# DEVELOPMENT OF A GENE-ACTIVATED SCAFFOLD FOR THE DOWNREGULATION OF ANTI-CHONDROGENIC SIGNALING PATHWAYS IN THE PROGRESSION & DEVELOPMENT OF OSTEOARTHRITIS

Domhnall Kelly[1,2], Tom Hodgkinson[1], James Dixon[3], Kevin Shakesheff[3], Caroline Curtin[1], Caitriona O'Driscoll[2,4], Fergal O'Brien[1,2]

[1]Tissue Engineering Research Group, RCSI, University of Medicine & Health Sciences, Ireland; [2]Centre for Research in Medical Devices (CÚRAM), NUI Galway, Ireland; [3]School of Pharmacy, University of Nottingham, UK; [4]Pharmacodelivery Group, School of Pharmacy, University College Cork, Ireland

**Background:** Biomaterial-mediated delivery of siRNA therapeutics provides a method of silencing specific genes known to hinder the tissue regeneration process while also providing a structural support for cell proliferation and matrix deposition [1]. The objective of this study was to use a cell penetrating peptide with a glycosaminoglycan-binding domain (GET) as a non-viral gene delivery vector [2], combined with a previously optimised collagen-hyaluronic acid (coll-HyA) with proven chondrogenic benefit [3] for the development of an advanced delivery system. The overall goal of this study is the manipulation of the early osteoarthritic microenvironment in 2D and 3D cell culture systems, allowing for favourable conditions more conducive to successful stem cell recruitment and chondrogenic differentiation.

**Methods:** Varying formulations of GET-siRNA complexes were analysed based on size, charge, morphology and encapsulation efficiency using dynamic light scattering, transmission electron microscopy, nanoparticle tracking analysis and gel retardation assays. A fluorescent transfection indicator was used to assess cellular uptake using live cell imaging and flow cytometry. Reporter gene knockdown was assessed using RT-qPCR. Optimised formulations of GET-siRNA were used to deliver siRNA against the p65 subunit of the NF-κB pathway (RELA/p65) to human MSCs in a simulated pro-inflammatory environment (Media + IL-1β/TNF-α). RT-qPCR was used to assess expression of downstream chondrogenic and OA-associated mediators. GET-p65 siRNA formulations were incorporated into porous coll-HyA scaffolds and scaffold architecture was assessed using SEM. hMSCS were cultured on gene-activated scaffolds (3D *in vitro*) in the presence of inflammatory cytokines and activity of downstream mediators was assessed.

**Results:** An optimized formulation of GET with siRNA resulted in nano-complexes of favourable size (146.0 ± 23.63 nm), zeta potential (32.32 ± 5.5 mV), and encapsulation efficiency (87.8 ± 0.1%). *In vitro* (2D) screening demonstrated efficient cellular uptake of GET-siRNA nano-complexes and successful reporter gene knockdown in a sustained yet transient manner. Successful knockdown of the p65 subunit attenuated cytokine mediated activation of the NF-κB pathway, preventing nuclear translocation, dampening downstream catabolic mediator expression (MMPs 3, 9, 13), and recapitulating chondrogenic transcription factor activity (SOX9). SEM imaging and release assay highlighted successful incorporation of GET-siRNA nano-complexes within the coll-HyA scaffolds. hMSCs seeded (3D) on gene-activated scaffolds in the presence of inflammatory cytokines further demonstrated successful silencing of the NF-κB pathway (71.11 ± 0.22% @ Day 3) and a significant reduction in downstream catabolic mediators (MMPs 3, 9, 13), corroborating results observed in 2D culture systems.

**Conclusions:** This study demonstrates the successful development of an advanced gene-activated scaffold delivery system capable of manipulation of the early OA microenvironment through the controlled delivery of therapeutic siRNA, creating a promising environment more conducive to stem cell recruitment and chondrogenic differentiation.