DEVELOPMENT OF A GENE-ACTIVATED SCAFFOLD TARGETING ANTI-CHONDROGENIC SIGNALING PATHWAYS IN THE PROGRESSION OF OSTEOARTHRITIS

Domhnall C. Kelly1,2, Tom Hodgkinson1, James E. Dixon3, Caroline M. Curtin3, Caitríona M. O’Driscoll2, Fergal J. O’Brien1,2
1Tissue Engineering Research Group, Dept. of Anatomy & Regenerative Medicine, RCSI University of Medicine & Health Sciences; 2Centre for Research in Medical Devices (CURAM), NUI Galway; 3School of Pharmacy, University of Nottingham; 4Pharmaceutical Delivery Group, School of Pharmacy, University College Cork, Ireland

ACKNOWLEDGEMENTS

CONTACT DETAILS

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 an environment more conducive to stem cell recruitment and chondrogenic differentiation.

BACKGROUND

- Inflammation is a driving factor in the progression of osteoarthritis (OA) and subsequent tissue degradation
- In response to injury, activation of the NF-KB pathway creates a positive feedback loop which leads to cartilage destruction

HYPOTHESIS

Combining non-viral siRNA delivery with collagen-hyaluronic-based (Coli-Hya) platforms optimized to enhance the chondrogenic potential of MSC, will enable safe, effective and durable cartilage regeneration, in addition to enhancing the intrinsic mechanisms of tissue repair

AIMS

1. Optimization of a non-viral delivery system for siRNA
2. Validation of therapeutic siRNA in OA simulated conditions
3. Development of advanced scaffold-based siRNA delivery system

RESULTS

Electrostatic interactions between GET (cationic) and siRNA (anionic) allows for the formation of GET-siRNA complexes at the (A) nanoscale (<300nm) with (B) a positive zeta potential, demonstrating (C) successful encapsulation of the nucleic acid cargo. An (D) optimized formulation of GET-siRNA (N/P 6) demonstrates successful (E) cellular uptake and (D) reporter gene silencing in a transient manner over 7 days

Figure 1: Dynamic light scattering (DLS) was used to determine (A) size and (B) zeta potential of varying formulations of GET-siRNA. (C) Gel retardation assay confirms successful encapsulation and release following heparin challenge. (D) TEM imaging of GET-siRNA N/P 6 indicates spherical morphology and minimal aggregation of complexes. (E) Successful cellular uptake indicates cytosplasmic localization of complexes. (F) In vitro stability of GET-siGAPDH demonstrates transient silencing with knockdown highest at Day 3.

CONCLUSION

(A) p65 knockdown prevents activation and (B) nuclear translocation of NF-κB dimer, (C) attenuating the downstream expression of catabolic mediators in the presence of inflammatory cytokines (IL-1β and TNF-α) in hMSC monolayer

Figure 2: (A) PCR analysis of p65 mRNA expression levels in stimulated cytokine media & following GET-siRNA silencing. (B) Ab staining indicates that p65 silencing inhibits expression and pro-inflammatory cytokine induced nuclear translocation. (C) PCR analysis of the mRNA expression levels of catabolic (MMP-3, MMP-9, MMP-13) downstream effectors of NF-KB signaling and in the presence of pro-inflammatory cytokines (IL-1β and TNF-α).

Coli-Hya scaffolds demonstrate (A) successful retention of GET-siRNA exhibiting (B) a delayed release profile (C) facilitating knockdown of target gene expression and silencing of downstream “anti-chondrogenic” mediators

Figure 3: (A) SEM and fluorescence microscopy demonstrate successful incorporation of GET-siRNA into scaffolds. (B) Successful retention of GET complexed siRNA compared to uncomplexed siRNA loaded into scaffolds. (C) Gene-activated scaffolds facilitated knockdown of the p65 subunit with highest silencing observed at Day 3. mRNA expression levels of catabolic downstream effectors demonstrate silencing effects at Day 3 and Day 7 in the presence of inflammatory cytokines representative of an OA microenvironment.