

# Polyvinyl alcohol–polyvinyl pyrrolidone thin films provide local short-term release of anti-inflammatory agents post spinal cord injury

Noelle Comolli,<sup>1\*</sup> Olivia Donaldson,<sup>1</sup> Nathan Grantier,<sup>1</sup> Victoria Zhukareva,<sup>2</sup> Veronica J. Tom<sup>2\*</sup>

<sup>1</sup>Department of Chemical Engineering, Villanova University, Villanova, Pennsylvania

<sup>2</sup>Department of Neurobiology and Anatomy, Drexel University College of Medicine, Philadelphia, Pennsylvania

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**Abstract:** Spinal cord injury (SCI) triggers a large inflammatory response that results in exacerbated tissue damage. Locally delivering anti-inflammatory drugs could mitigate this secondary wave of degeneration. The mitogen-activated protein kinase family members p38 and c-Jun N-terminal kinase (JNK) play important roles in the inflammatory response and cell death. We propose that the use of polymer thin films, made of polyvinyl alcohol and polyvinyl pyrrolidone blends (PVA–PVP), can be used to provide local release of inhibitors to p38 and JNK post-SCI. Release studies performed *in vitro* confirmed the inhibitors could be released from the film for up to 7 days. The thin film was also tested for its surgical feasibility using a cervical contusion model of SCI in adult female rats. Films with or without the inhibitors were placed subdurally over the injury site immediately following SCI. Animals were sacrificed 5 days post-SCI and spinal cord tis-

sue above and below the injury site was harvested. Additionally, films were removed for analysis. Scanning electron microscopy confirmed the anti-fouling properties of the PVA–PVP film. Tissue histology confirmed that the films themselves did not generate a large immune response, but they did compress the tissue slightly at its placement above the injury site. Finally, quantitative Western blot analysis determined the films loaded with p38 and JNK inhibitors delivered bioactive agents to the injury site and resulted in a significantly decreased amount of pro-cell death proteins. These data indicate that PVA–PVP films can be used to effectively deliver drugs to a SCI site. © 2012 Wiley Periodicals, Inc. *J Biomed Mater Res Part B: Appl Biomater* 100B: 1867–1873, 2012.

**Key Words:** MAP kinases, PVA-PVP thin films, drug delivery, spinal cord injury

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## INTRODUCTION

Spinal cord injury (SCI), frequently caused by trauma such as accidents or falls, results in the interruption of proper nervous system function and loss of sensation and/or mobility. SCI is defined by a loss of function, such as mobility or feeling, owing to damage in the spinal cord. Approximately, 1.3 million Americans are living with SCI (Christopher and Dana Reeve Foundation), and around 11,000 new cases are reported each year (NIH, 2001), which is equivalent to one new SCI every 41 min.<sup>1</sup> Lifetime expenses for SCI patients can total more than 2 million US dollars.<sup>1</sup> About 53% of SCI patients are quadriplegic and the remainder are paraplegic, experiencing complete or incomplete loss of motor function.<sup>2</sup>

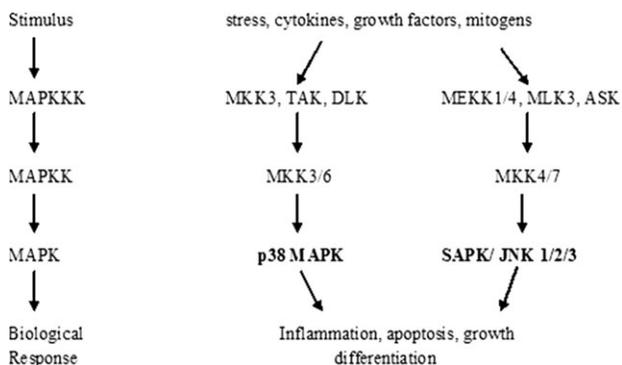
The damage caused by a SCI is broken into two categories: the primary injury and the secondary injury. The primary injury results from the actual mechanical trauma to local tissue, causing damage to the neural cells and blood

vessels. This results in tearing or severing of axons, which are the connections that relay signals in the nervous system. The lesion site expands over time owing to a wave of secondary degeneration from the influx of inflammatory cells, release of free radicals, and progressive cell death of both neurons and glia.<sup>3,4</sup> Thus, tissue in the vicinity of the impact that was spared from the initial injury may later be lost. The amount of tissue that is spared from injury correlates well with retention of function.

One pathway that appears to play a critical role in apoptosis in both central nervous system development and neurodegenerative disorders is the mitogen-activated protein kinase pathway (MAPK).<sup>5,6</sup> MAPK is a family of serine/threonine kinases that includes extracellular signal-regulated protein kinases (ERK), c-Jun N-terminal kinases (JNK), and p38 kinases. They play important roles in relaying external stimuli in the environment to the nucleus by phosphorylating downstream proteins, including transcription factors

\*Both authors contributed equally to this work.

**Correspondence to:** N. Comolli; e-mail: Noelle.comolli@villanova.edu



**FIGURE 1.** MAPK pathway related to SCI. MAPKs are stimulated by stress or growth factors and, in the case of SCI, these can lead to increased inflammation and cell death (apoptosis).

(Figure 1). ERK is thought to be activated by factors that promote cell growth, differentiation, and survival. In contrast, the p38 and JNK kinases are activated by cellular stress factors that promote apoptosis. Therefore, it is thought that for the most part, ERK, p38, and JNK have opposing roles in apoptosis; ERK is anti-apoptotic and JNK and p38 are proapoptotic.<sup>5,7,8</sup> p38MAPKs are also linked to the regulation of inflammation via activation of proinflammatory cytokines.

We would like to locally deliver inhibitors to the proapoptotic proteins p38 and JNK to a SCI site in an attempt to promote neuroprotection following SCI. However, the standard technique to deliver compounds to an injury site is intrathecal delivery via a catheter attached to an osmotic mini pump. There are several technical issues with the osmotic mini pumps that would limit their practical use to deliver neuroprotective agents very shortly after injury, including their need to be “primed” and incubated in saline for at least 8 h prior to use and possible interference with delivery if any air bubbles are present within the catheter. Additionally, chronic intrathecal catheter placement will, itself, result in damage to tissue.<sup>9</sup> Therefore, we wanted to develop a novel method to reliably and locally deliver small organic inhibitors to p38 and JNK to the injury site.

A simple approach would be to create a polymer thin film to place over the injury site postsurgery to provide very local release of the inhibitors. A film has a significant advantage over osmotic mini pumps because it can be kept frozen with the inhibitor already loaded and is ready for immediate, on-demand use. There is no time-consuming preparation, which, in the context of an emergency surgery close to the time of injury, will likely not be possible or optimal. Additionally, the film could serve as an easy mark for the surgeon to reaccess the site upon any necessary subsequent surgeries. It is critical that this thin film is flexible so that it can be placed easily on the top of the injury site, and both nonfouling and nonintegrating with the local tissue, and hence it can be easily removed without causing further damage or scarring.

Polyvinyl alcohol (PVA) and polyvinyl pyrrolidone (PVP) are commonly used polymers in biomaterial applications.

Both PVA and PVP have been previously used for controlled drug releases as well as many hydrogel applications such as artificial cartilage.<sup>10</sup> The blend of PVA–PVP has beneficial properties, as PVP is hydrophilic and the blend has anti-fouling properties.<sup>10,11</sup> The blend has been used in several other applications, such as the delivery of nitric oxide and the replacement of degenerated spinal disks. The PVA–PVP blend used has been previously shown to result a linear release of nitric oxide over a 24-h period, demonstrating that the hydrogel blend can also provide a protected controlled release of a drug.<sup>11,12</sup> PVA is currently used as artificial cartilage, as dialysis membrane, as artificial skin, for the dressing of wounds, cardiovascular device, and for the controlled release of active substances.<sup>10,12–16</sup>

As the previous blends of PVA–PVP have shown anti-fouling properties as well as controlled short-term release of therapeutics, it was proposed that thin films made of PVA–PVP could be used as a local delivery device for anti-inflammatory drugs following SCI. The thin films were placed directly above the injury site to provide a controlled release of p38 and JNK inhibitors with the goal of attenuating secondary degeneration after SCI.

## MATERIALS AND METHODS

### PVA/PVP thin film synthesis

The hydrogel thin films were created by combining polyvinyl alcohol (PVA, MW = 1,450,000, Fluka Analytical), polyvinyl pyrrolidone (PVP, MW = 55,000, Sigma Aldrich) at a ratio of 99:1, respectively. The polymers are mixed with distilled water and autoclaved to create a solution that is 10 wt % polymer. The inhibitors (p38i [SB203580 in dimethyl sulfoxide, DMSO] and JNKi [SP600125 in DMSO]) were added to the polymer solution to create a final concentration of 250  $\mu$ M each. Both inhibitors were purchased from LC Laboratories (Woburn, MA) and the DMSO from ACROS. Thin film molds were created with the dimensions of 24  $\times$  4  $\times$  1 mm using uncoated glass and Teflon strips (McMaster Carr). Gels were crosslinked in these molds using two freeze–thaw cycles (24 h freeze [–20°C], followed by 1 h thaw [25°C]). After the freeze–thaw cycles, gels were removed and stored in sterile phosphate-buffered solution (PBS, Invitrogen) for surgical procedures, or nonsterile PBS (Fisher Scientific) for environmental scanning electron microscopy and *in vitro* release studies.

### Surgical feasibility studies

All procedures complied with Drexel University’s Institutional Animal Care and Use Committee and National Institutes of Health guidelines for experimentation with laboratory animals. After all survival surgeries, animals were given ampicillin (200 mg/kg) and buprenorphine (0.1 mg/kg) postoperatively and placed on a thermal barrier to recover. They were returned to their cages once they became alert and responsive. Adult female Sprague–Dawley rats ( $n = 3$ ; Charles River) were injured as described previously.<sup>18–20</sup> Briefly, animals were injected intraperitoneally with ketamine (60 mg/kg) and xylazine (10 mg/kg). The right, dorsal surface of cervical level (C) 5 and C6 was exposed by

laminectomy. The vertebral column was stabilized by clamping the C3 and C7 vertebral bodies with forceps fixed to the base of an Infinite Horizon Impact Device (Precision Systems and Instrumentation). The animals were situated on the platform, and the 1.6-mm stainless steel impactor tip was positioned over the midpoint (medial to lateral) of the right side of C5. The animals were impacted with a 200 kdyne force with displacement of tissue to a depth of 1600–1800  $\mu\text{m}$ . Immediately after the impact, an incision was made in the dura over the injury site. Thin films not loaded with any drug were placed underneath the dura and over the caudal end of the injury site and C6. The dura was sutured shut over it using a 10-0 suture. The overlying musculature was closed using 4-0 sutures, and the skin was closed using wound clips.

Five days postinjury, animals were euthanized and transcardially perfused at room temperature using 4% paraformaldehyde (Fisher Scientific) in 0.1M PBS (Sigma Aldrich), pH 7.4. Room temperature solutions were used instead of the usual ice-cold solutions to protect the scaffold's structural integrity prior to fixing. Spinal cords were removed, postfixed in 4% paraformaldehyde for 3 days at 4°C followed by cryoprotection in 30% sucrose at 4°C for 5 days. The tissue was embedded in M-1 media (Thermo Shandon, Pittsburgh, PA), fast frozen with dry ice, and stored at  $-70^{\circ}\text{C}$  until processed.

Spinal cord tissue blocks were cut in the transverse plane at 20- $\mu\text{m}$  thickness. Sections were collected on poly-L-lysine-coated glass slides and stored at 4°C until analyzed. Slides with tissue sections were washed in PBS with 0.2% Triton-X, and nonspecific staining was blocked via incubation in 10% goat serum (Invitrogen, Carlsbad, CA) for 1 h at room temperature. Sections were incubated in primary antibody over night in a humidified chamber. Primary antibodies included mouse monoclonal anti-ED1 (Chemicon, 1:100) for the evaluation of macrophages and rabbit anti-GFAP (Dako, 1:1000) for the analysis of scar tissue formation. Tissue sections were washed in PBS followed by incubation in the dark for 2 h at room temperature in secondary fluorescent antibody. All secondary antibodies were used at 1:200 (Jackson Laboratories, Bar Harbor, ME) with 2% goat serum. Sections were again rinsed with PBS and cover slipped with Vecta Shield mounting media (Vector Labs, Burlingame, CA) and stored flat at 4°C until imaged. All images were taken using a Leica DM5500 fluorescent microscope (Leica Microsystems, Bannockburn, IL).

Thin films were removed and stored in sealed containers for Environmental Scanning Electron Microscopy (ESEM). ESEM was performed on thin films pre- and post-implantation ( $n = 3$  of each) using a FEI XL30. Samples were viewed without drying or coating.

#### ***In vitro* release studies**

Thin films ( $n = 6$ ) containing both inhibitors were placed in 20 mL of PBS and kept at 37°C for 10 days. Samples (0.5 mL) were removed at desired times, placed in high-performance liquid chromatography (HPLC) vials and frozen ( $-20^{\circ}\text{C}$ ) until testing. To maintain a constant volume, 0.5

mL of fresh buffer was added when each sample was removed. Samples were taken at 5 min, 10 min, 30 min, 1 h, 2 h, 4 h, 1 day, 2 days, 4 days, 7 days, and 10 days. The samples were tested using the Shimadzu Reverse Phase High-Performance Liquid Chromatography (RP-HPLC, Shimadzu USA Manufacturing, Canby, OR, Model No. SIL-20ACHT). The samples were run through a Shimadzu C-18 column with 70% methanol and 30% water at a constant flow rate for 15 min. The overall concentration of the inhibitor was determined with UV absorbance readings at 254 nm. Release data were normalized to the initial inhibitor concentration in both cases.

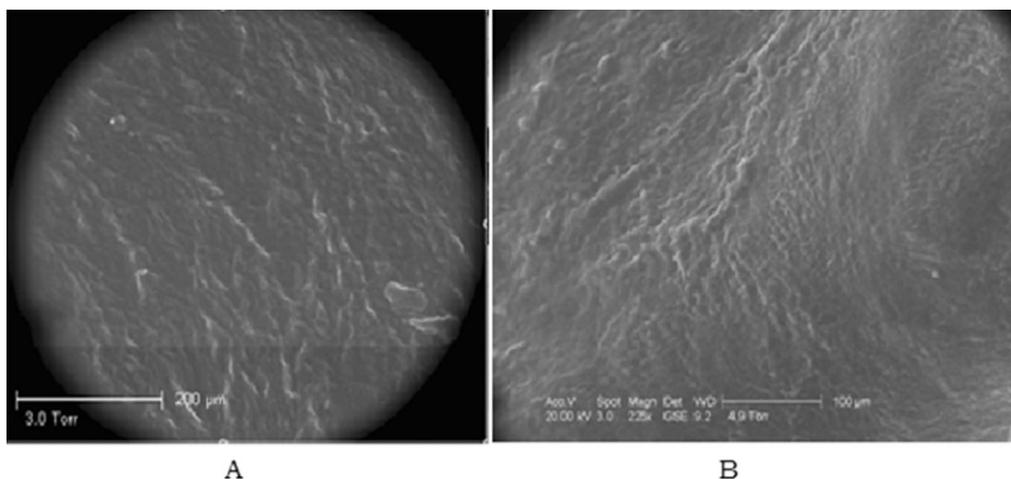
#### **Quantitative Western blotting studies**

Adult female Sprague–Dawley rats were unilaterally contused at C5 using the IH impactor, as described above. Immediately following injury, an incision was made in the dura and thin films either containing vehicle (10% DMSO in physiological saline,  $n = 6$ ) or the combination ( $n = 6$ ) of 250  $\mu\text{M}$  SB203580 (p38 MAPK inhibitor; p38i) + 250  $\mu\text{M}$  SP600125 (JNK inhibitor; JNKi) in DMSO were placed subdurally, as described above. The dura was sutured shut and the animals were allowed to recover. The animals were sacrificed 7 days following injury. Fresh tissue from the injury site, rostral to the injury site, and caudal to the injury site was collected and processed for quantitative Western blotting. Control tissue from normal, noninjured spinal cord was also harvested and processed. Quantitative Western blots were used to determine if treatment with inhibitors of p38 and JNK significantly decreases the levels of downstream signaling molecules. Proteins identified were caspase-3 (both the inactivate 50-kD protein and the cleaved, activated 20-kD protein), phosphorylated MAPKAPK-2 (downstream of active p38) and phosphorylated JNK (downstream of active JNK). Quantitative values were obtained via densitometric values of desired bands at the appropriate molecular weights using the Chemilmager 4400 (Alpha Innotech). The ratios of measured values of the proteins of interest to  $\beta$ -actin were calculated and analyzed for statistical significance using analysis of variance and post hoc Tukey's tests. Differences with  $p < 0.05$  were considered significant. In brief, 100  $\mu\text{L}$  aliquot of the tissue sample prepared for the Western blot analysis was taken and run on the HPLC (as described in *in vitro* release studies section).

## **RESULTS**

#### **Surgical feasibility of the thin film**

A preliminary study was done on a C5 contusion injury with nondrug-loaded PVA–PVP films. Five days postimplantation, the films and tissues were harvested. The film morphology was assessed using ESEM (Figure 2). There are no visible signs of pore formation or cracking. Furthermore, no cellular or protein adhesions are evident on the surface postimplantation. Tissue sections at the injury site were stained for activated inflammatory cells (macrophage marker ED-1) as well as glial scar formation (astrocyte marker GFAP) on both sides to determine the effect of the film itself on the local tissue (Figure 3). There were some



**FIGURE 2.** Surgical feasibility. Scanning electron microscope images of the PVA-PVP film surface pre- (A) and 5 days post- (B) *in vivo* application show that the cells/proteins do not adhere to the film, indicating that the film does not integrate with host tissue.

ED1+ macrophages (red; Figure 3) present within the injury site 5 days after injury, as expected. However, there did not appear to be an increased inflammatory response to the presence of the thin film as the activated macrophages accumulated within the lesion cavity and were not present on the dorsal surface of the spinal cord, which was in direct contact with the film. The GFAP+ astrocytes (green; Figure 3) around the thin film did not appear to react to the thin film, either, and astrocytes at the injury site looked similar to astrocytes surrounding a lesion site that was not treated with a film.

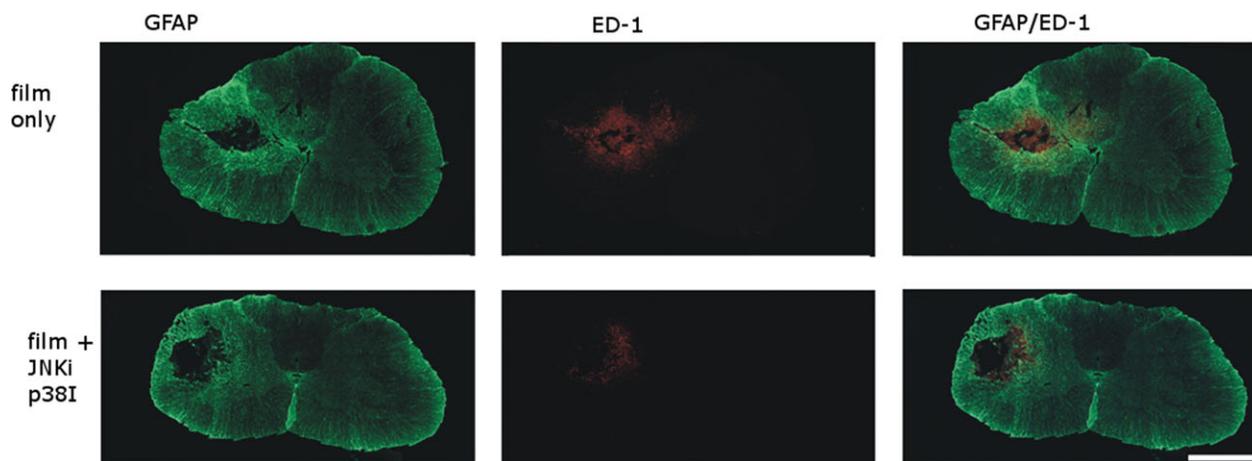
#### Release of inhibitors

Release of the inhibitors *in vitro* from the thin films was evaluated over 1 week time period via RP-HPLC analysis of samples at desired time points. Release data were normalized to the initial concentration of each inhibitor. The results (Figure 4,  $n = 4$ , error bars equal standard deviation) indi-

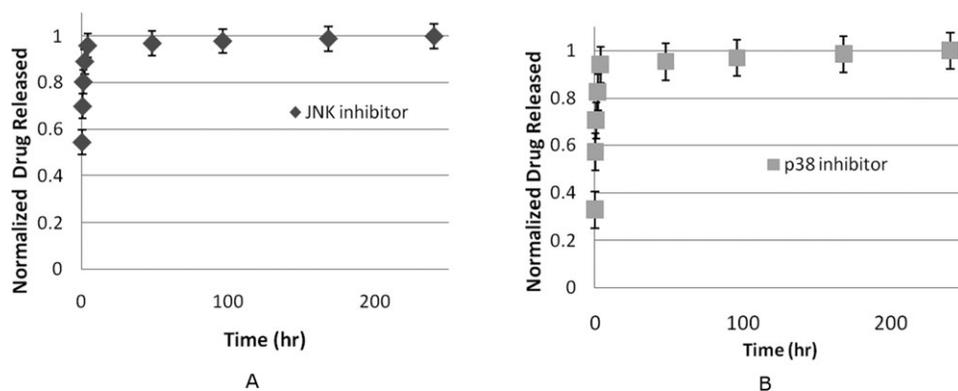
cate that there was an initial burst of inhibitor for both JNKi (A, 54%) and p38i (B, 32%) followed by a slow release over the next 2–4 days (JNKi reached 97% in 2 days, p38i reached 97% at 4 days). *In vivo* release was characterized at the end-point only by RP-HPLC analysis of lysed tissue sections at the injury site as well as above and below the injury site. These data (Figure 5) reveal that there is some inhibitor present at that final time point (5 days) in the local tissue. These data do not indicate that release is still occurring, only that some inhibitor is still present in the tissue at 5 days postimplantation. This indicates that the slow release combined with the initial burst seen *in vitro*, when translated *in vivo*, is providing lingering effects in the local tissue for at least 5 days.

#### Quantitative Western blot analysis

Western blot analysis was performed to determine the downstream effects on JNK and p38 MAPKs from the

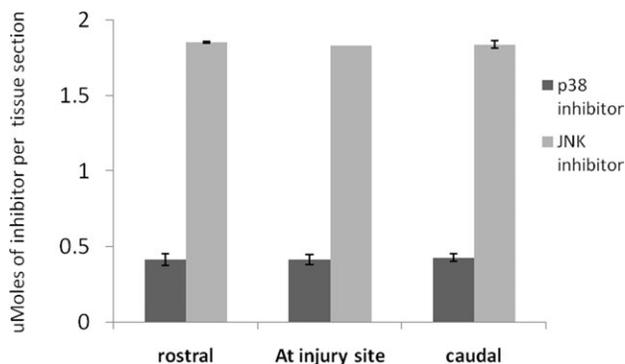


**FIGURE 3.** Transverse sections of spinal cord tissue at a contusion epicenter that was treated with either control thin film (top panel) or inhibitor-loaded film (bottom panel). Five days post-SCI and film placement, there is a glial scar (green, astrocytes) at the lesion site and activated inflammatory cells (red, ED-1) within and in tissue adjacent to the lesion cavity. The arrow indicates the placement of the film over the injury (circle) does create an indentation, likely owing to the lack of mechanical stability because of the cyst that formed at the injury site. [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]



**FIGURE 4.** *In vitro* release profiles of Jnk and p38 inhibitors from the PVA-PVP film. PVA-PVP films loaded with JNK and p38 inhibitors were placed in PBS at 37°C for 7 days. Samples were taken at desired times and analyzed via RP-HPLC. *In vitro* release studies confirmed that the JNK (A) and p38 (B) inhibitors were released from the film. Standard deviations are for  $n = 4$  samples.

treatment of a C5 contusion injury via inhibitor-loaded PVA-PVP thin films. The results (Figure 6) provide the amount of pMAPKAPK-2 (A), pJNK (B), caspase-3 20 kD (active form, C) and caspase-3 50 kD (precursor, D). Results in all cases show that treatment with films loaded with p38i and JNKi significantly decreased the amount of these signaling molecules, as compared to the control films, indicating the release of bioactive JNK and p38 inhibitors (significant difference have  $p < 0.05$ , as indicated by \*). In animals treated with p38i and JNKi-loaded films, there was 35% less phosphorylated MAPKAPK2 (kinase downstream of p38) in tissue at the injury site and 37% less phosphorylated MAPKAPK2 in tissue just caudal to the injury site than in animals treated with vehicle-loaded film. Additionally, there was significantly less phosphorylated JNK1 and JNK2 in animals treated with p38i+JNKi in tissue at the injury site [Figure 6(B); 29 and 43% decrease, respectively, from vehicle-treated injured tissue] and just caudal to the injury site [Figure 6(B); 33 and 30% decrease, respectively, from vehicle-treated tissue].



**FIGURE 5.** *In vivo* release of JNK and p38 inhibitor. Spinal cord tissue from animals treated with films loaded with either p38i or JNKi and sacrificed 5 days post-SCI was run through HPLC to quantify the amount of inhibitor present in the tissue. Results are shown from sections sampled at the injury site, rostral (above), and caudal (below) the injury. Although these results do not indicate the amount of inhibitor released, they do confirm that inhibitors were released *in vivo*. Standard deviations are for  $n = 4$  samples.

## DISCUSSION

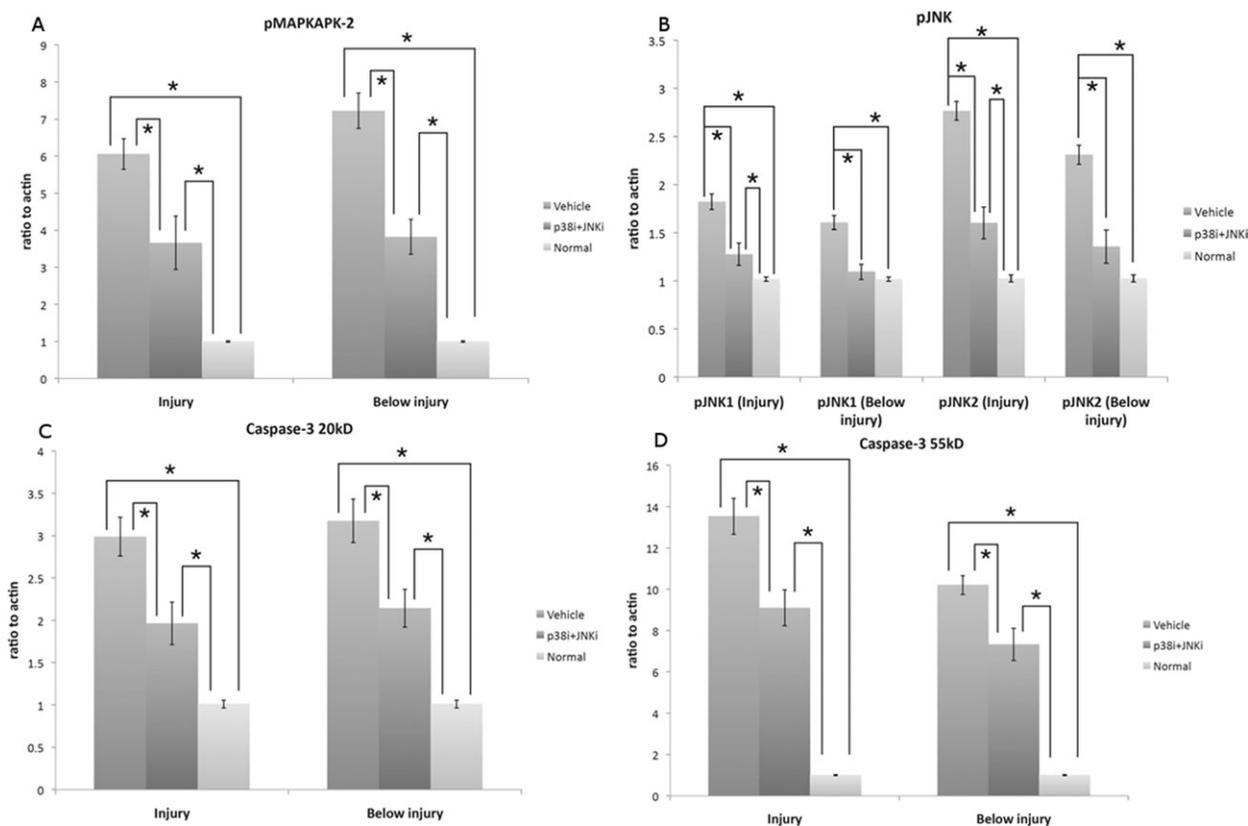
### Surgical feasibility of the thin film

We needed to first determine if it was technically feasible to place the PVA-PVP thin films over a fresh contusion injury and if the PVA-PVP films were biocompatible. Five days later, thin films loaded with just vehicle were acutely placed over a SCI site, animals were sacrificed, and both spinal cord tissue and the thin films were harvested. The lack of cellular or protein adhesions (Figure 2) indicates that the polymer film simply rested on top of the tissue with no integration. Comparing the images of the film prior to implantation [Figure 2(A)] and after implantation [Figure 2(B)], it is clear that the surface morphology did not change during implantation. Although we did not look at a more chronic time point to assess for cellular adhesions, the fact that we did not find any evidence of adhesions at 5 days suggests that cells are minimally capable of adhering to the film, if at all.

To determine the effect of the thin film itself sitting over the spinal tissue, we examined the histology of the tissue that was in direct contact with the film. There is evidence of compression of the tissue over the injury site (indicated by the arrow in Figure 3), but this is to be expected from any treatment at that site owing to the lack of mechanical stability of the fluid cyst (circled, Figure 3) created by the injury. This did not appear to result in a heightened inflammatory or gliotic response in dorsal tissue that was in direct contact with the film, as the activated inflammatory cells were localized to the lesion cavity and not in tissue adjacent to the film.

### Release of inhibitors from the thin film

*In vitro* studies confirmed that it was possible to release both JNKi and p38i from the PVA-PVP thin films over a period of 1 week (Figure 4). The release has an initial burst, followed by a slow release for a few days. As the goal of the p38i and JNKi treatment is to prevent inflammation after injury, the desired dosage would be a high initial dose followed by a lower dose for up to 1 week. Therefore, the *in vitro* release profile of inhibitor from these films was appropriate and fit our *in vivo* needs.



**FIGURE 6.** Quantitative Western blot results postthin film placement. Five days after SCI, tissue from animals treated with control films ( $n = 6$ ) or films loaded with p38i and JNKi ( $n = 6$ ) was collected, and analyzed using Western blot to determine the amount of pMAPKAPK-2 (A), pJNK (B), caspase-3 20 kD (active form, C) and caspase-3 50 kD (precursor, D). The results are shown as the ratio of the protein of interest to actin. Results in all cases show that treatment with films loaded with p38i and JNKi diminished the activity of these signaling molecules, as indicated by decreased levels of activated pMAPKAPK-2 (A) and pJNK (B) relative to control films. Additionally, the films loaded with the inhibitors also resulted in lower levels of the inactive (D; 50 kD) and active (C; 20 kD) forms of the pro-apoptotic cell death protein caspase-3 that vehicle-loaded films. These data indicate that bioactive JNK and p38 inhibitors were released from the film and that their use provides neuroprotection following SCI.  $*p < 0.05$ .

Looking at the results of *in vivo* release was more difficult. The actual amount of inhibitors released was quantified using remaining tissue extracts from the Western blot analysis and run on an HPLC. Quantification of the final end points was conducted to see if any remaining released inhibitor could be detected. The results (Figure 5) indicate that JNKi and p38i were detectable from the tissue samples 5 days post-SCI. These results, however, do not indicate whether or not these released inhibitors are active; they only confirm that the inhibitors were released *in vivo*.

#### ***In vivo* efficacy of thin film delivery**

To better determine if the p38 and JNK inhibitors that were released had any biological effects, we used Western blot analyses to directly determine if use of the inhibitor-loaded films decreased levels of proteins that are downstream of p38 and JNK activity. Phosphorylated mitogen-activated protein kinase-activated protein kinase 2 (pMAPKAPK-2) is a protein kinase that is activated by active p38. The addition of p38i should, therefore, decrease the levels of pMAPKAPK-2. Injury increased levels of pMAPKAPK-2 at the injury site and in tissue below the injury site. However, in animals with the inhibitor-loaded thin films, there was significantly

less pMAPKAPK-2 [Figure 6(A)] than in animals with the vehicle-loaded films. This indicates that the p38 inhibitor is released from the film *in vivo* and remains biologically active throughout release.

Similarly, following SCI, there was an increase in protein levels of phosphorylated, activated isoforms of JNK (pJNK1 and pJNK2) at the injury site and below the injury [Figure 6(B)]. However, levels of pJNK1 and pJNK2 were significantly lower at these locations in animals treated with the inhibitor-loaded films than the vehicle-loaded films. This indicated that the JNK inhibitors released from the films are also biologically active *in vivo*.

We wanted to determine if use of the JNK and p38 inhibitor-loaded thin films was neuroprotective and resulted in diminished levels of active caspase-3, a “death protein” that plays a critical role in cell death. There are two forms of caspase-3. A 50-kD caspase-3 precursor is considered biologically inactive. However, when it is cleaved to result in a 20-kD protein, it becomes activated and results in the cell’s death. Levels of both active and inactive caspase-3 are shown in Figure 5. There are normally very low levels of both the 20- and the 50-kD forms of caspase-3, as indicated by the low levels in

normal, uninjured tissue. Seven days after injury, there were significantly higher levels of both the 50- [Figure 6(D)] and the 20-kD forms of caspase-3 [Figure 6(C)] than in uninjured tissue, suggesting that injury results in the upregulation of these pro-cell death proteins. In animals that were treated with films loaded with p38 and JNK inhibitors, however, this increase in caspase-3 expression was attenuated. There was significantly less inactive (50 kD) and active (20 kD) caspase-3 in animals treated with JNKi and p38i than in animals treated with the control films. The inhibitor-loaded films resulted in a 35% decrease of active caspase-3 levels at the injury site and 37% decrease caudal to the injury site, compared to vehicle-loaded film. This is another demonstration of the effectiveness of the film to release inhibitors of the MAPK signaling cascade. Furthermore, it indicates that use of these films can be neuroprotective and decrease progressive cell death after SCI.

### CONCLUSIONS

The PVA-PVP thin film has been shown to create a perfect temporary (5–7 days) patch for local delivery of anti-inflammatory agents after SCI. Studies confirmed that the JNK and p38 inhibitors were released *in vitro* for up to 1 week. *In vivo* results confirmed it was not only released but also still biologically active, and therefore able to block the activation of enzymes further down the inflammatory (pMAPKAPK-2, pJNK1, pJNK2) and cell death (caspase-3) cascades. This is important, as the slowing of the inflammatory response and diminishing levels of proteins that result in cell death can potentially result in more tissue sparing after injury. This can have an impact on SCI patients' lives as the amount of tissue spared strongly correlates with function.

The PVA-PVP film does not degrade or integrate with the local tissue. In fact, endogenous cells appeared to not adhere at all, allowing the patch to be easily placed during the first surgery and removed during later surgeries. Although this is not ideal for all applications, for SCI, where multiple surgeries postinjury are common for treatment, this is a desired characteristic. This film is an excellent platform for short delivery applications where the absence of adhesion/cellular integration is preferred and multiple surgeries are routine. Placement of the film after the original surgery allows not only local controlled drug delivery but also keeps the surgical site clean of scar tissue and easily identifiable for subsequent surgical treatments. Ultimately, the use of p38i and JNKi-loaded PVA-PVP thin films to result in neuroprotection will be combined with a strategy to promote axonal regeneration<sup>18–20</sup> to further promote repair after SCI.

### ACKNOWLEDGMENTS

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### REFERENCES

- Palmer S, Kriegsman K, Palmer J. Spinal Cord Injury: A Guide for Living. Baltimore: Johns Hopkins University Press; 2000; xiv, p 290.
- Latham R. Spinal Cord Injury: Hope Through Research, Bethesda, MD: National Institute of Neurological Disorders and Stroke; 2003.
- Hagg T, Oudega M. Degenerative and spontaneous regenerative processes after spinal cord injury. *J Neurotrauma* 2006;23: 264–280.
- Beattie MS, Farooqui AA, Bresnahan JC. Review of current evidence for apoptosis after spinal cord injury. *J Neurotrauma* 2000; 17:915–925.
- Harper SJ, Wilkie N. MAPKs: new targets for neurodegeneration. *Expert Opin Ther Targets* 2003;7:187–200.
- Borsello T, Forloni G. JNK signalling: A possible target to prevent neurodegeneration. *Curr Pharm Des* 2007;13:1875–1886.
- Xia Z, Dickens M, Ringeaud J, Davis RJ, Greenberg ME. Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. *Science* 1995;270:1326–1331.
- Tanoue T, Nishida E. Molecular recognitions in the MAP kinase cascades. *Cell Signal* 2003;15:455–462.
- Jones LL, Tuszynski MH. Chronic intrathecal infusions after spinal cord injury cause scarring and compression. *Microsc Res Tech* 2001;54:317–324.
- Fussell G, Thomas J, Scanlon J, Lowman A, Marcolongo M. The effect of protein-free versus protein-containing medium on the mechanical properties and uptake of ions of PVA/PVP hydrogels. *J BiomaterSciPolym Ed* 2005;16:489–503.
- Tom VJ, Sandrow-Feinberg HR, Miller K, Santi L, Connors T, Lemay MA, Houle JD. Combining peripheral nerve grafts and chondroitinase promotes functional axonal regeneration in the chronically injured spinal cord. *J Neurosci* 2009;29:14881–14890.
- Seabra AB, de Oliveira MG, Poly(vinyl alcohol) and poly(vinyl pyrrolidone) blended films for local nitric oxide release. *Biomaterials* 2004;25:3773–3782.
- Abd El-Mohdy H, Ghanem S. Biodegradability, antimicrobial activity and properties of PVA/PVP hydrogels prepared by  $\gamma$ -irradiation. *J Polym Res* 2009;16:1–10.
- Ratner BD. Biomaterials Science: An Introduction to Materials in Medicine. San Diego: Academic Press; 1996. xi, p 484.
- Joshi A, Fussell G, Thomas J, Hsuan A, Lowman A, Karduna A, Vresilovic E, Marcolongo M. Functional compressive mechanics of a PVA/PVP nucleus pulposus replacement. *Biomaterials* 2006; 27:176–184.
- Colombo P, Bettina R, Santia P, Peppas NA. Swellable matrices for controlled drug delivery: gel-layer behaviour, mechanisms and optimal performance. *Pharm Sci Technol Today* 2000;3: 198–204.
- Sandrow HR, Shumsky JS, Amin A, Houle JD. Aspiration of a cervical spinal contusion injury in preparation for delayed peripheral nerve grafting does not impair forelimb behavior or axon regeneration. *Exp Neurol* 2008;210:489–500.
- Tom VJ, Houle JD. Intraspinal microinjection of chondroitinase ABC following injury promotes axonal regeneration out of a peripheral nerve graft bridge. *Exp Neurol* 2008;211:315–319.
- Tom VJ, Kadakia R, Santi L, Houle JD. Administration of chondroitinase ABC rostral or caudal to a spinal cord injury site promotes anatomical but not functional plasticity. *J Neurotrauma* 2009;26:2323–2333.
- Houle JD, Tom VJ, Mayes D, Wagoner G, Phillips N, Silver J. Combining an autologous peripheral nervous system “bridge” and matrix modification by chondroitinase allows robust, functional regeneration beyond a hemisection lesion of the adult rat spinal cord. *J Neurosci* 2006;26:7405–7415.