Targeted Non-Viral Delivery of Mini-Intronic Plasmids for Rheumatoid Arthritis

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Introduction

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 (RTN) 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 nanoparticles into synoviocytes, 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].

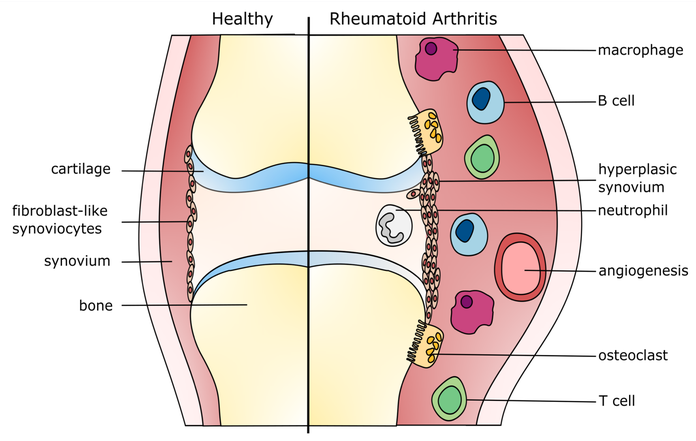

Figure 1: Pathophysiological changes to the joint during rheumatoid arthritis.

Methods

Receptor targeted nanoparticles (RTNs)

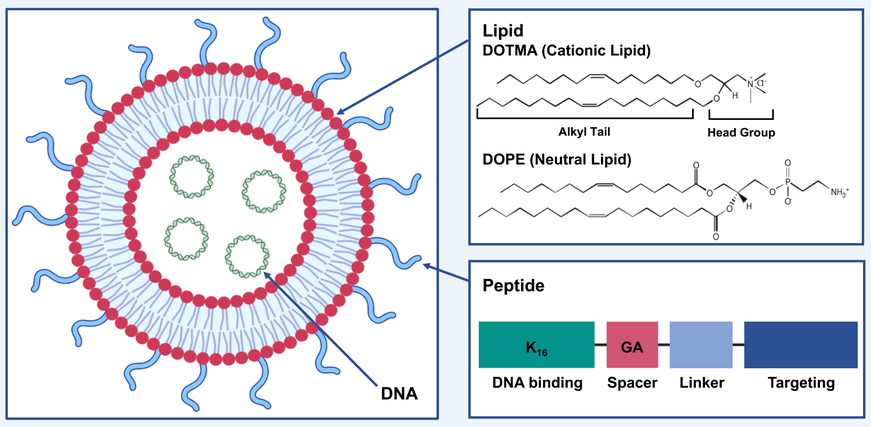

Figure 2: RTNs were comprised of luciferase or eGFP reporter plasmid cargo encapsulated with lipid and peptide. RTNs were optimised for delivery to HIG-82 synoviocytes. Optimised RTNs were formulated of the lipids DOTMA and DOPE (1:1 ratio), a peptide (Table 1) and plasmid DNA at a ratio of 1:4:1 lipid: peptide: DNA.

Peptide Name	Description	Linker	Peptide Sequence
ME27	Positive control, integrin binding	Cleavable	K_{16}RVRRGACRGDCLG
Pep E	Positive control	No linker	K_{16}GACSERSMNFCG
KG-32	Positive control	Cleavable	K_{16}RVRRGACYGLPHKFCG
KG-31	Positive control	Hydrophobic	K_{16}XSXGACYGLPHKFCG
KS-34	**Synoviocyte targeting**	**Cleavable**	**K_{16}RVRRGASFHQFARATLAS**
KS-33	**Synoviocyte targeting**	**Hydrophobic**	**K_{16}XSXGASFHQFARATLAS**
KT-34	Negative control	Cleavable	K_{16}RVRRGAARPLEHGSDKAT
KT-33	Negative control	Hydrophobic	K_{16}XSXGAARPLEHGSDKAT

Table 1: Peptides consist of a 16 lysine DNA binding domain (green), a linker region (pink), a spacer (pale blue), and a targeting sequence (dark blue). Linkers are RVRR (cleavable – furin and cathepsin B consensus sequences) and XSX (hydrophobic), where X is epsilon amino hexanoic acid. Synoviocyte targeting peptides are shown in bold, where the targeting sequence was taken from Mi *et al* (2003) [2].

RTN cell transfection protocol

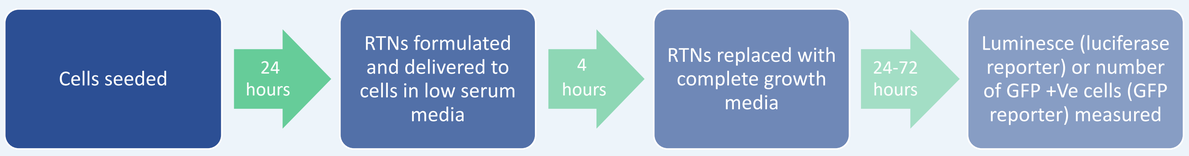

Figure 3: Cell lines were HIG-82 (rabbit synoviocyte), C28/I2 (human chondrocyte) or HepG2 (human hepatocyte). For transfection cells were seeded into 96 (luciferase) or 24 (eGFP) well plates. RTNs were formulated as per Figure 1 with 250 ng (luciferase) or 500 ng (GFP) DNA/well. Luminescence measured with a plate reader and GFP with flow cytometry as measures of transfection efficiency.

Results

RTNs containing synoviocyte targeting peptide (KS-34) efficiently transfect HIG-82 synoviocytes and peptides with cleavable linkers give superior transfection efficiency to hydrophobic

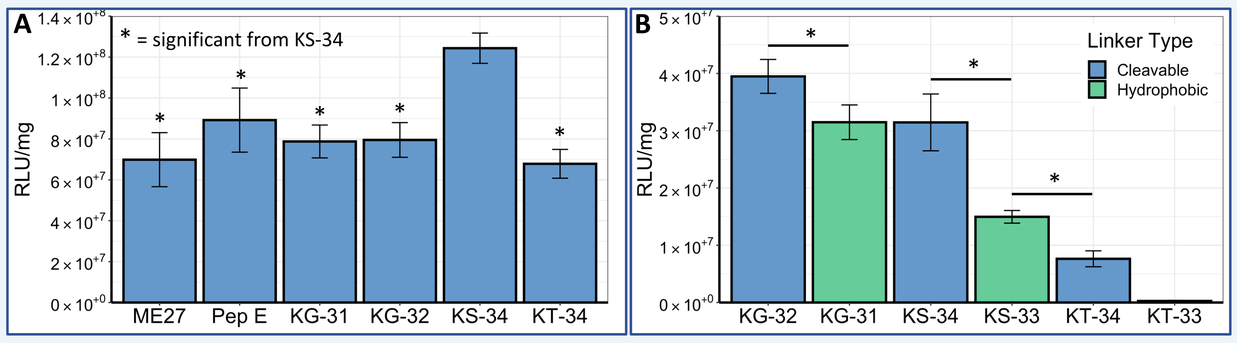

Figure 4: Comparison of A) different peptides and B) different peptide linkers used in C18/DOPE RTNs for delivery of a luciferase reporter plasmid to HIG-82 cells. RLU/mg = relative luciferase units per mg protein. * = p-value <0.05 as determined by one-way ANOVA with Tukey's multiple comparisons.

RTNs containing synoviocyte targeting peptide efficiently transfect synoviocytes, but show poorer efficiency in chondrocytes and hepatocytes, as compared to positive control

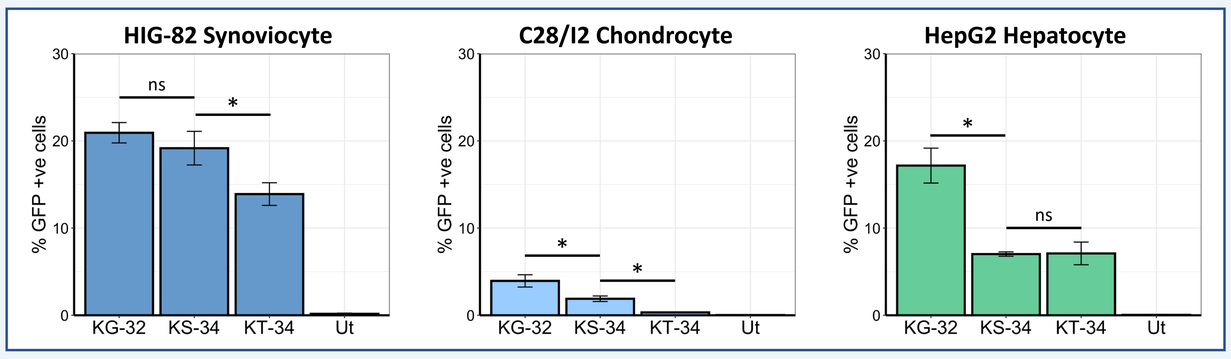

Figure 5: Number of GFP +ve HIG-82, C28/I2 and HepG2 cells 48 hours post-transfection of an eGFP reporter plasmid with KG-32, KS-34 or KT-34 RTNs, or untransfected (Ut) cells. RTNs with synoviocyte peptide KS-34 showed good transfection efficiency in HIG-82 synoviocytes compared to positive control KG-32. Conversely, KS-34 RTNs yielded much poorer transfection efficiency in chondrocytes and hepatocytes compared to RTNs with positive control peptides, 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.

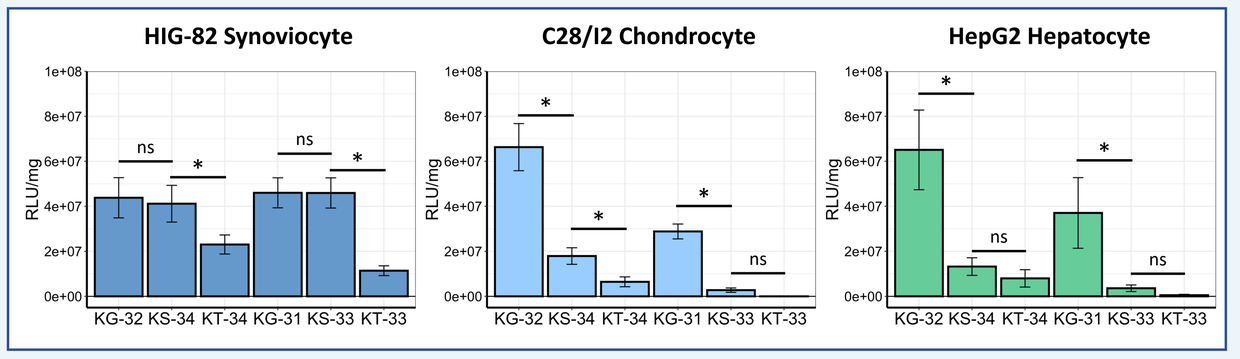

Figure 6: A luciferase reporter plasmid was delivered to HIG-82s, C28/I2s and HEPG2s with RTNs as in figure 5. Results are consistent with eGFP transfections; synoviocyte targeting peptides (KS) showed good transfection in HIG-82s, but not C28/I2s or HepG2s and positive control (KG) peptides showed good 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.

Mini-intronic plasmid (MIP) cloning protocol

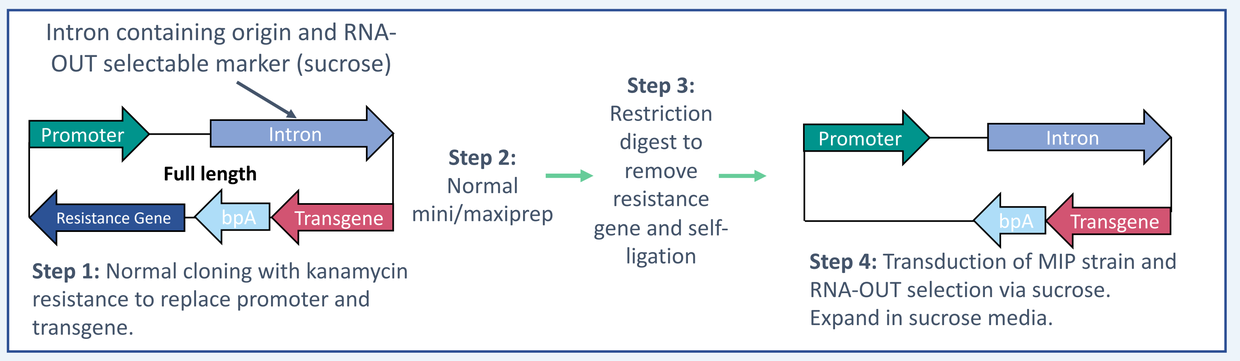

Figure 7: Cloning protocol used to produce 2 MIPs containing a firefly luciferase reporter gene and either a CMV, or an EF1α promoter.

RTN transfection of HIG-82 synoviocytes with MIPs

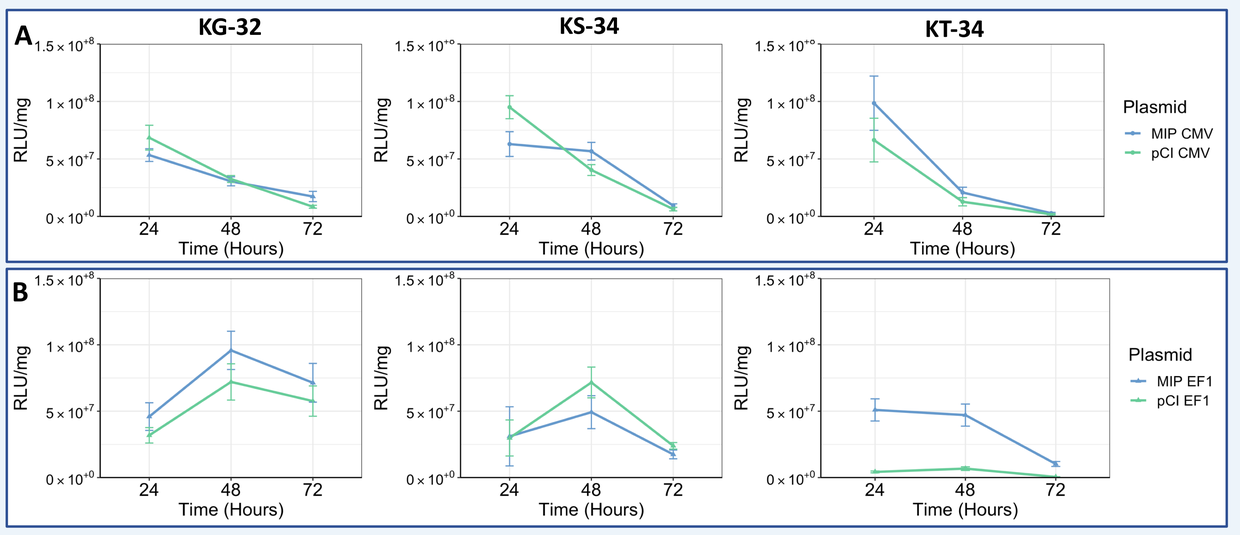

Figure 8: Luciferase activity of HIG-82 cells 24-72 hours post-transfection with RTNs delivering MIPs with either a CMV (A) or an EF1α (B) promoter as compared to equimolar ratios of conventional plasmids (pCI) containing an identical promoter and luciferase gene. Neither MIP constructs showed a clear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

RTN transfection of HEK293Ts with MIPs

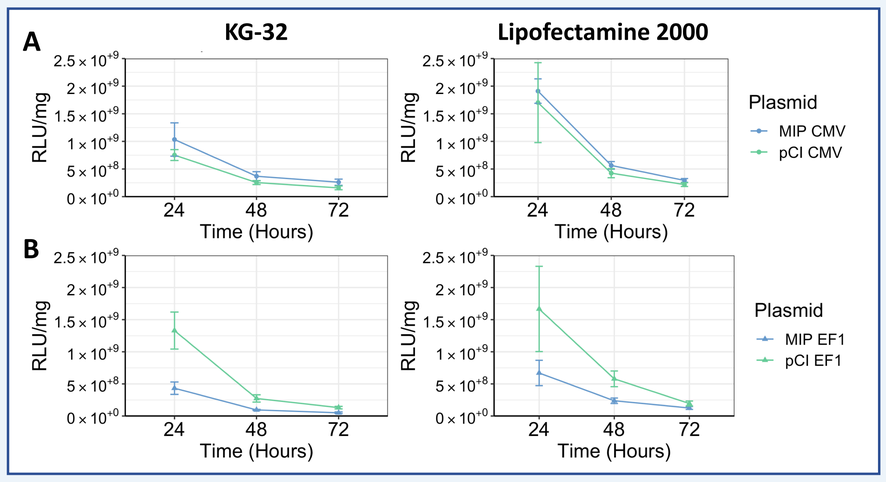

Figure 9: MIPs and pCI plasmids were delivered to HEK293T cells as in figure 8 with RTNs, as well as with the commercially available lipid transfection reagent lipofectamine 2000 to recapitulate the conditions used successfully by Lu *et al* [1]. Again, there was no clear benefit in transfection efficiency of MIPs over their plasmid counterpart at any time point, and MIP EF1α actually performed worse than pCI EF1α.

Conclusions

- 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.

References

[1] Lu, J., Zhang, F. & Kay, M. A. Mol. Ther. 21, 954–963 (2013)
[2] Mi, Z. et al. Mol. Ther. 8, 295–305 (2003)