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

1. 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

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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 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.

2. Materials and methods

2.1. 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 h 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.

2.2. 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 h at −20 °C and then exposed while frozen to UV light (365 nm, 50 mW/cm^2; 2 min). The formed hydrogel was lyophilized and then stored with desiccant until use.

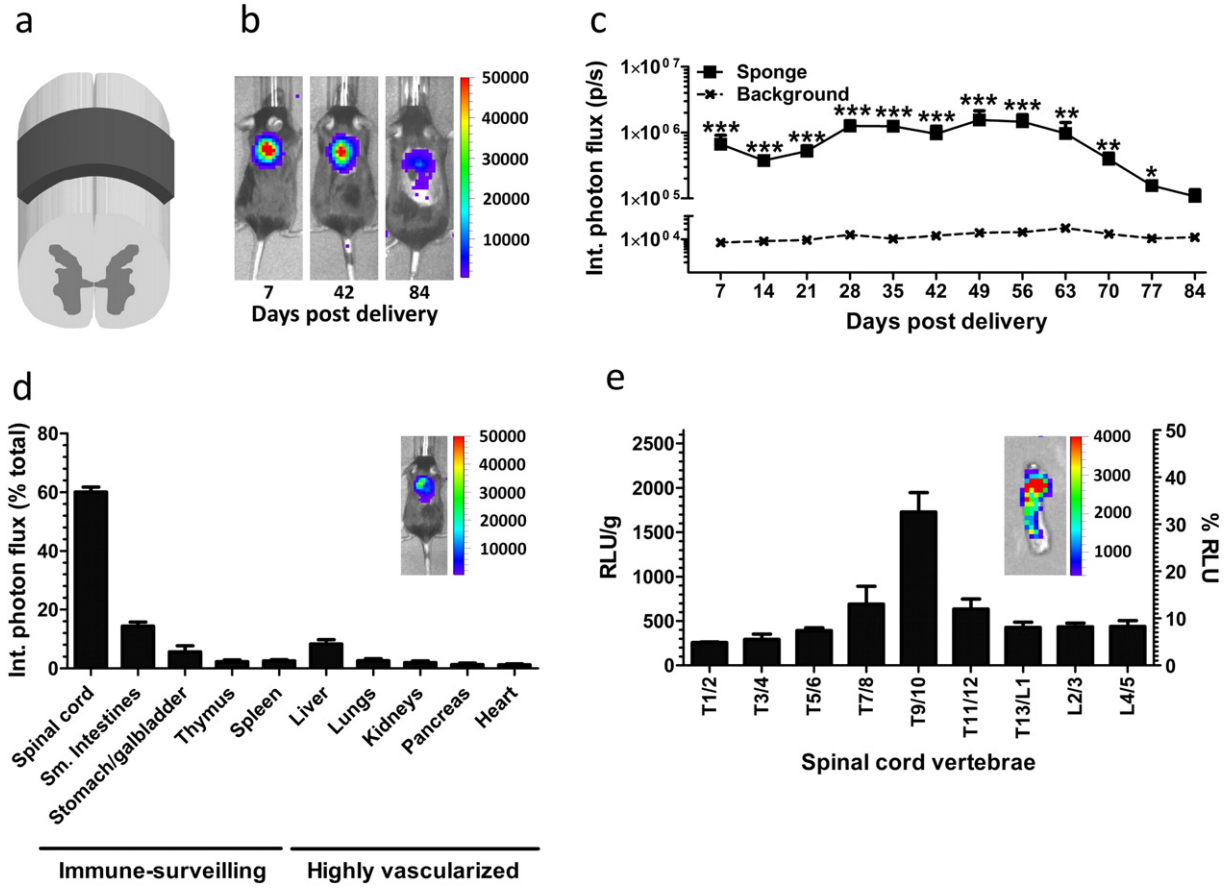

Fig. 1. Spatio-temporal profile of transgene expression in the uninjured spinal cord using sponge-mediated lentivirus delivery. (a) Schematic of sponge placement inside the intrathecal space surrounding the intact spinal cord. (b) Visualization of luciferase expression over time when placed around the 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.