Rheumatoid arthritis (RA) is an autoimmune disorder of the joint characterised by inflammation, synovial hyperplasia and increased vascularisation (Figure 1). Synovial fibroblasts are aggressive cells in RA pathogenesis, contributing to inflammation and cartilage degradation. Current treatment with disease modifying anti-rheumatic drugs increases the risk of serious infection. Further, some patients do not respond to drugs whilst others develop resistance leading to loss of efficacy. Hence, we aim to develop a gene therapy that modulates RA disease without life-threatening immunosuppression.

Our approach involves developing non-viral vectors called receptor-targeted nanoparticles (RTNs) to selectively deliver therapeutic genes to the inflamed synovium, thereby improving efficacy and reducing systemic side-effects. The RTN comprise a cationic lipid that self-assembles with the anionic DNA backbone and a neutral lipid DOPE to aid DNA endosomal escape (Figure 2). In addition, formulations include a peptide containing a cationic, 16-lysine domain for electrostatic DNA packaging and a synoviocyte targeting ligand to provide selective uptake of synoviocytes into synovioocytes, separated by a cleavable or hydrophobic linker to alter RTN stability in the endosome (Table 1). Further, we aim to develop a DNA vector that provides high and sustained therapeutic gene expression while minimising inflammatory response based on mini-intronic plasmids (MIPs). MIPs lack bacterial propagation sequences, making them safer and potentially less inflammatory, and have been shown previously to provide greater and more prolonged gene expression than conventional plasmids [1].

### Methods

#### Receptor targeted nanoparticles (RTNs)

RTNs were comprised of luciferase or eGFP reporter plasmid cargo encapsulated with lipid and peptide. RTNs were optimised for delivery to HIG-82 synoviocytes. Optimised RTNs were formulated of the lipids DOTMA and DOPE (1:1 ratio), a peptide (Table 1) and plasmid DNA at a ratio of 1:6 lipid: peptide: DNA.

#### RTN cell transfection protocol

- **Cells seeded**: 6, 24 and 48 hours
- **RTNs formulated and delivered to cells in low serum media**: RTNs replaced with cationic growth media
- **Luminescence/luciferase reporter** (in nanoluminescence units [NLU]) of GFP+GFP/viral reporter (measured)

#### Results

RTNs containing synoviocyte targeting peptide (KS-34) efficiently transfect HIG-82 synoviocytes and peptides with cleavable linkers give superior transfection efficiency to hydrophobic RTNs containing synoviocyte targeting peptide efficiently transfect synoviocytes, but show poorer efficiency in chondrocytes and hepatocytes, as compared to positive control

### References


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**Figure 1**: Pathophysiological changes to the joint during rheumatoid arthritis.

**Figure 2**: RTNs were comprised of luciferase or eGFP reporter plasmid cargo encapsulated with lipid and peptide. RTNs were optimised for delivery to HIG-82 synoviocytes. Optimised RTNs were formulated of the lipids DOTMA and DOPE (1:1 ratio), a peptide (Table 1) and plasmid DNA at a ratio of 1:6 lipid: peptide: DNA.

**Figure 3**: C4 cells were HIG-82 (rabbit synovium), C28/2 (human chondrocyte) or HepG2 (human hepatocyte). For transfection cells were seeded into 96-well plates (KS-16, KS-34 or KS-48 well plates). RTNs were formulated as per the RTN protocol (Figure 2) with 100 ng of luciferase or 500 ng of GFP DNA/well. Luminescence measured with a plate reader and GFP with flow cytometry as measures of transfection efficiency.

**Figure 4**: Comparison of A) different peptides and B) different peptide linkers used in C8/D8 RTNs for delivery of a luciferase reporter plasmid to HIG-82 cells. RLU/μg = relative luciferase units per μg protein. * p-value < 0.05 as determined by one-way ANOVA with Tukey’s multiple comparisons.

**Figure 5**: Number of GFP+ GFP of HIG-82, C28/2 and HepG2 cells 48 hours post-transfection of an eGFP reporter plasmid with KG-32, KS-34 or KG-34 RTNs, or untransfected (0 μl) cells. RTNs with synoviocyte peptide KS-34 showed good transfection efficiency in HIG-82 synoviocytes compared to positive control KG-32. Conversely, KS-34 RTNs yielded much poorer transfection efficiency in chondrocytes and hepatocytes compared to RTNs with positive control peptide, which transfected all cell types, indicating a degree of targeting specificity for synoviocytes. * p-value < 0.05 as determined by one-way ANOVA with Tukey’s multiple comparisons.

**Figure 6**: A luciferase reporter plasmid was delivered to HIG-82s, C28/2s and HepG2s with RTNs as in Figure 5. Results are consistent with eGFP transfections; synoviocyte targeting peptides (KS) showed good transfection in HIG-82s, but not C28/2s or HepG2s and positive control (KS) peptides showed good transfection efficiency in all cell types. This trend was seen for peptides with both cleavable and hydrophobic linkers. * p-value < 0.05 as determined by one-way ANOVA with Tukey’s multiple comparisons.

**Figure 7**: Cloning protocol used to produce 3 plasmids containing a firefly luciferase reporter gene and either a CMV or an EF1α promoter.

**Figure 8**: Luciferase activity of HIG-82 cells 24-72 hours post-transfection with RTNs delivering MIPs with either a CMV (A) or an EF1α (B) promoter as compared to equivalent ratios of conventional plasmids (pCI) containing an identical promoter and luciferase gene. Neither MIP construct showed a clear benefit over the corresponding pCI plasmid on transfection efficiency.

**Figure 9**: Luciferase activity of KG-32 cells 24-72 hours post-transfection with RTNs delivering MIPs with either a CMV (A) or an EF1α (B) promoter as compared to equivalent ratios of conventional plasmids (pCI) containing an identical promoter and luciferase gene. Neither MIP construct showed a clear benefit over the corresponding pCI plasmid on transfection efficiency.

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**Conclusion**

- Synoviocyte targeting RTNs (KS-34 peptide) show similar or superior transfection efficiency in HIG-82 cells to positive control RTNs previously demonstrating good efficiency in multiple cell types. However, KS-34 RTNs provide the additional benefit of synoviocyte selectivity, providing the basis for a targeted gene therapy.
- RTNs containing peptides with cleavable linkers showed superior transfection efficiency to their hydrophobic counterparts, regardless of targeting sequence, presumably due to improved endosomal escape.
- Contrary to results previously reported [1], the MIPs produced here showed no benefit on the amount or length of transgene expression over conventional plasmids following RTN delivery to HIG-82 cells.