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Sponge-mediated Lentivirus Delivery to Acute and Chronic Spinal Cord Injuries

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Abstract

The environment within the spinal cord after injury, which changes in the progression from the acute to chronic stages, limits the extent of regeneration. The delivery of inductive factors to promote regeneration following spinal cord injury has been promising, yet, few strategies are available to allow delivery during acute or chronic injury that would facilitate screening of candidate therapies. This report investigates the intrathecal delivery of lentiviruses for long-term expression of regenerative factors. Lentivirus-filled sponges were inserted into the intrathecal space surrounding the spinal cord, with transgene expression observed within multiple cell types that persists for 12 weeks for both intact and injured spinal cord, without any apparent damage to the spinal cord tissue. Sponges loaded with lentivirus encoding for Sonic hedgehog (Shh) were investigated for acute (delivered at 0 weeks) and chronic (at 4 weeks) injuries, and for multiple locations relative to the injury. In an acute model, sponges placed directly above the injury increased oligodendrocyte and decreased astrocyte presence. Sponges placed caudal to the injury had reduced impact on oligodendrocytes and astrocytes in the injury. In a chronic model, sponges increased oligodendrocyte and decreased astrocyte presence. Furthermore, the effect of Shh was shown to be mediated in part by reduction of Bmp signaling, monitored with an Msx2-sensitive reporter vector. The implantation of lentivirus-loaded biomaterials intrathecally provides the opportunity to induce the expression of a factor at a specified time without entering the spinal

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cord, and has the potential to promote gene delivery within the spinal cord, which can influence the extent of regeneration.

Keywords

Gene delivery; intrathecal delivery; lentivirus; macro-porous sponge; spinal cord injury

Introduction

Injury to the spinal cord initiates a sequence of events characterized by dynamic changes in cell populations [1, 2], production of regenerative and inhibitory proteins [3, 4], and development of a glial scar [1, 2, 5, 6]. Immediately after injury, inflammatory and immune cells are recruited [7, 8], and can facilitate regeneration through the removal of cellular debris resulting from the injury [9] and through stimulating the expression of growth factors [10–12]. However, the immune cells can also secrete inflammatory factors that lead to cell death. Soon after injury, spared axons and myelinating cells attempt to migrate into the injury site, but the accumulation of glial scar prohibits their entry [13, 14]. The thickness of the glial scar eventually declines [2]; however, the concurrent reduction in growth factor expression [3] in the chronic phase prohibits further regeneration.

Regeneration within the injured spinal cord is limited, in part, by the cellular environment, and modification of this environment with trophic factors, matrix degrading enzymes, and other factors can significantly enhance axon growth. Previously we reported over-expression of neurotrophin-3 and brain-derived neurotrophin factor after spinal cord injury increased the number of axons at the injury site; however, few of these axons were myelinated, limiting their regenerative potential [15]. In turn, Sonic hedgehog, which has a known role for promoting white matter sparing [16, 17], progenitor proliferation [18, 19] and oligodendrocyte-mediated myelination in the injured spinal cord [17], was over-expressed either immediately (acute) or four weeks after (chronic) injury induction, and assessed for its ability to promote regeneration.

Methods for the delivery of these inductive factors for extended times include approaches such as osmotic pumps, implantation of cells engineered to secrete a factor of interest, or gene therapy. While these approaches have been widely used, they have constraints. Osmotic pumps may injure underlying tissue [20] and transplanted cells may not survive in significant numbers [21]. Gene therapy vectors have been employed for the long-term expression of regenerative factors and/or expression of factors that block or reduce of inhibitory cues in the environment. However, the method of gene delivery into the injured spinal cord dictated the efficacy of the vector. Non-viral vectors delivered to the injured spinal cord promoted transgene expression for only 3 weeks [22]; while delivery of lentiviral vectors increased the expression level and duration resulting in axon growth and myelination after injury [17]. Vectors delivered intrathecally would often clear from the injury site, resulting in lower expression [23, 24] relative to intraparenchymal delivery. Those delivered inside the spinal cord tissue would often require injections at multiple sites [25, 26] to accommodate the small injection volumes that minimize tissue injury.

In this report we investigate the ability of macro-porous sponges implanted into the intrathecal space to deliver lentivirus to either an acute and chronic injury, and the potential of this delivery strategy to facilitate regeneration. Utilizing materials to implant gene therapy vectors inside the intrathecal space surrounding the spinal cord can minimize the potential injury to the spinal cord and reduce vector clearance to localize delivery to the site of interest. The transduced cell types, and the extent and duration of transgene expression were investigated for sponges placed rostral or caudal to the injury, which was used to tailor the location of peak expression. Furthermore, we investigated the efficacy of sonic hedgehog encoding lentivirus delivered from sponges to the influence the presence of myelinating oligodendrocytes relative to the presence of glial scar-producing astrocytes at the injury site, and to enhanced oligodendrocyte myelination relative to Schwann cell myelination.

Materials and Methods

Bridge fabrication

Seven-channeled poly(lactide-co-glycolide) (PLG) bridges for a mouse spinal cord hemisection lesion were formed based on a previously established technique [17, 27]. In brief, PLG (75:25 ratio of D,L-lactide to L-glycolide, inherent viscosity: 0.76 dL/g; Lakeshore Biomaterials, Birmingham, AL) and 63–106 μm salt were mixed in a 1:1 ratio and coated around seven cylindrical sucrose fibers, which were packed into a mold, equilibrated under 800 psi of carbon dioxide for 16 hours and then released at 60 psi/min to foam into the final structure. After sectioning to length and leaching for 15 min in distilled water, the resulting bridges were dried until use.

Porous PEG sponge fabrication

Porous PEG sponges were fabricated as done previously [28]. PEG-acrylate (4 arm, 20,000 Da; Laysan biomaterials; 10% w/v) was dissolved into PBS with photoinitiator (Irgacure 2959, Ciba; 0.5% w/v), frozen for 16 hours at -20°C and then exposed while frozen to UV light (365 nm, 50 mW/cm²; 2 min). The formed hydrogel was lyophilized and then stored with desiccant until use.

Lentivirus production

Lentivirus was prepared from human embryonic kidney cells (HEK 293T) cells according to established methods [17]. Cells were cultured in Dulbecco's modified eagle medium containing 10% fetal bovine serum and 1% penicillin-streptomycin. Cells were transfected using Lipofectamine 2000 (Roche Biosciences, Palo Alto, CA, USA) with expression vectors plenti-CMV-Firefly luciferase, plenti-UbC- β -galactosidase, plenti-CMV-human SHH, or plenti-Msx2-TATA-Firefly luciferase (Panomics, Fremont, CA, USA), and the following packaging vectors: pMDL-GagPol, pRSV-Rev and pIVSVSV-G. Supernatant was collected after 48 hours, concentrated in PEG-it (System Biosciences, Mountain View, CA) for 24 hours, precipitated using ultracentrifugation, resuspended in PBS and stored at -80°C until use. Titer was determined using a qPCR lentivirus titer kit (Applied Biological Materials, Inc., Richmond, BC, Canada). All cell culture materials were purchased from Invitrogen. For assessment of Msx2 activity, the Msx2-reporting lentivirus was co-delivered with the therapeutic vector at 10% of total virus titer.

Spinal cord injury

All procedures were performed according to the Animal Care and use Committee guidelines at Northwestern University. Surgery was performed as previously described (n = 4–5 per condition) [17, 27]. Female mice (C57Bl6, 20g, Charles River) were anesthetized using isoflurane (2%). A dorsal laminectomy was performed at T9-T10 for bridge implantation into a 2.25 mm long hemisection lesion. The injury site was stabilized by suturing the muscles together and stapling the skin after covering the site with Gelfoam. Baytril (enrofloxacin 2.5 mg/kg SC, once a day for 2 weeks), buprenorphine (0.01 mg/kg SC, twice a day for 3 days), and lactate ringer solution (5 mL/100 g, once a day for 5 days) was administered post-operatively. Bladders were expressed twice daily until urinary function recovered.

Gene delivery

Lentivirus ($1-4 \times 10^7$ particles total) was pipetted onto a macro-porous sponge, made of gelatin (Gelfoam) or poly(ethylene glycol) (PEG), immediately before use. The sponge was placed either above (T9–T10) or caudal to (T12) the injury. The *in vivo* imaging system (IVIS), which quantifies photons emitted with luciferase, was used to monitor expression levels over time. IVIS was also used to examine location of expression throughout the body *ex vivo*. A luciferase assay (Promega, Madison, WI) was used to quantify luciferase expression along the spinal cord 2 weeks after sponge implantation. Extracted spinal cords were sectioned every 2 vertebrae along the length of the spinal column, and frozen at -80°C until use. Samples were then homogenized in 100 μL of $1\times$ cell culture lysis reagent, centrifuged at 14,000 rpm for 10 min at 4 C, and assessed using the assay.

Immunohistochemistry

Spinal cords were extracted 2 or 8 weeks after sponge implantation, frozen at -80°C , and sectioned coronally in 18 μm thick slices (N = 5). Two sections in the middle of the bridge were analyzed for cell and axon presence. Hoechst⁺ cells were quantified for transduction by selecting positive area using a color threshold, watershedding the selection, and summing the number of particles using FIJI/ImageJ. The following antibody sources and concentrations were used: Ki67 for proliferation (1:400, AbCam, AB16667), Gfap for astrocytes (1:400, AVEs, GFAP), Sox2 for progenitors and astrocytes (1:400, Abcam, AB97959), Olig2 for oligodendrocyte-lineage cells (1:200, Millipore, MAB450), NeuN for neurons (1:200, Millipore, MAB377), Nf200 for neurofilaments (1:400, Sigma Aldrich, N4142), MBP for myelin (1:400, Santa Cruz, SC-13914), CD13 for endothelial cells (1:100, Santa Cruz, SC-15360), CD8 for cytotoxic T cells (1:400, BD Biosciences, BD-553027), CD4 for helper T cells (1:400, Novus Biologicals, AP-MAB0707), cellular fibronectin (1:100, Abcam, AB6328), F4/80 for macrophages and monocytes (1:400, AbD Serotec, MCA497GA), P0 for myelinating Schwann cells (1:400, AVEs, Pz0), P75 for immature Schwann cells (1:200, Promega, G3231), and luciferase for transgene expression (1:75, Promega, G7451). Secondaries used were Alexa Fluor 488, 555, 647 anti-IgG and fluorescein anti-IgY antibodies diluted 1:500. Samples were counterstained with Hoechst 33528.

Results

Delivery to the Uninjured Spinal Cord

Transgene expression was investigated following the implantation of lentivirus-loaded macroporous sponges implanted intrathecally (Fig 1a). Lentivirus delivery using sponges was initially characterized on uninjured spinal cords. Transgene expression persisted over 12 weeks (Fig 1b,c). At 2 weeks, the majority of transgene expression was expressed in the spinal cord, with low levels of expression observed in other tissues that may result from the movement of transduced cells from the initial site of delivery (Fig 1d). For expression within the spinal cord, most expression was directly under the site of implantation or in the adjacent segments (Fig 1e). By 12 weeks, the levels of transgene expression had decreased, with the greatest reduction in the spinal cord (Suppl. Figure 1), which may result from immune-mediated gene silencing [29].

We also investigated multiple materials for their ability to promote transduction locally (Fig 2a). Lentivirus from porous sponges made with poly(ethylene glycol) (PEG) achieved expression levels similar to those obtain with gelatin (PEG averaged 14% greater across time relative to gelatin, Fig 2b). Sponges that had been rehydrated were also evaluated, and expression averaged 63.8% across time relative to that obtained for gelatin, Fig 2c), which may result from a loss of virus during the additional rehydration step. Enhanced localization of virus to the implantation site was investigated through modifying gelatin hydrogels with heparin and chitosan, which have been previously been employed to localize gene delivery from materials [17]; however, the addition of these lentivirus immobilizing agents [17] significantly ($p < 0.05$) reduced transgene expression (Suppl. Figure 2), likely due to insufficient release of virus from the sponge.

After intrathecal implantation into an uninjured mouse, transduced cells were observed in all regions of the spinal cord directly underneath the implantation site (Suppl. Figure 3): the grey and white matter, the central canal, and the dorsal root, and to a lesser extent, rostral and caudal to the site (Suppl. Figure 4), consistent with the luciferase measurements. Additionally, transgene expression was lower in the spinal cord tissue opposite the implantation site (Suppl. Figure 3), suggesting lentivirus transport through the tissue may be a limiting factor for this delivery method. Damage to the spinal cord due to sponge-mediated delivery was not detected in the histological sections. Immediately underneath the sponge, multiple cell types were identified as transduced in the grey and white matter of the spinal cord, including, Gfap⁺ astrocytes (Fig 3a–d), Sox2⁺ progenitors (Fig 3e–g), Olig2⁺ oligodendrocytes (Fig 3e,f,h), CD8⁺ cytotoxic T cells (Fig 3i–k), CD13⁺ endothelial cells (Fig 3i,j,l), and Neun⁺ neurons (Fig 3m–o). As some markers labeled multiple cell types, we assessed their co-localization. Cell markers for glia (Gfap⁺, Sox2⁺ and Olig2⁺) sparingly overlapped (Suppl. Figure 5). CD13 cells rarely overlapped with F4/80 cells (Suppl. Figure 6). In the grey matter, the majority of transduced cells were Neun⁺ (28.6%), Olig2⁺ (25.4%), and CD8⁺ (15.7%) (Fig 3k,h,o). In the white matter, these cells were Gfap⁺ (31.1%), Olig2⁺ (13.1%), and CD13⁺ (10.0%) (Fig 3c,h,k). P75⁺ and P0⁺ Schwann cells were also transduced (Suppl. Figure 5), but only resided in the dorsal roots of the uninjured spinal cords.

Delivery to the Injured Spinal Cord

Lentivirus loaded sponges implanted intrathecally around injured spinal cords induced transgene expression. A lateral hemisection was created in the spinal cord, with insertion of a microporous PLG bridge. Sponges were placed caudal or above the injury either immediately (acute) or four weeks (chronic) after induction of the injury (Fig 4). All conditions resulted in transgene expression that persisted for the duration of the study. By 2 weeks of implantation, transgene expression in acutely injured spinal cords was almost 3-fold higher than uninjured spinal cords, and expression was 1.4-fold higher for implantation into a chronically injured mouse relative to the injured cord. Multiple doses of lentivirus were attempted to enhance expression in the chronically injured or intact cord (Suppl. Fig 7); however, the second delivery of lentivirus did not increase expression in either case.

The cell types transduced by lentivirus in the spinal cord were subsequently investigated. Two weeks after injury, immune and inflammatory cells had been recruited to the injury, evidenced by the presence of F4/80⁺ macrophages and CD4⁺ helper T cells. Nf160⁺ axons had elongated into the injury as well. P75⁺ Schwann cells were also found throughout the injury; however, few had matured, indicated by a lack of P0⁺ staining. Glial cells had not entered the injury by this time. CD13⁺ endothelial cells were observed in the bridge, although more were found at lesion boundary, possibly hindered in mobility by the scar-producing Gfap⁺ astrocytes also present at the lesion boundary at this time. At six weeks post injury, glial cells (Gfap⁺, Olig2⁺, and Sox2⁺) and P0⁺ mature Schwann cells had started to enter the bridge. Additionally, cells types that were recruited to the injury at two weeks persisted: F4/80⁺, CD4⁺, Nf160⁺, P75⁺, and CD13⁺ cells. However, Neun⁺ nerve somas were not found at or adjacent to the injury site at either time-point. The cell types transduced after spinal cord injury reflected the cell types present at, or caudal to, the injury. (Suppl. Fig 8). P75⁺ Schwann cells (Fig 5a–d) and Gfap⁺ astrocytes (Fig 5e–h), which were present at the injury and lesion border respectively, were transduced in these regions. Distal to the injury site, Gfap⁺ astrocytes (Fig 5i–l) and Neun⁺ neurons (Fig 5m–p) were transduced, similar to the uninjured spinal cord. The cell types present at the bridge were similar in the acute and chronic time point, as were the cell types that were transduced.

The ability of Sonic hedgehog to promote spinal cord regeneration in acute and chronic injuries was subsequently investigated. Sponges loaded with Sonic hedgehog-encoding lentivirus were implanted intrathecally directly above the injury. Co-delivery of a reporter construct to quantify the activity of Msx2, a well-established effector of Bmp signaling [30] that is inhibited by Sonic hedgehog signaling [31, 32], demonstrated a significantly decreased activity with Sonic hedgehog expression relative to control beyond day 45 (Fig 6). The activity was reduced by 50.2% towards the end of the study, supporting a prolonged effect of lentiviral delivery. Additional effects of Shh signaling were investigated through the cell types present at the injury. With expression of Shh, Olig2⁺ cells populated the injury with few Gfap⁺ cells were present, consistent with Sonic hedgehog's role for directing progenitor cell differentiation [33, 34]. In contrast, animals receiving the control sponge had many Gfap⁺ cells and few cells that were Olig2⁺ (Fig 6). Consistent with the increased presence of Olig2⁺ cells, substantial numbers of axons myelinated by oligodendrocytes were observed for sponges loaded with Shh-encoding lentivirus and placed at the injury (Fig 7).

The presence of Olig2⁺ oligodendrocytes and their myelination of axons were less apparent for sponges placed caudal to the injury. The types of glia populating the injury were similar in acutely delivered (assessed at 8 weeks post-injury and post-delivery) and chronically delivered (12 weeks post-injury, or 8 weeks post-delivery) spinal cords, which is surprising as the microenvironment of the acutely injured spinal cord has been shown previously to support the differentiation of progenitors to scar-producing astrocytes instead of myelinating oligodendrocytes [35]. However, this observation may reflect instead a late recruitment and differentiation of these cells, as few glia were present 4 weeks after injury (Fig 6d).

Discussion

Local, long-term delivery of therapeutics to the injured spinal cord has been difficult to achieve without re-injuring the spinal cord. Osmotic pumps have been extensively used for continuous delivery of candidate therapies [36–39]. Most frequently, the catheters are positioned to deliver to the intrathecal space [40–43]. However, long-term placement of the needle inside the intrathecal space has been shown to stimulate the production of scar tissue at the injection site and injure the spinal cord below the needle [20]. The resulting health complications for the patient motivate the creation of delivery strategies that cause less tissue damage. As an alternative, needles have recently been positioned to deliver therapies intraventricularly [42], intramuscularly [44], or more frequently, intravenously [41, 42, 44]. While these methods reduce the risk of spinal cord trauma, they do not localize the therapeutic [43]. Furthermore, the off-site implantation of these therapies requires increased dosages to achieve therapeutic effects [43, 44], which could potentially trigger an immune response.

Herein, we report sponge-mediated lentivirus delivery as a novel method for providing long-term, localized expression of regeneration-promoting factors with reduced risk for damaging spinal cord tissue. Our injury model implants a porous poly(lactide-co-glycolide) bridge inside a hemisection lesion that define the boundaries of the injury, permitting the evaluation of cells recruited to the injury. Robust transgene expression was achieved using a variety of macro-porous materials inserted intrathecally. The delivery effectiveness was reduced with modifications to immobilize the lentivirus [17], suggesting the mechanism of delivery involves diffusion through the micrometer-sized pores of the sponge to the adjacent spinal cord tissue. Placing the sponge inside the intrathecal space surrounding the spinal cord permitted lentivirus delivery to the uninjured spinal cord without detectable damage to the tissue. Lentivirus delivered in this manner achieved expression profiles similar to previously reported lentivirus delivery methods [17]. Viral vectors are emerging as safe and effective delivery vehicles in a range of human trials, with vehicles based on lentivirus contributing to clinical success. Ongoing efforts to engineer vectors will likely be necessary to overcome challenges associated with applications such as spinal cord injury [45]. In the uninjured spinal cord, sponge-mediated delivery induced transgene expression in spinal cord near the implantation site for weeks, with transduction primarily of Neun⁺ neurons and Gfap⁺ astrocytes, consistent with reports of other delivery schemes for intact spinal cord [46–48]. High expression was also achieved when sponges were intrathecally implanted around injured spinal cord, without signs of additional injury arising from the sponge. Sponges could be placed above or caudal to the injury, which would impact the concentration and

gradient of the target protein relative to the injury. Cell types transduced in injured spinal cords were shown to be independent of delivery time (within 4 weeks after injury), similar to previous reports of viral-mediated gene delivery [49, 50]. However, the cell type transduced was dependent on the proximity to the injury. At the injury, P75⁺ Schwann cells were the primary type transduced. Adjacent to the injury, both P75⁺ Schwann cells and Gfap⁺ astrocytes were transduced to the greatest extent, consistent with bridge-mediated lentivirus delivery to the injured spinal cord [15]. Finally, in regions distal to the injury, primarily Gfap⁺ astrocytes and Neun⁺ neurons were transduced, similar to the uninjured spinal cord.

Sonic hedgehog has emerged as a potential therapy for enhancing regeneration following spinal cord injury. In an untreated spinal cord, an injury induces the expression of Bmp, directing the differentiation of nearby progenitors to scar-producing astrocytes [35] which inhibit the elongation and myelination of axons [13, 14] that can re-establish the neural circuitry required for regeneration. Expression of Sonic hedgehog in this study reduced the activity of the transcription factor activity of Msx2, a known effector of Bmp [30], in the injured spinal cord, which suggests a reduction in Bmp signaling, a feature previously seen in developmental studies [31, 32]. This change in transcription factor activity corresponded with an increase in the presence of Olig2⁺ oligodendrocytes and a reduction in the presence of scar-forming Gfap⁺ astrocytes at the injury site. Furthermore, the increase in Olig2⁺ oligodendrocyte presence was correlated with an increase in oligodendrocyte-mediated myelination of axons and a concurrent decrease in Schwann cell-mediated myelination of axons, consistent with our previous report [17]. Over-expression of Sonic hedgehog also promoted oligodendrocyte presence and myelination with a delay in lentivirus delivery. However, implantation of the lentivirus loaded sponge caudal to the injury reduce the impact on oligodendrocytes and astrocytes. Off-site expression of neurotrophins has been reported to enhance axon growth compared to expression at the injury site [51, 52], which likely reflects differences in the mechanisms of action for these neurotrophins relative to Sonic hedgehog.

Lentivirus delivery was delayed for up to four weeks after spinal cord injury in order to assess the efficacy of Sonic hedgehog in a clinically-relevant setting. In the clinic, the administration of therapeutic interventions occurs once the level and nature of the injury have been established. Clinical assessment of spinal cord injured patients, which also determine which treatment interventions are desirable, can delay treatment up to one month after injury [53]. However, the effect of delayed treatment on therapeutic efficacy has been less extensively characterized for non-cell based therapies, such as gene delivery, than for cell-based therapies. The time-window for cell transplantation has been well-established as 10 days to 30 days for many cell sources [21, 35, 54, 55], with few exceptions [56]. In contrast, few studies have assessed the effects of timing for non-cell based therapies. Interventions delayed less than one week have typically shown little to no reduction in improvement [57, 58]. However, the outcome of treatments delivered in the sub-acute (2–4 weeks after injury) to chronic phase (beyond four weeks), as assessed in our study, was more variable: some worsening with the delay [59, 60], while other treatments appeared to be unaffected [49, 52]. Such changes in the efficacies of these therapies with a delay in administration may reflect the dynamic response to the injury, with changing cell

populations at the sub-acute and chronic phases. As evidenced in the literature, the change in composition of scar tissue [1, 2, 5, 6] and of protein expression levels [3, 4] during this time-frame is drastic, motivating the assessment of time as a variable in pre-clinical studies.

In conclusion, we developed a sponge-mediated lentivirus delivery platform that can be used to provide long-term expression in the injured spinal cord similar to previously established delivery methods with minimal risk of injuring spinal tissue. With this platform, lentivirus encoding for Sonic hedgehog was delivered and it enhanced the presence of oligodendrocytes and decreased the presence of astrocytes. Changing sponge placement from above to caudal to the injury site reduced the impact on oligodendrocytes and astrocytes, while implanting the sponge four weeks after injury was found to maintain the impact of Shh on oligodendrocytes and astrocytes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Spatio-temporal profile of transgene expression in the uninjured spinal cord using sponge-mediated lentivirus delivery. (a) Schematic of sponge placement inside the intrathecal space surrounding the intact spinal cord. (b) Visualization of luciferase expression over time when placed around the uninjured spinal cord using the *in vivo* imaging system (n=5). (c) Quantification of luciferase expression over time. (d) Distribution of expression throughout the body at 2 weeks (n=5). (e) Localization of expression along the spinal cord at 2 weeks (n=5). * indicate significant difference ($p < 0.05$) to background level. Doubling and tripling of asterisks indicate significant differences ($p < 0.01$ and $p < 0.001$, respectively). RLU: relative light units.

Figure 2.

Transgene delivery using macro-porous materials. (a) From left to right, Gelfoam®, a lyophilized macro-porous PEG sponge, and a rehydrated macro-porous PEG sponge, the latter of which is outlined with a dashed white line to indicate the border. Transgene expression at multiple time points for (b) Gelfoam®, (c) a lyophilized macro-porous PEG sponge, or (d) a hydrated macro-porous PEG sponge is placed around an uninjured spinal cord ($n=5$ for b–d). Differences in expression between the materials were not significant ($p < 0.05$).

Figure 3.

Transduction of (a–d) Gfap⁺ and Ki67⁺, (e–h) Sox2⁺ and Olig2⁺, (i–l) Cd8⁺ and Cd13⁺, and (m–o) Neun⁺ cells in the uninjured spinal cord. Blue: Hoechst⁺ cells. Red: Luciferase⁺ transduced cells. Green: Gfap⁺, Sox2⁺, Cd8⁺, and Neun⁺ cells, respectively. Grey: Ki67⁺, Olig2⁺, Cd13⁺, and Nf200⁺ cells, respectively. + indicate significant difference (p < 0.05). Arrows (green+) and arrowheads (grey+) indicate transduced cells. GM: grey matter. WM: white matter. + indicate significant difference (p < 0.05) between grey and white matter. Images are representative of sections from 5 mice, and quantification in bar graphs is with n=5. Scale bar in the top row applies to all panels in the corresponding column.

Figure 4.

Lentivirus delivery to (a,c,d,g,h) chronic and (b,e,f,i,j) acute spinal cord injuries. (a,b) Schematic revealing timing of spinal cord injury (SCI), lentivirus delivery (LV) and extractions for acute and chronic spinal cord conditions. Schematic of sponge placement for (c,d) chronic and (e,f) acute spinal cord injuries (c,e) caudal to or (d,f) above the injury site. Transgene expression when placed around (g,h) chronic and (i,j) acute spinal cord injuries (g,i) caudal to or (h,j) above the injury site. WPI: weeks post injury, WPD: weeks post delivery. n=5 for all conditions.

Figure 5.

Transduction of cells when lentivirus-filled sponges are placed above acutely-injured spinal cords. (a–e) P0⁺ and P75⁺ cells at the injury site, Gfap⁺ and Olig2⁺ cells (f–j) adjacent to and (k–o) distal to the injury site, and Nf200⁺ and Neun⁺ cells distal to the injury site. Dashed line indicates the interface between (f–j) the host tissue and the implanted PLG bridge, with the bridge above the line in all panels, and (k–t) the grey and white matter, with the white matter below the line in all panels. Blue: Hoechst⁺ cells. Red: Luciferase⁺ transduced cells. Green: P0⁺, Gfap⁺, Gfap⁺, and Nf200⁺ cells, respectively. Grey: P75⁺, Olig2⁺, Olig2⁺, and Neun⁺ cells, respectively. Similar transduction profiles were seen in the other injured spinal cord conditions (data not shown). Dash lines indicate the interface of the bridge and the host tissue, with the bridge located above the line in all panels. Arrows (green +) and arrowheads (grey+) indicate transduced cells. Images are representative of sections from 5 mice. Scale bar in the left column applies to all images in the row.

Figure 6.

Oligodendrogenesis after spinal cord injury. (a) Schematic illustrating the role of Sonic hedgehog (Shh) and Bone morphogenetic proteins (Bmp) in regulating *Msx2* expression and progenitor differentiation in the spinal cord. (b) Visualization and (c) quantification of *Msx2* expression in the injured spinal cord over time when Shh-encoding lentivirus or a control lentivirus (β -galactosidase) was delivered. (d–m) Gfap⁺ and Olig2⁺ cells in the injured spinal cord when sponges expressing (f,h,i,k,m) Shh, (e,g,j,k) control, or (d) no lentivirus was delivered (e–i) chronically or (j–m) acutely. Chronically delivered samples were extracted (e–h) 12 weeks or (i) 8 weeks post injury with (d) the no lentivirus condition extracted at 4 weeks to indicate the presence and state of glial cells at the injury site prior to delivering lentivirus. Acutely delivered samples were all extracted (j–m) 8 weeks post injury. Blue: Hoechst⁺. Red: Olig2⁺. Green: Gfap⁺. WPI: weeks post injury. Arrows (red+) and arrowheads (green+) indicate the presence of oligodendrocytes and astrocytes, respectively. *n* = 4 for all conditions. Scale bar in the left column applies to all images in the row.

Figure 7.

Myelination after spinal cord injury. (a–j) Mbp⁺ and P0⁺ axons (Nf200⁺) in the injured spinal cord when sponges expressing (c,e,g,i,j) Shh, (b,d,f,h) control, or (a) no lentivirus was delivered (b–f) chronically or (g–j) acutely. Myelination by oligodendrocytes is identified as colocalization of Mbp⁺/Nf200⁺/P0⁺ staining. Chronically delivered samples were extracted (b–e) 12 weeks or (f) 8 weeks post injury with (a) the no lentivirus condition extracted at 4 weeks to indicate the presence and myelination of axons at the injury site prior to delivering lentivirus. Acutely delivered samples were all extracted (g–j) 8 weeks post injury. Blue: Nf200⁺. Red: Mbp⁺. Green: P0⁺. Arrowheads in the 3 boxes at the bottom indicate the colocalization of Mbp⁺, Nf200⁺, and P0⁺ staining in the dashed outline. WPI: weeks post injury. Images are representative of 4 mice. Scale bar in left column applies to all images in the row. Scale bar in the left column applies to all images in the row.