Development of a biomaterial-based siRNA delivery system to promote neuronal regrowth following spinal cord injury

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Background: Following spinal cord injury (SCI), paralysis ensues as neurons are unable to regrow their axons through the lesion cavity that forms at the injury site. We propose that biomaterial-mediated RNA therapy might be a promising avenue for treating SCI by delivering RNA molecules to injured neurons from an optimal implantable scaffold to regulate the production of growth-related proteins. This strategy could enable bridging of the lesion cavity while also providing physical support for, and enhancement of, axonal regrowth. In particular, delivery of siRNA has the ability to silence intrinsic neuronal proteins inhibitory to growth (e.g. phosphatase and tensin homolog (PTEN)). In this study, we developed an advanced scaffold-based system designed to deliver siRNA cargoes in a controlled and localized way to injured neurons, aiming to promote improved axonal regrowth after SCI.

Methods: A novel non-viral nanoparticle, GAG-binding enhanced transduction (GET)[1], was employed to deliver the siRNA to neurons. Building on our advanced biomimetic, hyaluronic acid-based scaffold with proven potential for SCI applications[2], we sought to utilise this scaffold platform by simultaneously delivering these siRNA-nanoparticles to neurons to promote neuronal regrowth and recovery following SCI.

Results: PTEN, a well-known inhibitor of neuronal regrowth was selected for knockdown using siRNA. First, the non-viral GET vector demonstrated effective delivery of siRNA into mature neurons and *PTEN*-siRNA nanoparticles successfully inhibited *PTEN* expression in primary neurons. Next, the hyaluronic acid-based scaffolds[2] were successfully functionalised with siRNA-nanoparticles. siRNA-activated scaffolds had the capability to retain the siRNA-nanoparticles within their architecture, enabling successful transfection of neurons. Finally, by incorporating *PTEN*-siRNA nanoparticles into our scaffold we enhanced the ability of the scaffold to stimulate growth in damaged cortical neurons, without changing the beneficial properties of the scaffold.

Conclusions: In conclusion, this study highlights the successful development of an advanced biomaterial-based siRNA-activated scaffold tailored for SCI repair applications and highlight its potential as an effective strategy for stimulating axonal regrowth across the lesion site as a novel therapeutic to treat SCI.

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1. Dixon, J.E. et al. PNAS 113, E291-299 (2016).
2. Woods, I. et al. AHM, e2101663 (2021).