

# What happens to stem cells when we inject them into mice

## The fate of Mesenchymal Stem Cells after Intravenous Administration



Grace Dobbs – [o.g.dobbs1@ncl.ac.uk](mailto:o.g.dobbs1@ncl.ac.uk) 140037324 – BSc Biomedical Sciences  
Supervised by Tracy Heng and Senora Mendonca



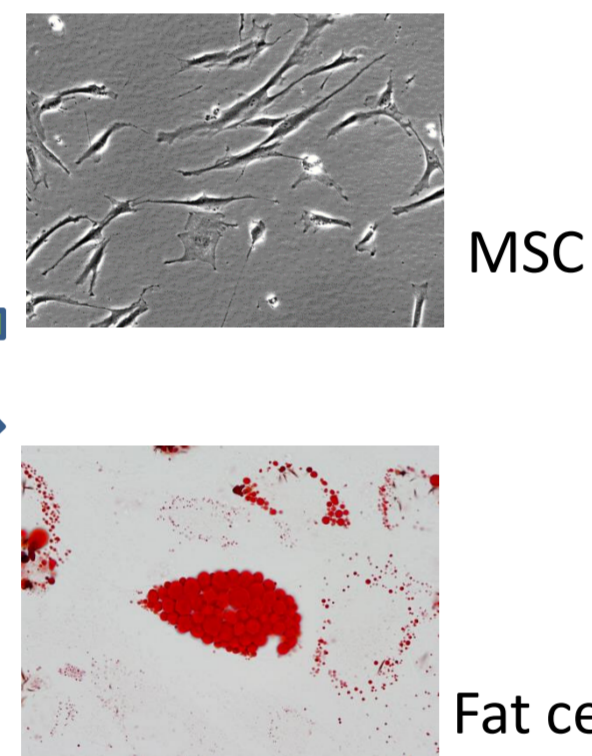
### Introduction

Mesenchymal stem cells (MSCs) are a type of multipotent stromal (connective) cell found in the bone marrow<sup>1</sup>

They can be characterised by :

- Their ability to adhere to plastic
- Expression of specific surface proteins
- Differentiation into osteoblasts (bone cells), adipocytes (fat cells) and chondroblasts (cartilage)<sup>2</sup>

Here the cell type changes showing differentiation



The research lab I joined focusses on the promise of MSCs in the treatment of asthma. Previous experiments have shown that after injecting human MSCs into an asthma mouse model, they will localise to the lungs and suppress airway inflammation. My work is an expansion of this previous study by researching if mouse MSCs behave in a similar way to human MSCs once injected into the mouse.

### Aims

- Create the fluc-GFP plasmid which expresses the fluorescence gene needed for tracking
- Transfection of 293T cells with the fluc-GFP plasmid to produce virus
- Transduction of HeLa cells (control cells) with the fluc-GFP plasmid and create HeLa cells which express GFP and calculate the virus titre to work out how much virus is needed to infect our cells of interest
- Compare the fate of hMSC vs mMSC when injected

### Methods

Creating the pfluc-GFP plasmid

Preparation of agar plates and bacterial colonies

Picking of single bacteria colony and seed into media

Inoculate growing bacteria

Purify the DNA using Qiagen maxi prep kit

Sequence plasmid DNA

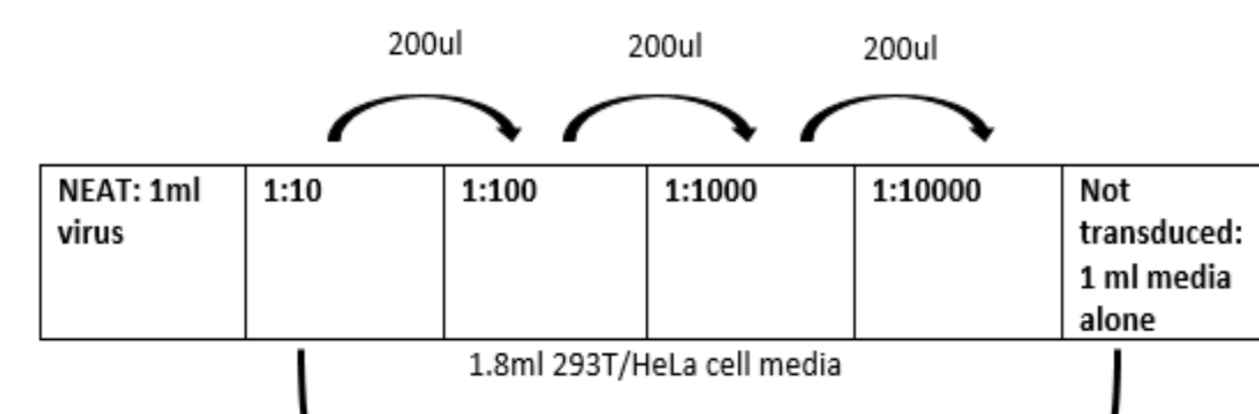
Transduction and transfection

Day before transfection plate  $5 \times 10^6$  293 T cells into a 10cm dish

Transfect 293 T cells with 3 plasmids; the pfluc plasmid we created, pSPAX2, pMD2G

Plate HeLa cells in a 6 well plate at 100,000 per well

Transduce HeLa cells with the viral supernatant collected from the transfected 293 T cells and titrate the virus across the wells

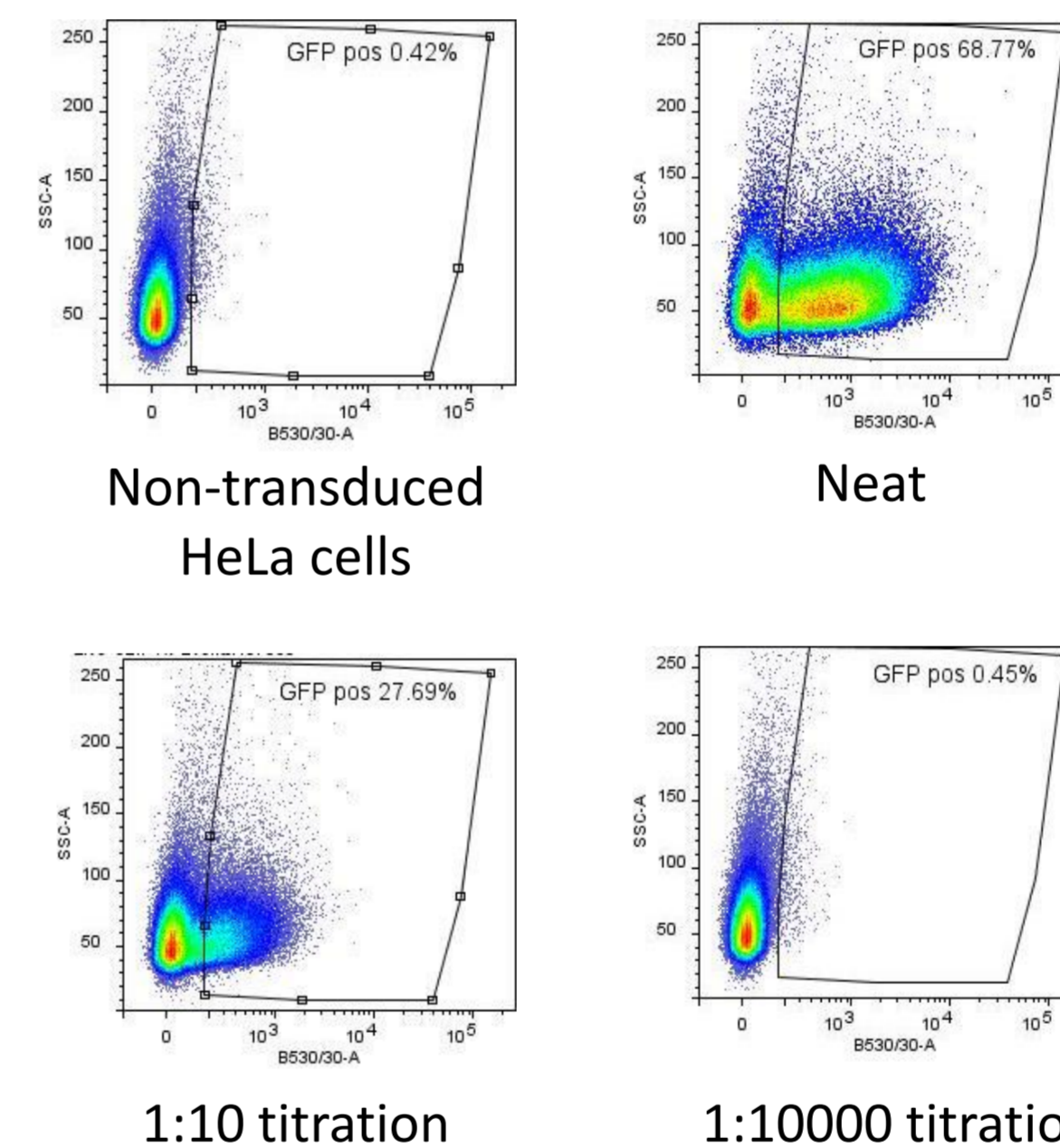


We also used this same method for the transduction of the hMSC

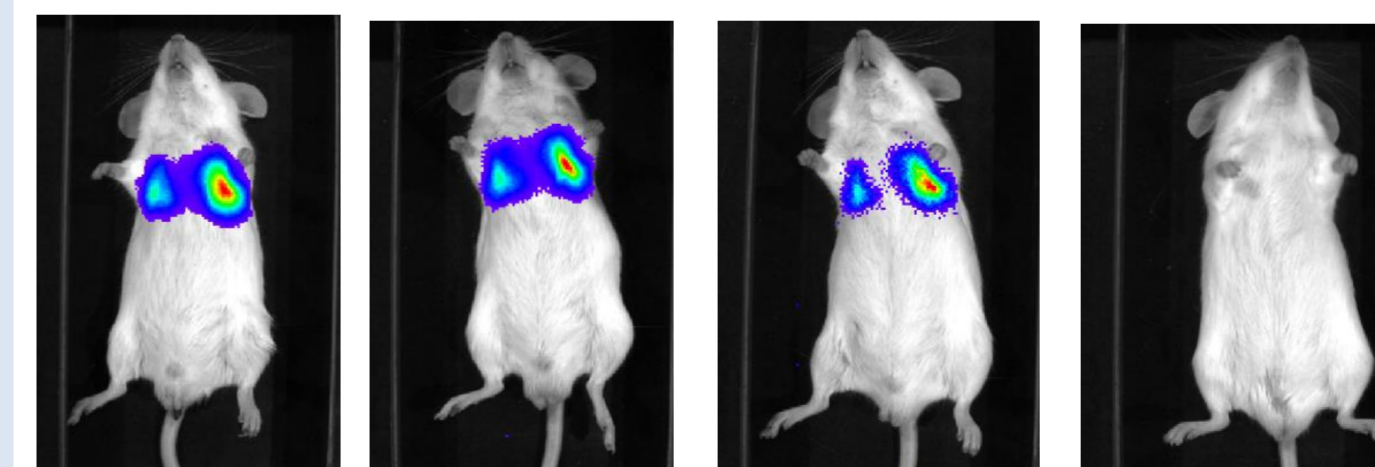
### Results

We confirmed the sequence of the fluc-GFP plasmid

The transduced HeLa cells expressed the fluc-GFP as seen in the FACs plots below which show the % of cells which are now fluorescing. The plots show that the higher the titration of virus, the more successful the transduction is, and more cells fluoresce.



After transducing the hMSC we injected them into mice and tracked them to the lungs



Day 0 (post injection) 4h post injection 24h post injection 48h post injection

By imaging the mice, we see the MSCs localise in the lungs but then are cleared within 48 hours

All procedures and experiments conducted were done in accordance with the guidelines of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and approved by the Monash University Animal Ethics committee

### Discussion

From our project, the injected hMSCs cleared from the lungs within 48 hours but their therapeutic benefit lasted longer and reduced the hallmarks of asthma

This indicates that whilst the hMSC are in the lungs for those two days, they are interacting with surrounding immune cells to have their inflammatory effects and therefore it is the effects of these immune cells which reduce the inflammation

Unfortunately we had trouble expanding the mMSCs so were unable to complete the comparison aim

### What's next

- To transduce the mMSC and complete the comparison aim by seeing how long they survive in the lungs and if they have a greater therapeutic effect
- To investigate what cells the MSCs could be interacting with in the lungs to have their effects
- Whether increasing the life-time of the MSCs in the lungs has a greater benefit for reducing asthma

### Keywords/abbreviations

MSC = mesenchymal stem cells  
hMSC = human mesenchymal stem cells  
mMSC = mouse mesenchymal stem cells  
Fluc-GFP plasmid = DNA which codes for the green fluorescent protein  
HeLa cells = immortal cell line used as the control  
293 T cells = isolated from human embryonic kidneys which are reliable for use with transfection

### References

1. Mathias, L. J. et al. "Alveolar Macrophages Are Critical For The Inhibition Of Allergic Asthma By Mesenchymal Stromal Cells". *The Journal of Immunology* 191.12 (2013): 5914-5924
2. Dominici, M. et al. "Minimal Criteria For Defining Multipotent Mesenchymal Stromal Cells. The International Society For Cellular Therapy Position Statement". *Cytotherapy* 8.4 (2006): 315-317