Development of a novel process to create an effective 'off the shelf' gene therapeutic for incorporation into bioinks for 3D printing

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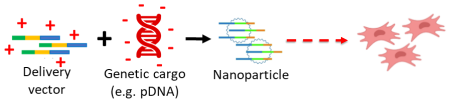
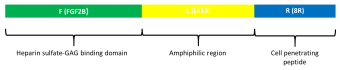
Introduction

- Regeneration of articular cartilage remains challenging due to its avascular and aneural nature and while some success in the repair of small cartilage defects has been reported with biomaterial scaffolds alone[1], **large cartilage defects** require additional intervention[2,3]
- 3D printed advanced bio-implants functionalised with cells or **therapeutic nucleic acid loaded nanoparticles (NPs)** consisting of a **delivery vector** complexed to **plasmid DNA (pDNA)** to promote cartilage repair provide a potential solution[4,5]



- Viral vectors have traditionally resulted in higher, more stable transfection than non-viral vectors but have associated safety concerns – as transient gene expression is sufficient for this application, safer **non-viral delivery vectors** were investigated
- The **glycosaminoglycan enhanced transduction (GET) peptide** has previously shown promise as a non-viral delivery vector in our research group[3] – it consists of 3 subunits; a heparin sulfate-GAG binding domain, an amphiphilic region which binds nucleic acids and a cell penetrating peptide



- Formulating these NPs as an **'off the shelf' preparation** for incorporation into bioinks improves the efficiency of the 3D printing process by negating the need to complex freshly prepared NPs prior to each print
- **Lyophilisation** will be investigated as a potential method to achieve this aim

Aims

Overarching aim:
Develop a novel process to create an effective 'off the shelf' gene therapeutic for incorporation into bioinks

Specific aim 1: Investigate the effects of lyophilisation on the size and charge of a GET and pDNA NP

Specific aim 2: Assess the transfection efficiency of lyophilised NPs in MSCs and their effects on cell metabolic activity

Specific aim 3: Assess the stability of the lyophilised NPs over time to determine a shelf life

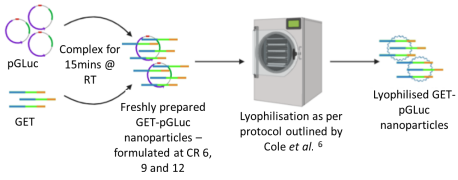
Specific aim 4: Formulate lyophilised NPs consisting of the GET peptide and therapeutic pDNA for cartilage repair (e.g. SOX trio)

Future Work

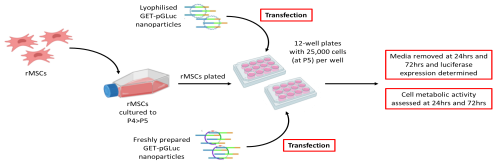
Future work will include lyophilisation cycle optimisation and stability studies. NPs formulated with therapeutic pDNA (e.g. SOX trio) will be assessed for their effect on cartilage repair.

Methods

1. NPs consisting of the GET peptide and pDNA for the reporter protein Gaussia luciferase (pGLuc) were formulated at increasing charge ratios (CRs) of peptide to pDNA based on their electrostatic interactions, of 6, 9 and 12. These NPs were then lyophilised in a Christ Epsilon benchtop freeze dryer using 5% trehalose as a lyoprotectant, as per the protocol outlined by Cole *et al.*[6]



2. NP size and polydispersity was measured via dynamic light scattering and NP zeta potential was measured via electrophoretic light scattering using the Zetasizer 3000 HS (Malvern).
3. Rat MSCs were transfected with both lyophilised and freshly complexed NPs to determine if the lyophilisation process affected transfection efficiency of the NPs. Cell metabolic activity and luciferase expression were determined at 24hrs and 72hrs post-transfection respectively.



Results

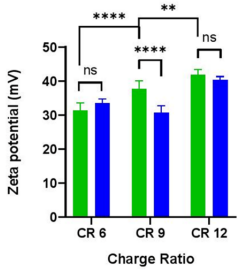


Figure 1: Effect of lyophilisation on NP charge

Outcome 1: The lyophilisation process has no significant effect on NP charge at CR 6 or CR 12

A significant difference of -7mV was noted at CR 9 however the NP still retained its overall cationic charge and this difference not correlate with a decrease in gene expression (see figure 4). Therefore this difference in charge did not affect cellular uptake of the NPs.

NP surface charge is a key determinant of cellular uptake with positively charged NPs, such as the GET-pDNA NPs, displaying improved cellular uptake compared to those with a negative charge[7].

References

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Results

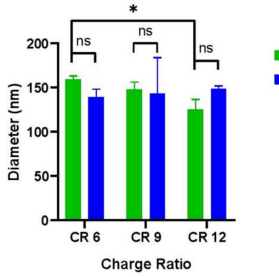

Figure 2: Effect of lyophilisation on NP size

Outcome 2: The lyophilisation process has no significant effect on NP size

This is an important finding as NP size is also a key factor in determining cellular uptake, influencing both if and how the NP enters the cell.

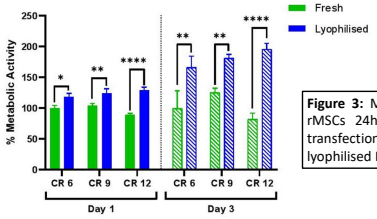

Figure 3: Metabolic activity of rMSCs 24hrs and 72hrs post transfection with fresh and lyophilised NPs

Outcome 3: There was a marked increase in the metabolic activity of the cells following transfection with the lyophilised NPs

This phenomenon may be partly attributed to the use of the sugar trehalose as a lyoprotectant, but indicates that the NPs are non-toxic to the cells.

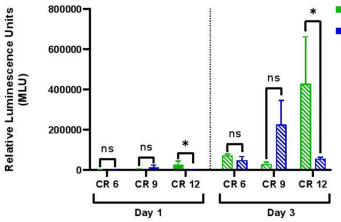

Figure 4: Transfection efficiency of fresh and lyophilised NPs 24hrs and 72hrs post transfection

Outcome 4: Comparable levels of transfection efficiency were achieved with freshly prepared and lyophilised NPs at CR 6 and CR 9

This indicates that the lyophilisation process does not affect the transfection efficiency of the NPs at lower charge ratios, however further optimisation will be required for higher CRs.

Conclusion and Impact

These results demonstrate that lyophilised **NPs are non-toxic** and can display **comparable levels of transfection efficiency** to their freshly prepared counterparts at lower CRs – paving the way for development of an 'off the shelf' gene therapeutic for incorporation into bioinks. These findings show promise in facilitating the development of a novel method to **enable long-term, stable storage of gene therapeutics** – an important step in enabling the clinical translation of gene therapeutic products for various tissue engineering applications.

Acknowledgements

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