Using Genetically Engineered Induced Pluripotent Stem Cell Derived Neural Stem Cells (iPSC-NSC) as Therapy for Ischemic Stroke.

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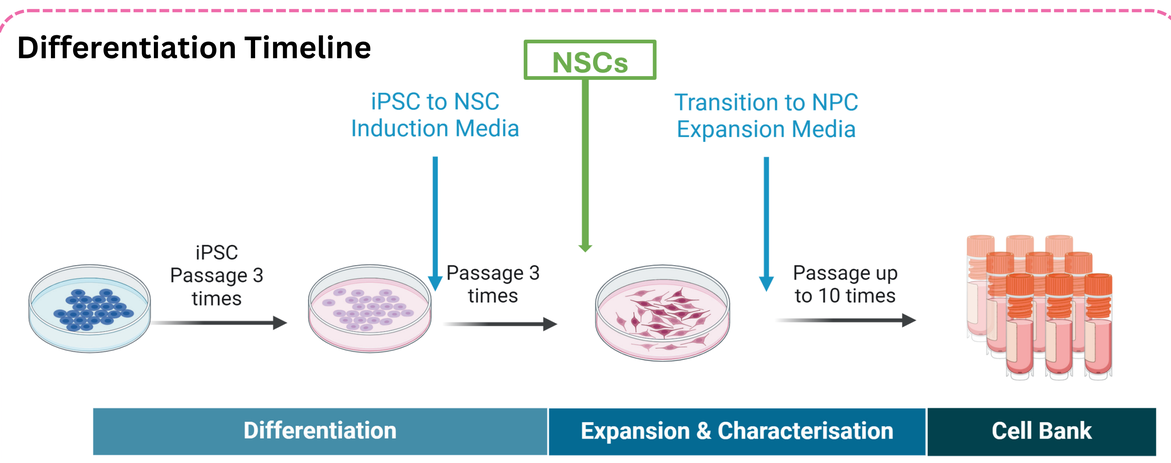
Background & Aim

- There are no neuroprotective or neuroregenerative therapeutic options for ischaemic stroke.
- Neural stem cells (NSC) are unique in their inherent ability to differentiate into functional neurons, but have limited migratory and survival capacities. Genetic engineering may help NSCs overcome these limitations and enhance their therapeutic potential.
- Intra-arterial (IA) delivery is advantageous for clinical translation as it is less invasive and fits in with clinical treatment workflow.

Aim: To investigate whether genetically modified NSCs could be an effective adjunct therapy for ischemic stroke.

Methods and Results

Step 1: Culturing iPSC derived NSCs



Characterisation

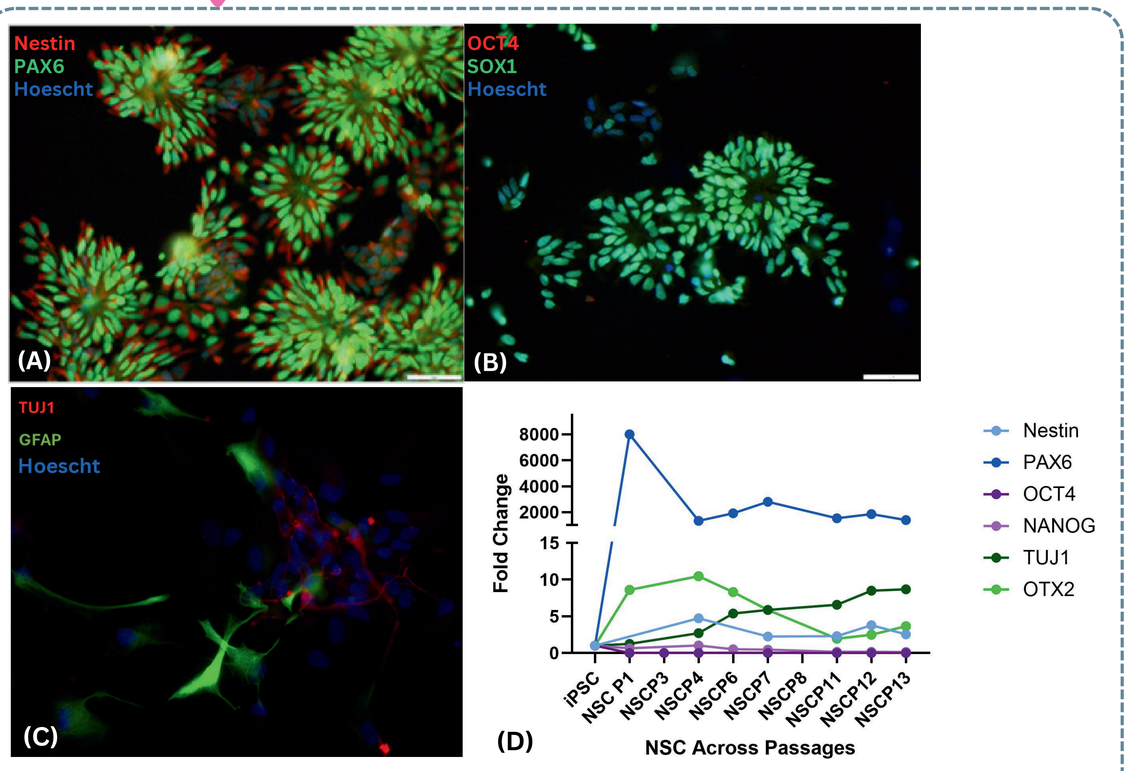
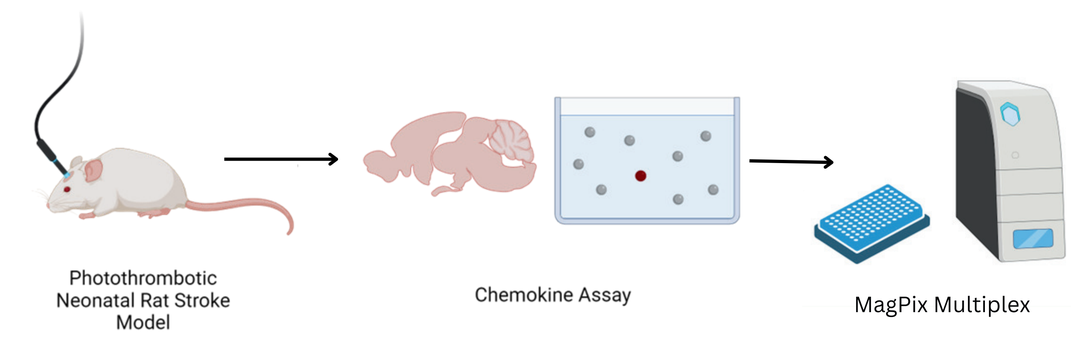


Figure 1: Characterisation of iPSC-NSCs. (A&B) NSC immunofluorescence. (C) Downstream differentiation. (D) qPCR of NSCs across passages.

Step 2: Genetically modifying iPSC-NSCs



Target Gene Selection

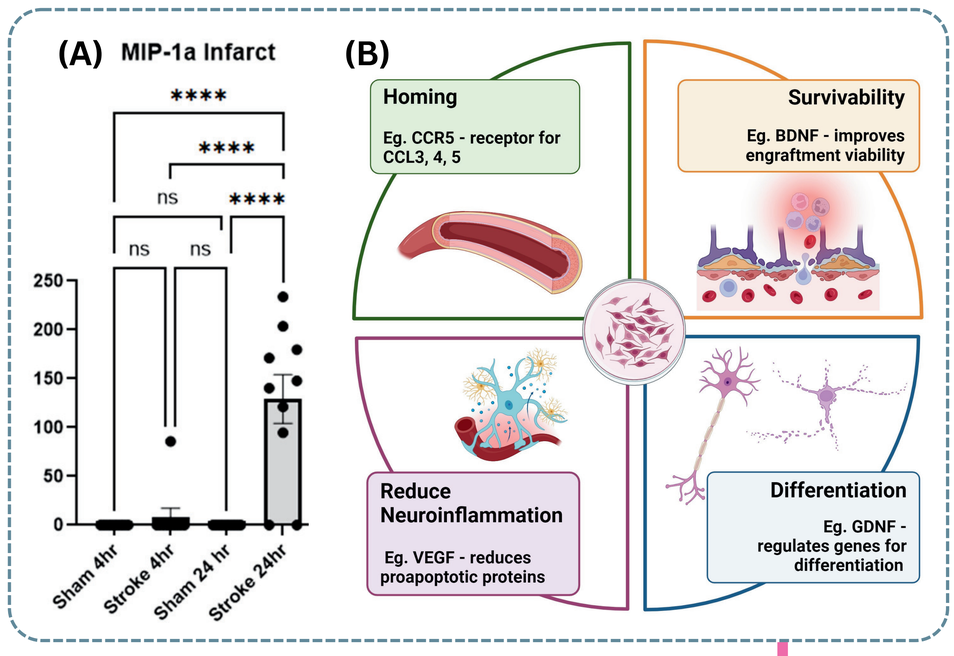


Figure 2: (A) MagPix data. MIP-1a (CCL3) is significantly elevated in the stroke model at 24 hours. ****P < 0.0001. (B) Examples of genetic modifications to enhance different aspects of NSCs.

Lentivirus Transduction Validation

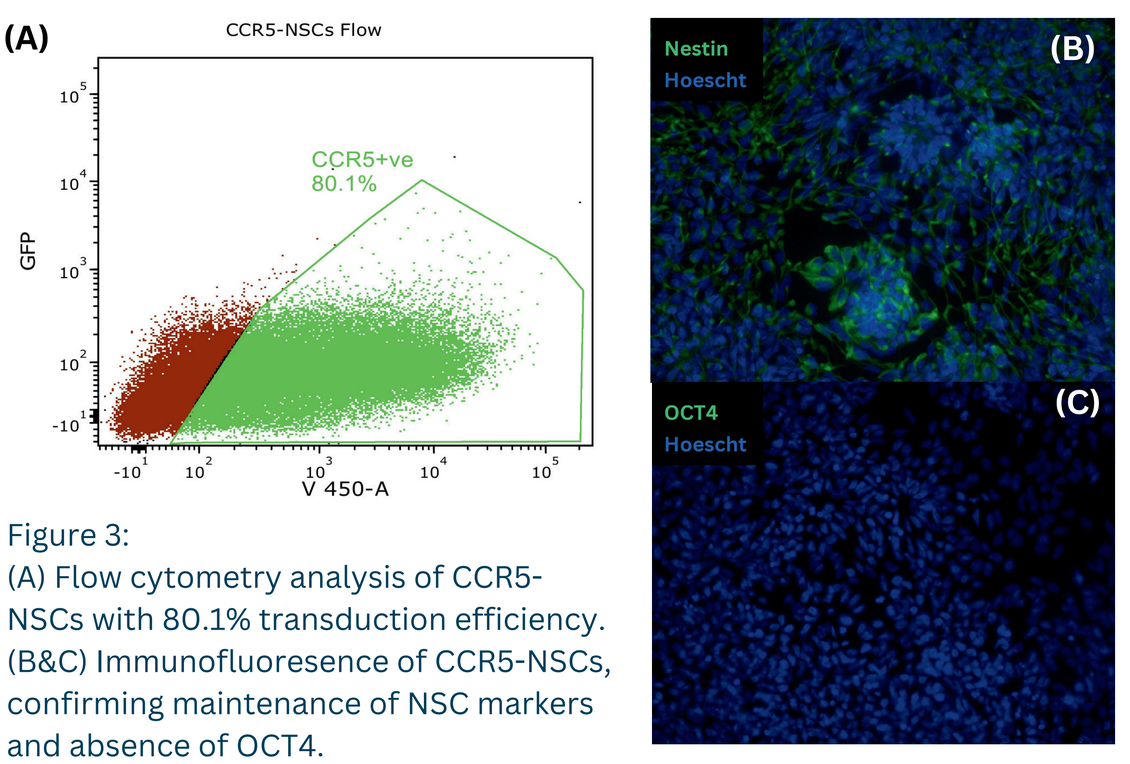
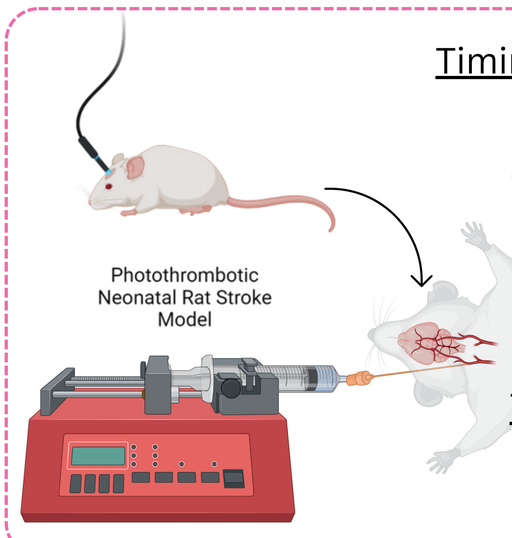


Figure 3:
(A) Flow cytometry analysis of CCR5-NSCs with 80.1% transduction efficiency. (B&C) Immunofluoresence of CCR5-NSCs, confirming maintenance of NSC markers and absence of OCT4.

Step 3: IA Delivery of cells & Therapeutic Efficacy



Timing of cell administration: 24-hours post-stroke.

Treatment groups: Modified NSCs, naive NSCs, vehicle and sham.

Treatment outcome assessment: histology and molecular studies of the brain and behavioural studies of animals.

Summary and Conclusions

We have successfully generated iPSC-NSCs and genetically modified them to enhance their therapeutic potential. We hope our *in vivo* results will show that genetically modified NSCs outperform their naive counterparts and improve stroke outcomes.

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