Acta Biomaterialia 32 (2016) 89–99

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Full length article

Sonic hedgehog delivery from self-assembled nanofiber hydrogels reduces the fibrotic response in models of erectile dysfunction ☆

Shawn Choe [a], Dorina Veliceasa [a], Christopher W. Bond [b], Daniel A. Harrington [c], Samuel I. Stupp [d,e,f,g], Kevin T. McVary [h], Carol A. Podlasek [a,i,*]

[a] Department of Urology, University of Illinois at Chicago, Chicago, IL 60612, United States
[b] Department of Allergy/Immunology, Northwestern University, Feinberg School of Medicine, Chicago, IL 60611, United States
[c] Department of Biosciences, Rice University, Houston, TX 77005, United States
[d] Simpson-Querrey Institute for BioNanotechnology, Northwestern University, Chicago, IL 60611, United States
[e] Department of Chemistry, Northwestern University, Chicago, IL 60611, United States
[f] Department of Materials Science and Engineering, Northwestern University, Chicago, IL 60611, United States
[g] Department of Biomedical Engineering, Northwestern University, Chicago, IL 60611, United States
[h] Division of Urology, Southern Illinois University School of Medicine, Springfield, IL 62794, United States
[i] Department of Physiology and Bioengineering, University of Illinois at Chicago, Chicago, IL 60612, United States

ARTICLE INFO

Article history:
Received 12 October 2015
Received in revised form 8 January 2016
Accepted 13 January 2016
Available online 14 January 2016

Keywords:
Self-assembling peptide amphiphile
nanofiber hydrogels
Sonic hedgehog
Penis
Collagen
Cavernous nerve injury

ABSTRACT

Erectile dysfunction (ED) is a serious medical condition in which current treatments are ineffective in prostatectomy and diabetic patients, due to injury to the cavernous nerve (CN), which causes irreversible remodeling of the penis (decreased smooth muscle and increased collagen), through a largely undefined mechanism. We propose that sonic hedgehog (SHH) and neural innervation, are indispensable regulators of collagen in the penis, with decreased SHH protein being an integral component of the fibrotic response to loss of innervation. We examined collagen abundance and morphology in control (Peyronie's), prostatectomy and diabetic patients, and in rat models of penile development, CN injury, SHH inhibition and under regenerative conditions, utilizing self-assembling peptide amphiphile (PA) nanofiber hydrogels for SHH delivery. Collagen abundance increased in penis of ED patients. In rats, collagen increased with CN injury in a defined time frame independent of injury severity. An inverse relationship between SHH and collagen abundance was identified; SHH inhibition increased and SHH treatment decreased penile collagen. SHH signaling in the pelvic ganglia (PG)/CN is important to maintain CN integrity and when inhibited, downstream collagen induction occurs. Collagen increased throughout penile development and with age, which is important when considering how to treat fibrosis clinically. These studies show that SHH PA treatment reduces collagen under regenerative post-prostatectomy conditions, indicating broad application for ED prevention in prostatectomy, diabetic and aging patients and in other peripheral nerve injuries. The PA nanofiber protein vehicle may be widely applicable as an *in vivo* delivery tool.

Statement of Significance

We use self-assembling peptide amphiphiles (PA) as biological delivery vehicles to prevent cavernous nerve (CN) injury induced erectile dysfunction (ED). These versatile hydrogels were molecularly pre-programmed for sonic hedgehog (SHH) protein delivery, either from an injectable solution with fast, in situ assembly into a soft hydrogel, or by highly aligned monodomain nanofiber bundles. We used PAs to examine a novel neuronal component to collagen regulation and the role of SHH in the fibrotic response to CN injury. SHH perturbation in the penis or the CN, selectively impacts collagen, with SHH inhibition increasing and SHH treatment suppressing collagen. These results suggest that SHH treatment by PA has translational potential to suppress collagen induction and remodelling, an irreversible component of ED development.

☆ Summary Sentence: Sonic hedgehog and neural innervation are important regulators of collagen in the penis.

* Corresponding author at: Department of Urology, M/C 955, University of Illinois at Chicago, 820 S. Wood St., CSN 515, Chicago, IL 60612, United States.
E-mail address: cap325@uic.edu (C.A. Podlasek).

http://dx.doi.org/10.1016/j.actbio.2016.01.014
1742-7061/

1. Introduction

The cavernous nerve, which provides innervation to the penis, becomes injured during prostatectomy surgery, in diabetic patients, and with aging, resulting in remodeling of penile morphology and erectile dysfunction (ED). The penis is composed of smooth muscle, collagen and elastic fibers. In response to peripheral nerve injury, smooth muscle and elastic fibers decrease while collagen increases in both ED patients [1,2] and animal models [3,4]. This is an irreversible process that underlies ED development. Aging and consumption of high fat diets are also associated with increased collagen abundance and thickening of fibers [5–7]. Current ED treatments are not effective to prevent the penile remodeling, nor long term ED development. The mechanism of how collagen induction occurs in response to CN injury is complex and remains largely undefined.

As is the case with other peripheral nerves, CN regeneration efforts have not translated into improved clinical outcomes, and there is minimal understanding of how neuronal changes impact tissue remodeling. Few factors have been identified in the penis that impact collagen induction. The most widely explored is the TGF-β pathway, which in patients, increases collagen abundance 2.5–4.5-fold [8] and in animal models increases with CN injury [9]. TGF-β-1-induced collagen synthesis is inhibited by cyclic AMP synthesis in human corpora cavernosal smooth muscle cells [10], suggesting a potential mechanism of how TGF-β may be regulated. TGF-β also functions downstream of the adenosine receptor A(2B)R which has been implicated to increase proliferation in corpora cavernosal fibroblast cells [11]. Accumulating evidence suggests that cross talk may occur between the sonic hedgehog (SHH) pathway and TGF-β signaling in several diseases including gastric carcinoma [12], melanoma bone metastasis [13] and pulmonary fibrosis [14]. It is thought that Hedgehog may mediate epithelial–mesenchymal crosstalk in pulmonary fibrosis, with SHH inducing TGF-β in lung fibroblasts while TGF-β induces SHH in cultured alveolar epithelial cells [15]. Thus, we hypothesize that SHH may also serve as an important mediator/regulator of collagen synthesis in normal and injured penis.

We have shown in previous studies that the SHH pathway is critical for the response of the penis and of the CN to denervation, regulating both penile and CN architecture, and smooth muscle apoptosis [16–23]. The SHH pathway has recently been suggested to also play an important role in the pathogenesis of fibrosis [24,14], with activation of the pathway present in fibrotic diseases such as sclerosis, interstitial pneumonitis, injury-related inflammations [25–27] and idiopathic pulmonary fibrosis [28]. In support of this idea, during development, the expression pattern of type XVIII collagen in the ureter bud is responsive to changes in SHH expression in the epithelium [29]. In the adult, hedgehog (Hh) signaling can regulate lung fibrosis [30], and expression of SHH and GLI were correlated with cerulean-induced fibrosis in the pancreas [31], suggesting that SHH may be a regulator of collagen. In this study we will examine if collagen production is responsive to SHH signaling in the penis and in the CN, and to CN regulation, and thus may provide a novel avenue for clinical intervention post prostatectomy and in diabetic patients.

We have described the use of self-assembling peptide amphiphiles (PA) as biological delivery vehicles, to prevent ED-related smooth muscle apoptosis in the penis [20,21]. These versatile hydrogel systems can be molecularly pre-programmed for SHH protein delivery, either from (1) an injectable solution with fast, *in situ* assembly into a soft hydrogel, or (2) highly aligned monodomain nanofiber bundles with increased mechanical integrity [32,33]. In both permutations, these PAs offer a customized, biodegradable, solution for delivering proteins in a controlled manner over extended periods, and are easily translatable to patients in the clinic. In this study we will examine a novel neuronal component to collagen regulation and the role of the SHH pathway in the fibrotic response to nerve injury. We will utilize these innovative PA systems for SHH delivery to the luminal surfaces of the corpora cavernosa (via *in situ* gelation), and SHH delivery to the injured CN from a manipulable supramolecular cable (via monodomain aligned nanofibers). These materials and their technology have potentially broad application to other peripheral nerves and the tissues that they innervate.

2. Materials and methods

2.1. Animals

199 Sprague–Dawley rats postnatal day 7 (P7) through P300 were obtained from Charles River. The study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal care protocol was approved by the Office of Animal Care and Institutional Biosafety at the University of Illinois at Chicago (OACIB) and by the IACUC committee at Northwestern University and animals were cared for in accordance with institutional approval.

2.2. Patient tissues

Corpora cavernosal tissue was obtained from 21 patients who underwent penile prosthesis implantation at Northwestern Memorial Hospital. Eight patients had a previous prostatectomy, five had diabetes, and eight control patients underwent corrective surgery for Peyronie's disease. Peyronie's disease is a condition of the penis characterized by the alteration in the appearance and cellularity of collagen within the tunica albuginea, which becomes fibrotic with disease progression. While Peyronie's patients have intromission difficulty, the underlying defect is tunical so the lacunar tissue remains fundamentally normal [34]. Peyronie's tissue is the best available control other than cadaver tissue and corporal tissue was obtained from a region away from the involved tunica. For the penile prostheses cases, the corporal tissue at the site of corporotomy was identified, minimally handled and a small wedge of lacunar tissue excised prior to any dilation or other corporal manipulation was performed. For the control Peyronie's cases, the corporal tissue at the site of tunical defect and the surrounding corpora in the vicinity of the planned grafting site were identified. Similarly these normal tissues were minimally handled and a small wedge of lacunar tissue excised prior to any other corporal manipulation or grafting was performed. The tissue was immediately handed to a lab technician present in the operating room and the tissue was snap frozen in liquid nitrogen or fixed in 4% paraformaldehyde overnight at 4 °C prior to paraffin embedding. Exclusion criteria included patients under 18 years of age. The complete study protocol was approved by the institutional review board of Northwestern University and written informed consent was obtained from all patients. For the prostatectomy patients it had been 1–17 years since surgery with an average of 6 years. For the diabetic patients, the onset of diabetes was between 7 and 24 years with an average of 12 years.

2.3. Hydroxyproline assay

Collagen abundance was quantified on frozen corpora cavernosal tissue using a modified hydroxyproline assay [35]. Each sample was examined in duplicate. The absorbance was read at 550 nm