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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Background: Articular cartilage facilitates the frictionless movement of synovial joints, however due to its avascular and aneural nature it has a limited ability to self-repair. While some success in the repair of small cartilage defects has been reported with the use of biomaterial scaffolds alone, larger defects often require additional therapeutic intervention. The development of 3D printed biomaterial scaffolds functionalised with cells or nucleic acid loaded nanoparticles (NPs) provide a potential solution and thus there is now an increased focus on the formulation of novel cell-laden and gene-activated bioinks for cartilage repair. The overarching aim of this study is to develop a novel process to create an 'off the shelf' gene therapeutic consisting of a non-viral delivery vector and therapeutic plasmid DNA (pDNA) NP which can be pre-prepared for incorporation into bioinks. This would negate the need to complex fresh NPs immediately prior to each print. This gene-activated bioink will then ultimately be used to 3D print an advanced bio-implant for cartilage repair.

Methods: In order to achieve an 'off the shelf' NP formulation, lyophilisation was investigated as a potential solution. NPs consisting of the non-viral delivery vector glycosaminoglycan enhanced transduction (GET) peptide and pDNA for the reporter protein Gaussia luciferase (pGLuc) were formulated at increasing ratios of peptide to pDNA based on their electrostatic interactions, defined as charge ratio (CR), of 6, 9 and 12. These NPs were formulated with 5% trehalose as a lyoprotectant, then lyophilised in a Christ Epsilon benchtop freeze dryer and 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, UK). Rat mesenchymal stem cells (rMSCs) were then transfected with both lyophilised NPs (L-NPs) and freshly complexed NPs (F-NPs) to determine if the lyophilisation process affected transfection efficiency of the NPs. Cell metabolic activity was assessed using the alamarBlue® assay (Thermo Fisher Scientific) and transfection efficiency was determined using the Pierce™ Gaussia Luciferase Flash Assay Kit (Thermo Fisher Scientific) at 24hrs and 72hrs post-transfection respectively.

Results: There was no significant difference in size between L-NPs and F-NPs at all CRs and there was no significant difference in zeta potential between L-NPs and F-NPs at CR 6 or CR 12. A significant difference in zeta potential of -7mV was observed at CR 9, however this did not correlate with a decrease in gene expression. Transfection with L-NPs resulted in a significant increase in rMSC metabolic activity, a finding which may be attributed to the use of trehalose as a lyoprotectant. We were able to obtain comparable levels of transfection efficiency with L-NPs and F-NPs at CR 6 and CR 9. Further optimisation should be carried out for the formulation at a higher CR and future work will also include stability studies.

Conclusions: This study has demonstrated that lyophilisation shows promise in enabling stable storage of gene therapeutic nanoparticles without affecting cellular uptake. (Acknowledgements – Funding: ReCAP: ERC Advanced Grant number 788753).