An Injectable Biomimetic Hydrogel containing Non-Viral P2X7R Silencing RNA Nanoparticles for the Treatment of Post-Traumatic Temporal Lobe Epilepsy

Anne M. O'Callaghan[1,2,3], Carly Douglas[4], David Loane[4], James E. Dixon[5], Adrian Dervan[1,2], Ian Woods[1,2], Tobias Engel[3,6], Fergal J. O'Brien[1,2]

[1] Tissue Engineering Research Group, Dept. of Anatomy & Regenerative Medicine, RCSI, Dublin; [2] Advanced Materials & Bioengineering Research (AMBER) Centre, RCSI, Dublin; [3] FutureNeuro, RCSI, Dublin; [4] Neurotrauma and Neuroimmunology, Trinity College Dublin, Dublin; [5] School of Pharmacy, University of Nottingham Biodiscovery Institute, University of Nottingham, Nottingham, UK; [6] Department of Physiology and Medical Physics, RCSI, Dublin

Background: Temporal lobe epilepsy (TLE) is the most prevalent acquired seizure disorder in adults, often caused by brain injury. Anti-seizure drugs are ineffective in 33% of patients (classed as having drug-refractory TLE) and exhibit additional short-comings including cognitive side effects and a failure to tackle the underlying disease pathophysiology. The purinergic receptor P2X7R, highly expressed on inflammatory microglia within injured brain regions, contributes to chronic inflammation. Notably, systemic P2X7R inhibition has shown disease-modifying effects in murine TLE models, however its expression throughout the body means focal delivery is crucial for effective translation of P2X7R-targeting therapies. Therefore, the key aim of this study was to develop an injectable hydrogel release system capable of delivering P2X7R-targeting silencing RNA (siRNA) to cortical epileptic lesions.

Methods: P2X7R-targeting siRNA was delivered to murine microglial cells using nanoparticles formed through complexation of siRNA with a non-viral peptide-based vector, and knockdown was validated by PCR. Microglial cell phenotype and pro-inflammatory cytokine release were quantified by PCR and ELISA respectively. To produce an injectable biomimetic hydrogel, hyaluronic acid (a key component of brain tissue) was reacted with methacrylic anhydride to produce methacrylated hyaluronic acid (MeHA). Injectable MeHA solutions were shown to crosslink in response to blue light and the resulting hydrogel's mechanical properties were analysed. Finally, the siRNA nanoparticles were encapsulated in the hydrogel and release was quantified using fluorescent reporter siRNA (siGlo).

Results: Using siGlo, an optimal siRNA nanoparticle concentration of 50nM was found to achieve high cellular internalisation with minimal cytotoxicity in microglia. Following non-viral P2X7R siRNA transfection, P2X7R knockdown was successfully demonstrated in reactive microglial cells. Knockdown was then shown to induce a resting phenotype, characterised by reduced expression of the pro-inflammatory microglial marker Iba1 as well as significant downstream reductions in the release of the neurotoxic cytokine IL-1β. MeHA hydrogels exhibited a stiffness compatible with neural cells, microarchitectures suitable for retaining encapsulated siRNA, and a robust degradation profile. Importantly, siGlo nanoparticles were released from the hydrogels in a controlled manner, and effective cellular uptake of hydrogel-released nanoparticles was observed.

Conclusions: This study demonstrates that injectable MeHA hydrogels, designed to mimic the composition and mechanical properties of brain tissue, can encapsulate and release non-viral P2X7R siRNA nanoparticles. Furthermore, these nanoparticles were also shown to efficiently down-regulate P2X7R gene expression in reactive microglia which significantly modified associated inflammatory signalling pathways. Future work will aim to confirm the disease-modifying effects of this system *in vivo* using a murine injury model of TLE. In summary, this gene-activated hydrogel targeting microglial P2X7R demonstrates potential to act as a novel disease-modifying treatment strategy for drug-refractory TLE patients.