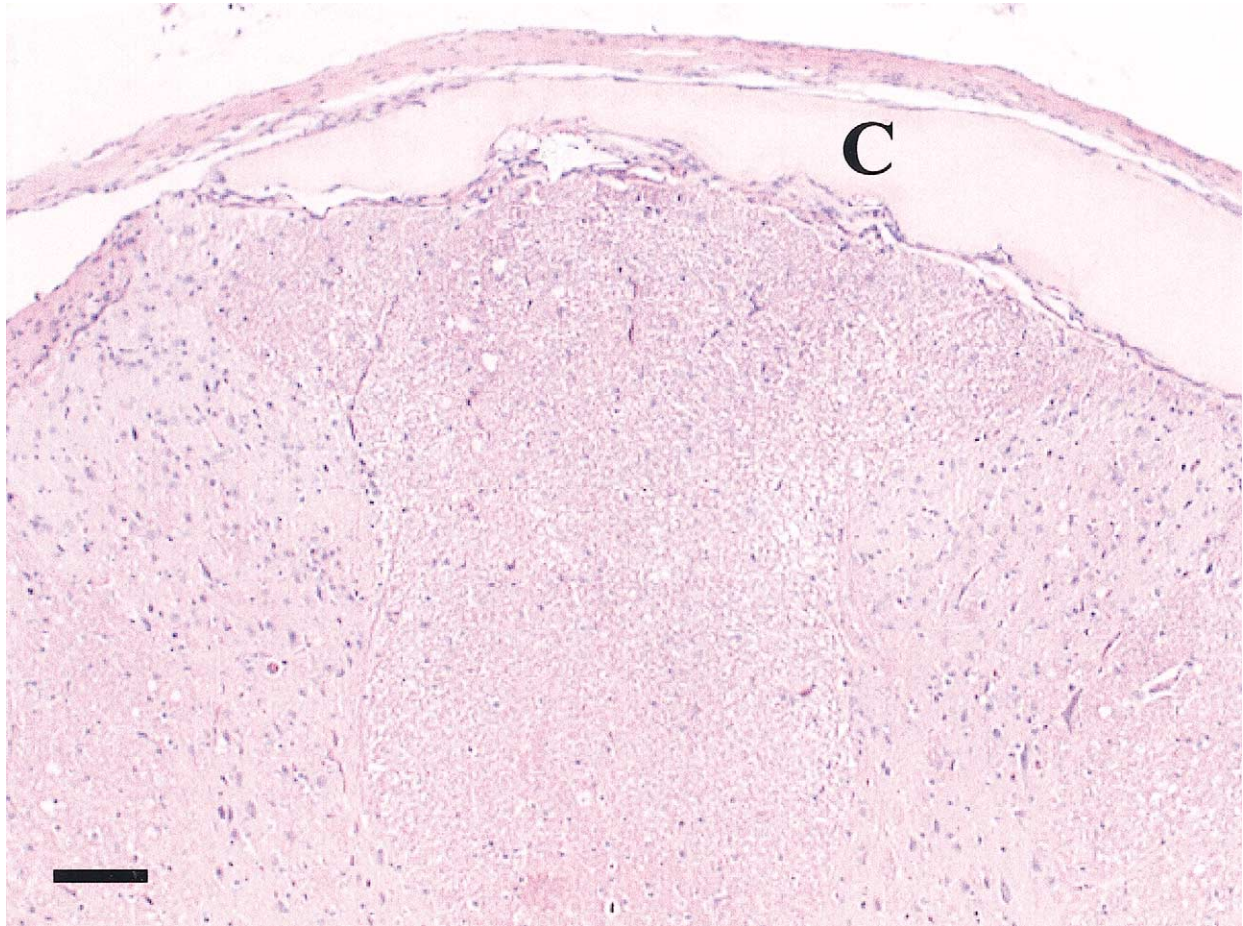
Fig. 7. Dorsal aspect of the spinal cord 56 days post-injection of the collagen (C) solution (H&E stain, scale bar = 100 μm). The presence of the collagen did not cause an inflammatory response.

Immunohistochemistry

Evaluation of the astrocytic reaction with GFAP showed that the injected collagen in uninjured animals did not cause astrocytosis at either 14 or 56 days (Figs. 5a and b). Fibrous-like processes were evident around the periphery of the spinal cord in the white matter. This response was similar in the control groups. In injured animals, protoplasmic astro-cytes were evident in both the collagen and the aCSF groups, but there were no differences between the groups at either 14 or 56 days post-implantation (Figs. 5c and d). Significant GFAP immunoreactivity was evident in the white and gray matter of injured cords.

In uninjured animals at both 14 and 56 days, collagen did not cause an inflammatory reaction in the spinal cord based on ED-1 immunohistochemistry, further demonstrating its safety in vivo (Figs. 6a and b). In the injured groups, collagen did not accentuate the inflammatory response at either 14 or 56 days compared to the aCSF groups (Figs. 6c and d). In both groups, macrophage and microglia were mainly present in the white matter of injured cords at 56 days. Macrophages were also present in the cystic cavities that formed after injury in both groups. These results sug-

gest that collagen in the SAS is well tolerated for 56 days further confirming its long-term biocompatibility. Additional evidence of the limited inflammatory response to injected collagen in the SAS is shown in Fig. 7.

Discussion

Several experimental strategies to treat SCI are being investigated. Among these, intrathecal delivery of therapeutic agents has been demonstrated to be beneficial. Previously, continuous delivery of therapeutic agents for SCI has mainly been achieved by the minipump technology. Although encouraging results have been attained, intrathecal drug delivery techniques still have significant limitations. An optimal DDS should provide delivery of the desired agent at the site of injury to prevent systemic side effects. Moreover, the DDS should not affect the spinal canal environment. Due to its minimally invasive nature, an injectable, biodegradable system can meet the criteria for an improved intrathecal DDS. In this study, the safety of a new intrathecal DDS for treatment of spinal cord injury was

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assessed and shown to be safe first by an in vitro model and then in vivo.

A spinal canal model was built as a first test to examine the effect of implanting a DDS in the SAS on CSF flow. In the model, flow was partially blocked by implanting a material, which simulated injection of the DDS in vivo. It has been shown that obstruction of the SAS can interfere with the CSF pathways creating uneven pressure in the spinal canal and possible damage to the spinal cord (Heiss et al., 1999). For example, pulsatile pressure waves resulting from blockage of the SAS are transmitted from the SAS into the spinal cord and can lead to syringomyelia, a chronic disorder that usually evolves slowly. For this reason, it was important to evaluate the effect of implanting an intrathecal matrix with a model. A limitation of the model is that only acute pressure differences (2- to 24-h periods) rather than long-term observations can be measured. Since conditions such as syringomyelia usually evolve slowly, the in vivo studies included evaluation of the long-term effects of a matrix implanted in the SAS. Also, due to the possibility that permanent blockage of the SAS could ultimately lead to syrinx formation, we formulated a biodegradable DDS with collagen as the delivery matrix, although other biodegradable, biocompatible, injectable polymers could have also been used.

Another limitation of the spinal canal model is its rigidity. The model's spinal cord and dural sheath were made from rigid materials, Tygon and acrylic plastic, respectively, which are different from the delicate spinal tissue. Blockage of the intrathecal space with a DDS may not only affect CSF flow but also cause cord compression if a rigid material is implanted. Our model served only as a starting point in determining the effects of implanting a DDS on CSF flow, and thus, in vivo evaluation of the DDS was necessary.

The in vivo assessment of the intrathecal DDS showed that it was well tolerated in the SAS. The persistence of the collagen matrix in the SAS 8 weeks post-implantation in both uninjured and injured animals suggests that it is a stable depot for long-term delivery of therapeutic agents. Uninjured animals were not affected functionally by implantation of the DDS. Moreover, the DDS was inert in the spinal canal, as demonstrated by histological and immunohistochemical techniques. The presence of the DDS did not cause syrinx formation. Furthermore, in moderately injured animals, the DDS did not accentuate the inflammatory response. Although the sample size per group was small, implantation of the DDS did not affect the neurobehavioral outcome scores in any of the 20 animals studied. These results suggest that the novel DDS is safe and may prove to be an effective, alternative method to deliver drugs intrathecally. Using this DDS, dispersion of therapeutic agents within the DDS can provide localized delivery at the injection site. Delivery of therapeutic agents is not limited to proteins since other strategies such as drug-containing microspheres or vectors can also be incorporated into the DDS

and used for localized delivery. The versatility of the DDS makes it an attractive alternative delivery system for compressive and contusive spinal cord injury models. Currently, the therapeutic value of delivering EGF and FGF-2 from the collagen matrices is being investigated.

Acknowledgments

References

Aebischer, P., Schluep, M., Deglon, N., Joseph, J.M., Hirt, L., Heyd, B., Goddard, M., Hammang, J.P., Zurn, A.D., Kato, A.C., Regli, F., Baetge, E.E., 1996. Intrathecal delivery of CNTF using encapsulated genetically modified xenogeneic cells in amyotrophic lateral sclerosis patients. Nat. Med. 2, 696–699.

Ankeny, D.P., McTigue, D.M., Guan, Z., Yan, Q., Kinstler, O., Stokes, B.T., Jakeman, L.B., 2001. Pegylated brain-derived neurotrophic factor shows improved distribution into the spinal cord and stimulates locomotor activity and morphological changes after injury. Exp. Neurol. 170, 85–100.

Basso, D.M., Beattie, M.S., Bresnahan, J.C., 1996. Graded histological and locomotor outcomes after spinal cord contusion using the NYU weight-drop device versus transection. Exp. Neurol. 139, 244–256.

Beardsley, D., Holman, S., Gantt, R., Robinson, R.A., Lindsey, J., Bazaral, M., Stewart, S.F., Stevens, R.A., 1995. Transient neurologic deficit after spinal anesthesia: local anesthetic maldistribution with pencil point needles? Anesth. Analg. 81, 314–320.

Burchiel, K.J., Hsu, F.P., 2001. Pain and spasticity after spinal cord injury: mechanisms and treatment. Spine 26, S146–160.

Heiss, J.D., Patronas, N., DeVroom, H.L., Shawker, T., Ennis, R., Kammerer, W., Eidsath, A., Talbot, T., Morris, J., Eskioglu, E., Oldfield, E.H., 1999. Elucidating the pathophysiology of syringomyelia. J. Neurosurg. 91, 553–562.

Holman, S.J., Robinson, R.A., Beardsley, D., Stewart, S.F.C., Klein, L., Stevens, R., 1997. Hyperbaric dye solution distribution characteristics after pencil-point needle injection in a spinal cord model. Anesthesiology 86, 966–973.

Jakeman, L.B., Wei, P., Guan, Z., Stokes, B.T., 1998. Brain-derived neurotrophic factor stimulates hindlimb stepping and sprouting of cholinergic fibers after spinal cord injury. Exp. Neurol. 154, 170–184.

Jones, L.L., Tuszynski, M.H., 2001. Chronic intrathecal infusions after spinal cord injury cause scarring and compression. Microscopy Res. Techn. 54 (5), 317–324.

Kojima, A., Tator, C.H., 2000. Epidermal growth factor and fibroblast growth factor 2 cause proliferation of ependymal precursor cells in the adult rat spinal cord in vivo. J. Neuropathol. Exp. Neurol. 59, 687–697.

Kojima, A., Tator, C.H., 2002. Intrathecal administration of epidermal growth factor and fibroblast growth factor 2 promotes ependymal proliferation and functional recovery after spinal cord injury in adult rats. J. Neurotrauma 19, 223–238.

Kumar, K., Kelly, M., Pirlot, T., 2001. Continuous intrathecal morphine treatment for chronic pain of nonmalignant etiology: long-term benefits and efficacy. Surg. Neurol. 55, 79–86.

Lee, T.T., Green, B.A., Dietrich, W.D., Yezierski, R.P., 1999. Neuroprotective effects of basic fibroblast growth factor following spinal cord contusion injury in the rat. J. Neurotrauma 16, 347–356.

Levin, E., Muravchick, S., Gold, M.I., 1981. Density of normal human cerebrospinal fluid and tetracaine solutions. Anesth. Analg. 60, 814–817.

Luna, L.G., U.S. Armed Forces Institute of Pathology, 1968. Manual of histologic staining methods of the Armed Forces Institute of Pathology, third ed. Blakiston Division McGraw-Hill, New York.

Mokri, B., Hunter, S.F., Atkinson, J.L.D., Piepgras, D.G., 1998. Orthostatic headaches caused by CSF leak but with normal CSF pressure. Neurology 51, 786–790.

Namiki, J., Kojima, A., Tator, C.H., 2000. Effect of brain-derived neurotrophic factor, nerve growth factor, and neurotrophin-3 on functional recovery and regeneration after spinal cord injury in adult rats. J. Neurotrauma 17, 1219–1231.

Rabchevsky, A.G., Fugaccia, I., Turner, A.F., Blades, D.A., Mattson, M.P., Scheff, S.W., 2000. Basic fibroblast growth factor (bFGF) enhances functional recovery following severe spinal cord injury to the rat. Exp. Neurol. 164, 280–291.

Ramer, M.S., Priestley, J.V., McMahon, S.B., 2000. Functional regeneration of sensory axons into the adult spinal cord. Nature 403, 312–316.

Rieselbach, R.E., DiChiro, G., Freireich, E.J., Rall, D.P., 1962. Subarachnoid distribution of drugs after lumbar injection. N. Engl. J. Med. 267, 1273–1278.

Rivlin, A.S., Tator, C.H., 1978. Effect of duration of acute spinal cord compression in a new acute cord injury model in the rat. Surg. Neurol. 10, 38–43.

Rivlin, A.S., Tator, C.H., 1977. Objective clinical assessment of motor function after experimental spinal cord injury in the rat. J. Neurosurg. 47, 577–581.

Robinson, R.A., Stewart, S.F., Myers, M.R., Lien, L.F., Rinaldi, J.R., Swisher, J.L., Drasner, K., 1994. In vitro modeling of spinal anesthesia. A digital video image processing technique and its application to catheter characterization. Anesthesiology 81, 1053–1060.

Shibayama, M., Hattori, S., Himes, B.T., Murray, M., Tessler, A., 1998. Neurotrophin-3 prevents death of axotomized Clarke's nucleus neurons in adult rat. J. Comp. Neurol. 390, 102–111.

Shoichet, M.S., Gentile, F.T., Winn, S.R., 1995. The use of polymers in the treatment of neurological disorders. Trends Polymer Sci. 3, 374–380.

Storkson, R.V., Kjorsvik, A., Tjolsen, A., Hole, K., 1996. Lumbar catheterization of the spinal subarachnoid space in the rat. J. Neurosci. Methods 65, 167–172.

Thorne, R.G., Frey II, W.H., 2001. Delivery of neurotrophic factors to the central nervous system: pharmacokinetic considerations. Clin. Pharmacokinet. 40, 907–946.

White, F.M., 1991. Viscous fluid flow, second ed. McGraw-Hill, New York.

Yaksh, T.L., 1999. Spinal drug delivery, first ed. Elsevier, Amsterdam.

Zuniga, R.E., Perera, S., Abram, S.E., 2002. Intrathecal baclofen: a useful agent in the treatment of well-established complex regional pain syndrome. Reg. Anesth. Pain Med. 27, 90–93.