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| **Development of a scaffold-mediated small-interfering RNA delivery system to promote neuronal recovery following spinal cord injury**  |
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| **Background:** Following spinal cord injury (SCI), paralysis ensues as neurons intrinsically lack the ability to significantly recover. Based on our lab’s extensive experience developing gene-activated biomaterial scaffolds for tissue regeneration, we propose that our novel scaffold1 developed with optimised composition, architecture and stiffness for SCI repair might provide localised delivery of therapeutic genetic cargoes to abate the effects of this neurotoxic environment. In particular, delivering siRNA to injured neurons has the ability to silence intrinsic neuronal molecules inhibitory to recovery following SCI. This study thus aimed to first identify a suitable genetic pathway to target to promote recovery before investigating whether our scaffold1 could effectively deliver siRNA to neurons to manipulate this pathway. Finally, an *in vitro* model of oxidative stress, which contributes to widespread neuronal apoptosis after SCI, was used to assess the therapeutic potential of this scaffold-mediated siRNA delivery system for SCI repair. |
| **Methods:** First, to identify a genetic pathway to target, bioinformatic analyses were used on data spanning the developmental timeframe of spinal cord neurons. Next, a novel non-viral nanoparticle, GAG-binding enhanced transduction (GET)2, was employed to deliver siRNA to neurons. Scaffolds were then loaded with siRNA-nanoparticles and were assessed for their ability in delivering siRNA to neurons to manipulate the target pathway. Finally, to assess the therapeutic potential of these scaffolds, neurons were stimulated with hydrogen peroxide (H202) to simulate oxidative stress. |
| **Results:** From the bioinformatic analyses undertaken, phosphatase and tensin homolog (PTEN), a potent inhibitor of neuronal recovery, was selected for non-viral siRNA targeting to enhance neuronal recovery. Next, a fluorescently-tagged siRNA was successfully complexed with GET nanoparticles and delivered to the cytoplasm of neurons. Subsequently, functional knockdown of the PTEN was achieved in neurons following delivery of PTEN siRNA using GET. Building on this, a siRNA-activated scaffold delivery system was successfully established by incorporating the GET-siRNA nanoparticles into the scaffold. PTEN siRNA delivered from the scaffold then successfully interfered with the expression of PTEN mRNA. Finally, neurons cultured on PTEN siRNA scaffolds and challenged with H2O2 showed enhanced metabolic activity and crucially showed a decrease in expression of key markers of apoptosis. |
| **Conclusions:** Taken together, we have successfully developed a PTEN siRNA activated scaffold delivery system that displays anti-apoptotic effects in an *in vitro* model of neurotoxic oxidative stress. Our results demonstrate a novel scaffold-based strategy to enhance the intrinsic ability of neurons to repair, ultimately suggesting it might be employed as a novel treatment for SCI.Funding: Irish Rugby Football Union Charitable Trust and SFI Centre for Advanced Materials and BioEngineering Research Center(AMBER)1. Woods, I. *et al.* *AHM*, e2101663 (2021).2. Dixon, J.E. *et al.* *PNAS* **113**, E291-299 (2016). |