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

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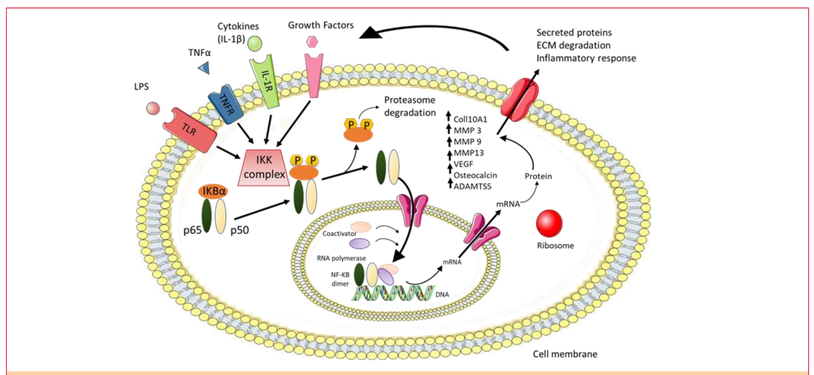
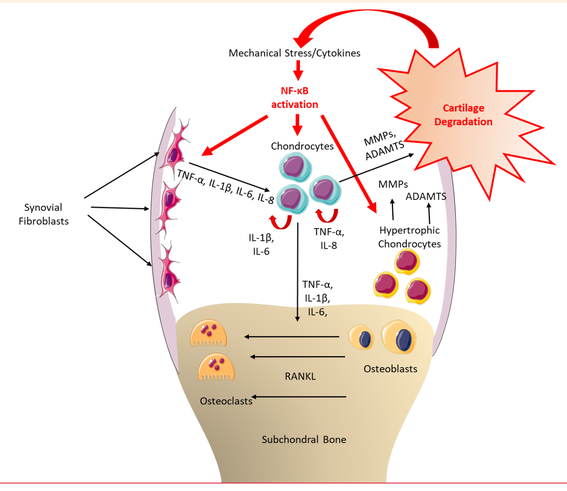
RCSI DEVELOPING HEALTHCARE LEADERS WHO MAKE A DIFFERENCE WORLDWIDE

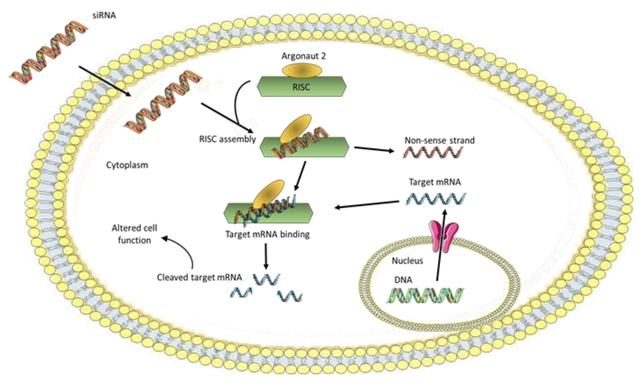
BACKGROUND



- 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

- Selective siRNA-mediated silencing targeting the NF-KB pathway can reduce chondrocyte apoptosis, hypertrophy and ECM degradation, halting the progression of OA





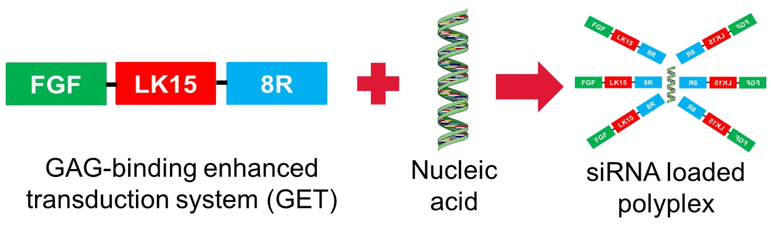
- Nucleic acids (siRNA) have an unfavourable biodistribution profile
- Undergo rapid degradation by nucleases
- To date, there has been a lack of a safe and efficient delivery system

HYPOTHESIS

Combining non-viral siRNA delivery with collagen-hyaluronic-based (Coll-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

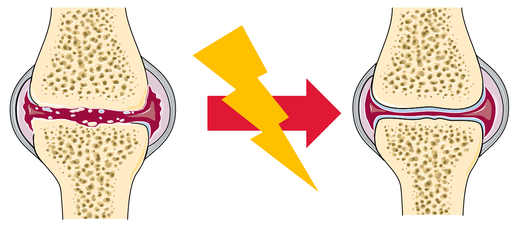
AIMS

1. Optimization of a non-viral delivery system for siRNA



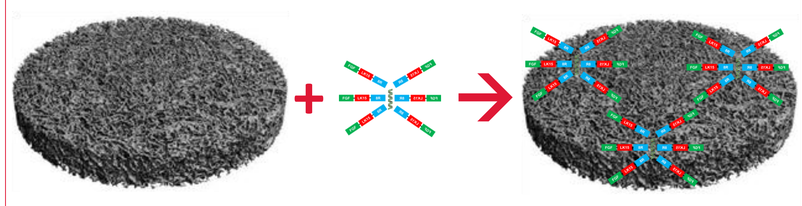
- Physicochemical characterisation
- Demonstrate protection of siRNA in presence of serum nucleases
- Assess 2D uptake & transfection efficiency

2. Validation of therapeutic siRNA in OA simulated conditions



- Assess knockdown at mRNA & protein level
- Silence NF-KB pathway activation
- Assess downstream effects on "anti-chondrogenic" mediators and effectors

3. Development of advanced scaffold-based siRNA delivery system



- Fabrication of gene-activated biomaterial-based delivery platform
- Assess therapeutic release rate
- Assess 3D uptake & transfection efficiency

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

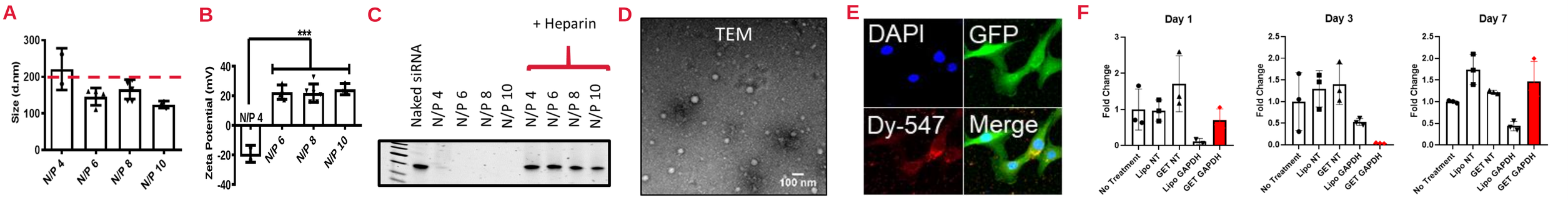


Figure 1: Dynamic light scattering (DLS) was used to determine (A) size and (B) zeta potential of varying formulations of GET-siRNA. (C) Gel retardation assay confirms successful encapsulation and release following heparin challenge. (D) TEM imaging of GET-siRNA N/P 6 indicates spherical morphology and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

(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

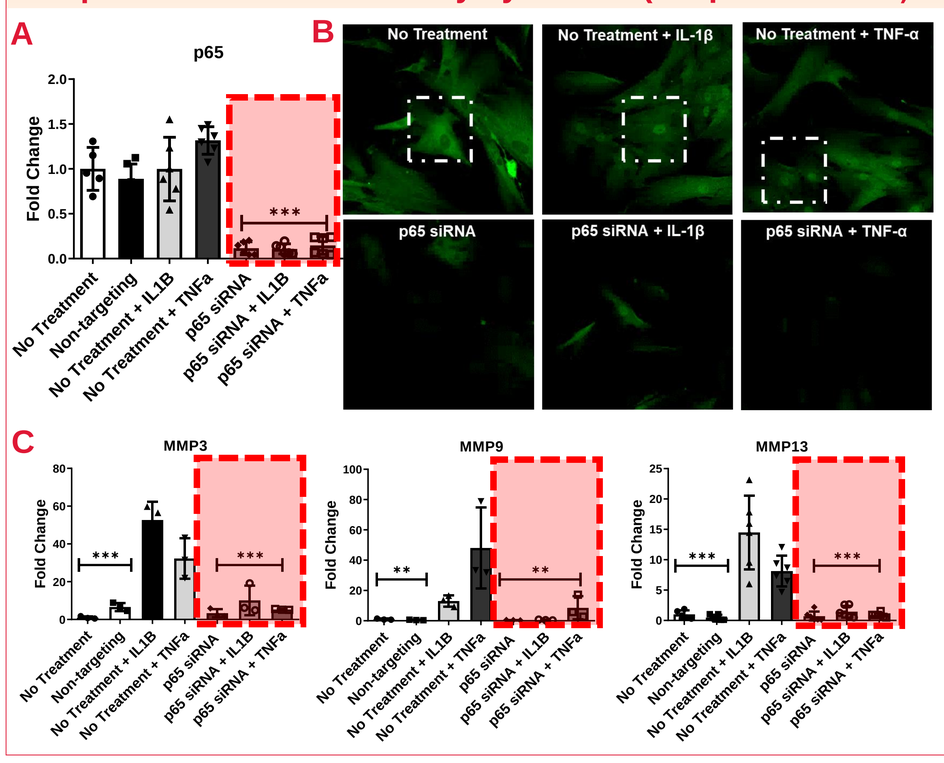


Figure 2: (A) PCR analysis of p65 mRNA expression levels in stimulated cytokine media & following GET-siRNA p65 silencing. (B) Ab staining indicates that p65 silencing inhibits expression and pro-inflammatory cytokine induced nuclear translocation. (C) PCR analysis of the mRNA expression levels of catabolic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

Coll-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

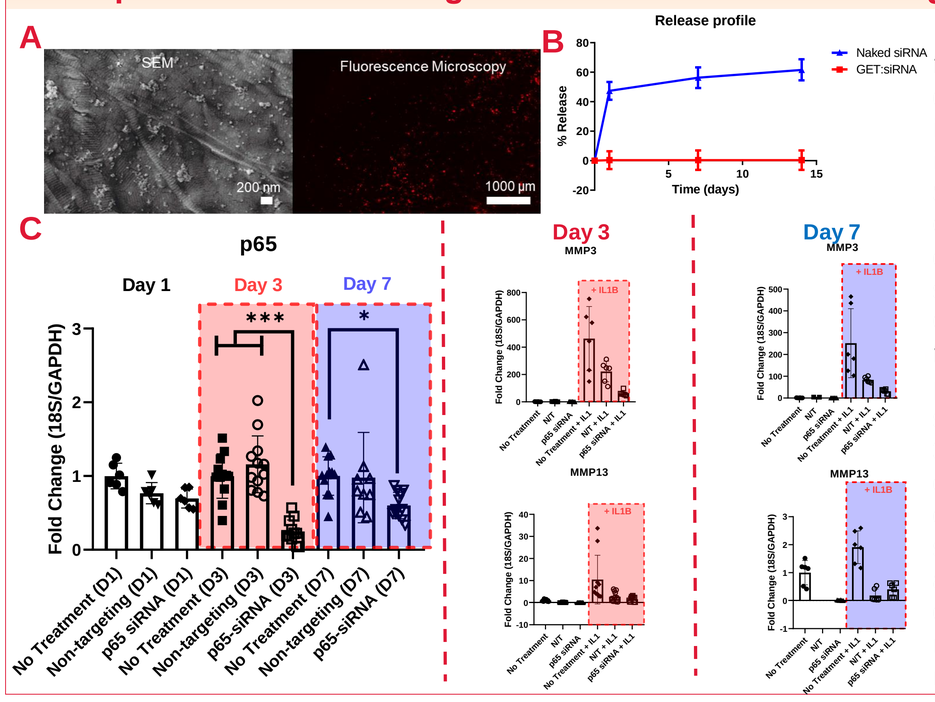


Figure 3: (A) SEM and fluorescence microscopy demonstrate successful incorporation of GET-siRNA into scaffods. (B) Successful retention of GET complexed siRNA compared to uncomplexed soak-loaded siRNA. (C) Gene-activated scaffolds facilitated knockdown of the p65 subunit with highest silencing observed 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.

CONCLUSION

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.

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