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Peptide amphiphile nanofiber hydrogel delivery of sonic hedgehog protein to the cavernous nerve to promote regeneration and prevent erectile dysfunction

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Abstract

Erectile dysfunction (ED) has high impact on quality of life in prostatectomy, diabetic and aging patients. An underlying mechanism is cavernous nerve (CN) injury, which causes ED in up to 80% of prostatectomy patients. We examine how sonic hedgehog (SHH) treatment with innovative peptide amphiphile nanofiber hydrogels (PA), promotes CN regeneration after injury. SHH and its receptors patched (PTCH1) and smoothened (SMO) are localized in PG neurons and glia. SMO undergoes anterograde transport to signal to downstream targets. With crush injury, PG neurons degenerate and undergo apoptosis. SHH protein decreases, SMO localization changes to the neuronal cell surface, and anterograde transport stops. With SHH treatment SHH is taken up at the injury site and undergoes retrograde transport to PG neurons, allowing SMO transport to occur, and neurons remain intact. SHH treatment prevents neuronal degeneration, maintains neuronal, glial and downstream target signaling, and is significant as a regenerative therapy.

Key words: Peptide amphiphile nanofiber hydrogel; Cavernous nerve injury (prostatectomy); Sonic hedgehog; Erectile dysfunction; Regeneration

Erectile dysfunction (ED) is a debilitating condition that has high impact on quality of life in 52% of men aged 40 to 70[1] and 22% of men under age 40.[2] Men at high risk for ED development are prostate cancer patients treated by prostatectomy, diabetic patients and aging men. A significant underlying cause of ED development is injury to the cavernous nerve (CN, peripheral nerve that provides innervation to the penis) that occurs in up to 82%-85% of prostatectomy patients,[3,4] and with progressive peripheral neuropathy in diabetic patients (75%).[5] Only a small portion (36%) of prostatectomy patients recover erectile function without intervention,[6] and PDE5 inhibitors are ineffective in the majority (69%) of prostatectomy patients,[7] thus improved treatments are needed. In response to CN injury, the downstream target of innervation, the corpora cavernosa of the penis, undergoes extensive remodeling, with abundant smooth muscle apoptosis[8] and increased collagen deposition.[9,10] This irreversible process makes the corpora cavernosal tissue unresponsive to normal signaling pathways, even when the CN undergoes limited regeneration. In order to prevent this adverse penile remodeling

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Summary Sentence: Sonic hedgehog delivered by peptide amphiphile nanofiber hydrogel maintains normal signaling between pelvic ganglia neurons and glia after cavernous nerve crush, preventing neuronal degeneration and apoptosis, and erectile dysfunction.

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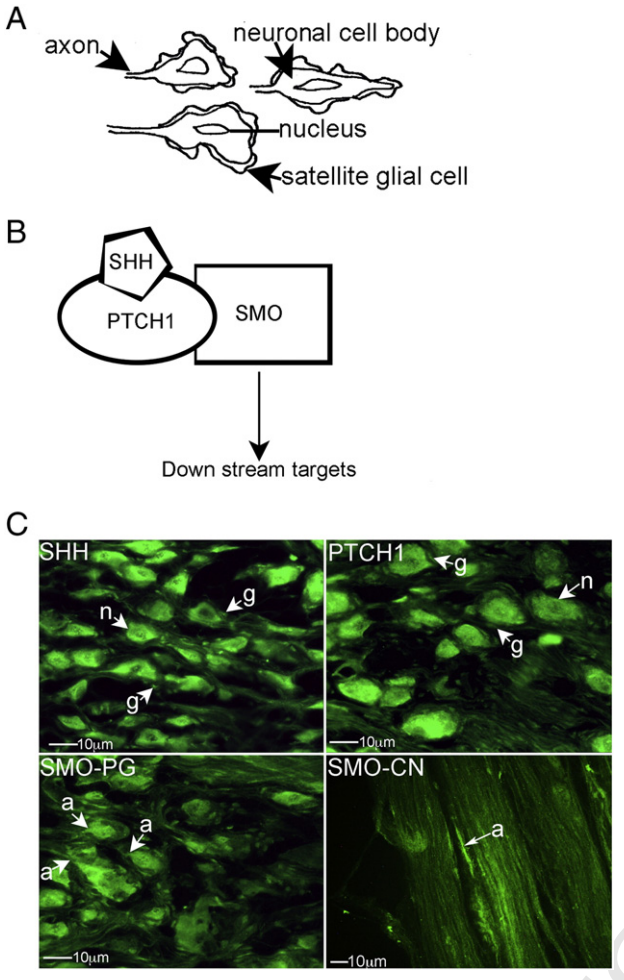

Figure 1. (**A**) Diagram showing the relationship between PG neurons and associated satellite glial cells. (**B**) Diagram of the SHH signaling pathway. (**C**) Immunohistochemical analysis of the PG assaying for SHH, PTCH1 and SMO proteins, and analysis of SMO protein in the CN (400× magnification). Arrows indicate staining. n = neuron. g = glial cell. a = axon.

and ED, protection and regeneration of the CN are critical. We have shown in previous studies that the sonic hedgehog (SHH) pathway is neuroprotective[11] and promotes CN regeneration by 60% at 6 weeks after CN injury when delivered by peptide amphiphile nanofiber hydrogels.[12] However the mechanism of how CN regeneration is significantly enhanced by SHH is unknown.

SHH is abundantly expressed in pelvic ganglia (PG) neurons.[13] Communication between neurons and associated glia is essential for development and maintenance of PG and CN architecture. The main components of the PG are the principal neurons (neuronal cell body and axon), sheathed by satellite glial cells (Figure 1, *A*), which are active partners in neuronal communication.[14] Bidirectional signaling selectively occurs between specific subpopulations of glia, neurons, and synapses[15] and is important for neuronal function. Injury to peripheral nerves is common and can be caused by neuropathy, trauma, repetitive compression,[16–18] or related surgery (such as prostatectomy). Regeneration begins in the axon stump distal to the site of injury within 24-36 hours. The distal nerve shows overall distortion of normal nerve anatomy, axonal swelling and axonal vacuolization which are microanatomical signs of Wallerian degeneration.[19] Local conditions influence how initial regenerative axon sprouts emerge from parent axons,[20] while CN injury triggers a cascade of events in PG neurons, including changes in expression of neurotransmitters, neurotrophic factors, cytokine production,[21] and SHH pathway signaling.[11,12] Following injury, the regenerative abilities of these important injured parasympathetic ganglion neurons and the factors in the environment that influence regeneration are poorly understood.[22]

Our previous studies show that SHH protein is decreased in the PG and CN with CN injury,[11] and when SHH is inhibited in the PG, demyelination of CN fibers and degeneration of non-myelinated fibers were observed,[12] causing downstream apoptosis of penile smooth muscle, induction of ED,[13] and identifying a critical role for SHH in maintaining CN architecture. SHH treatment of the CN at the time of injury by peptide amphiphile (PA) nanofiber hydrogels is both neuroprotective[11] and significantly enhances CN regeneration.[12] However the mechanism of how SHH treatment is beneficial and promotes regeneration is unknown. In other organs, Shh is important for nerve development[23,24] and in the central nervous system, SHH serves as an axon guidance molecule.[25,26] In the peripheral nervous system, adenoviral vector delivery of Shh to injured sciatic nerve, improved motor neuron survival after injury,[27,28] SHH inhibition caused motor neuron death at the axotomy site,[27] and there was impaired regenerative capacity in the absence of Shh.[28] Shh and its receptors, patched (PTCH1, part of the receptor SHH binds to) and smoothened (SMO, signals to downstream targets, Figure 1, *B*), are expressed in adult dorsal root ganglia neurons and glia and knockdown of Shh in adult sensory neurons resulted in decreased regenerative axon sprouting, and branching *in vitro*, suggesting a role for Shh in facilitating outgrowth. Shh is necessary for maintenance of astrocyte proliferation in the optic nerve.[29] However Shh protein is not sufficient to drive proliferation of astrocytes *in vitro* but it was *in vivo*, suggesting that its effect on astrocyte proliferation *in vivo* requires cell–cell interactions that do not operate in dissociated cultures.[30] Thus we propose that SHH interaction between PG neurons and glia is important to maintain normal PG/CN signaling and downstream penile architecture.

We have developed self-assembling peptide amphiphiles for *in vivo* delivery of SHH protein to the CN to promote regeneration and prevent ED.[12] These innovative and broadly applicable hydrogels are composed of highly aligned monodomain nanofiber bundles,[31,32] which allow *in vitro* assembly of linear hydrogel with SHH protein intercalated between and along the nanofiber bundles as they form. The flexible hydrogel can be picked up with forceps and placed on top of the CN *in vivo*. CN preservation and regeneration are enhanced as the SHH protein is released, gradually from the gel.[11,12] This type of PA hydrogel allows for customized, controlled protein delivery over extended periods with a biodegradable vehicle, and is easily translatable to prostatectomy patients in the clinic. In this manuscript we examine the mechanism of how SHH treatment by PA is neuroprotective and promotes CN regeneration after CN crush injury. SHH promotes CN regeneration by maintaining normal signaling between PG neurons and glia and to downstream