Combined biolistic and cell penetrating delivery for an effective and scalable intradermal DNA vaccine

Roizza Beth So[1], Gang Li[1], Victoria Brentville[2], Janet M. Daly[3], James E. Dixon1,[4]

[1] Regenerative Medicine & Cellular Therapies (RMCT), Biodiscovery Institute (BDI), School of Pharmacy, University of Nottingham, Nottingham, NG7 2RD, UK. [2] Scancell Ltd, University of Nottingham Biodiscovery Institute, University Park, Nottingham, NG7 2RD, UK. [3] One Virology, Wolfson Centre for Global Virus Research, School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington, Loughborough, LE12 5RD, UK. [4] NIHR Nottingham Biomedical Research Centre, University of Nottingham, Nottingham, UK.

Background: Physical-based gene delivery via biolistic methods involves precipitation of nucleic acids onto microparticles and direct transfection through membranes into cells in exposed tissue (e.g. skin) by high velocity acceleration, such as with the Helios gene gun system employing gold microparticles (MPs). The glycosaminoglycan (GAG)-binding enhanced transduction (GET) system exploits novel fusion peptides consisting of cell-binding, nucleic acid condensing, and cell-penetrating domains, which enable enhanced transfection across multiple cell types. In this study, we combined chemical (GET) and physical (gene gun) DNA delivery systems and hypothesized the combination would generate enhanced distribution and effective uptake in cells not initially transfected by biolistic penetration.

Methods: Initial transfection studies explored various formulations to determine the optimal GET-gold MP-DNA ratio. The next stage involved incorporating the GET-gold formulation into fireable gene gun bullets. Subsequent experiments explored the physicochemical characterization, optimisation of bullet contents and transfection experiments *in vitro* (monolayers, engineered tissue) and *in vivo*.

Results: Transfection experiments in cell monolayers and engineered tissue demonstrated these formulations transfected efficiently, including in DC2.4 dendritic cells. We incorporated these formulations into a biolistic format for gene gun by forming fireable dry bullets obtained via lyophilization (freeze drying). This system is simple and has enhanced scalability compared to conventional methods to generate bullets. Flushed GET bullet contents retained their ability to mediate transfection (17-fold and 13-fold greater reporter gene expression than standard spermidine bullets in the absence and presence of serum, respectively). Fired GET bullets in cells, collagen gels and mice showed increased reporter gene transfection compared to untreated controls, whilst maintaining cell viability *in vitro* and having no obvious toxicity *in vivo*. Lastly, a SARS-CoV-2 plasmid DNA vaccine with spike (S) protein-receptor binding domain (S-RBD) was delivered by gene gun using GET bullets. Specific T cell and antibody responses comparable to the conventional system were generated.

Conclusions: The non-physical and physical combination of GET-gold-DNA carriers using gene gun showed potential as an alternative DNA delivery method that is scalable for mass deployed vaccination and intradermal gene delivery.