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| **Nanoparticle-mediated silencing of Connexin43 for SCI applications** |
| Martyna Stasiewicz1,3, James E. Dixon4, Adrian Dervan1,3, Fergal J. O’Brien1,2,3 |
| 1 Tissue Engineering Research Group (TERG), Royal College of Surgeons, University of Medicine and Health Sciences, Dublin; 2 Trinity Centre for Biomedical Engineering, Trinity College Dublin, Dublin 2,Ireland; 3Advanced Materials & Bioengineering Research (AMBER) Centre, RCSI, 123 St. Stephen’s Green, Dublin 2, Ireland; 4The University of Nottingham Biodiscovery Institute, Nottingham, NG7 2RD, UK. |
| **Background:** Spinal cord injury (SCI) is a devastating traumatic injury characterized by formation of growth inhibiting scars around a lesion cavity in the damaged tissue. Consisting of astrocytes and invading fibroblasts, this potent barrier impedes the regrowth of injured axons but it also functions to protect undamaged tissue by restricting toxic inflammatory cell ingress. As removal of the scar increases the spread of injury, we hypothesized by reducing Connexin43 (Cx43) gap junctional protein which promotes scar cell interaction may help to destabilize scars and controllably reduce, rather than remove barrier function. To do so, we employed TERG-based expertise in scaffold fabrication and gene therapy to develop a biomimetic, gene-activated biomaterial scaffold capable of retaining and releasing nanoparticles complexed with silencing (si)RNA for non-viral delivery to scar-forming cells. |
| **Methods:** To induce scar-forming behaviours, astrocytes and meningeal cells were treated with SCI-associated cytokines IL1α, TNFα, C1q and TGFβ and rate of cell migration and expression of scar-associated Cx43 and collagen I assessed. For scaffold development, 3mg/mL hyaluronic acid and collagen IV scaffolds designed specifically for spinal cord applications were freeze-dried and soak-loaded with GAG-enhanced transduction (GET) peptide nanoparticles complexed with Cx43 siRNA. Cytokine-treated human astrocytes and meningeal fibroblasts were then seeded on the scaffolds. To test nanoparticle diffusion from the scaffold and ability to transfect surrounding cells, conditioned media was isolated from the gene-activated scaffold cultures and added to 2D cultures of astrocytes and meningeal fibroblasts. |
| **Results:** Treatment of astrocytes and meningeal fibroblasts with pro-fibrotic TGFβ induced scar-forming behaviours by upregulating Cx43 and scar-associated collagen I proteins, and enhanced rate of cell migration, while IL1α, TNFα, C1q treatment did not. Transfecting the injured astrocytes and meningeal fibroblasts with optimized GET: Cx43 siRNA nanoparticles abrogated TGFβ-induced upregulation of Cx43 and collagen I. The optimized siRNA concentration was then used to develop gene-activated scaffolds containing GET: Cx43 siRNA nanoparticle complexes for non-viral delivery to astrocytes and meningeal fibroblasts. When cultured on the gene-activated scaffolds, these cells demonstrated nanoparticle uptake and subsequent reduction in Cx43 junctional protein expression compared to controls. A similar downregulation of Cx43 protein expression was observed in astrocytes and meningeal fibroblasts treated with conditioned media isolated from gene-activated scaffolds, demonstrating the successful release of nanoparticles from the scaffold. Furthermore, Cx43 silencing increased separation between cells indicating “loosening” of cell-to-cell connections which is being further explored. |
| **Conclusions:** We developed a gene-activated scaffold capable of retaining and releasing GET: Cx43 siRNA nanoparticles, and successfully transfecting human astrocytes and meningeal fibroblasts. We determined that treatment with optimized GET: siRNA nanoparticles reversed the scar-forming behaviours induced by pro-fibrotic TGFβ treatment. In summary, this work outlines the development of a scaffold-based, non-viral gene therapy strategy targeting Cx43 junctional protein-mediated connections between scar forming cells as a novel biomaterials-based strategy for SCI applications. |