

# Production of CD3+ cells using ferrofluids for cell isolation, activation, expansion and subsequent transfection for adoptive cell therapy



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## Introduction

- Pilot study to investigate whether mAb-functionalized colloidal magnetic nanoparticles with solution-phase mAbs activate T cells for *in vitro* expansion and transfection
- Activation, expansion and transfection/transduction of immune cells – particularly T cells – have become particularly significant owing to their role in preparing genetically modified cells for adoptive immunotherapies
- In this study, we investigated whether T cells isolated using common-capture ferrofluids (CC-FFs) could be:
  - activated with solution-phase @CD28 mAb
  - expanded in static culture with IL-2-containing media
  - transfected with a GFP plasmid

## Methods

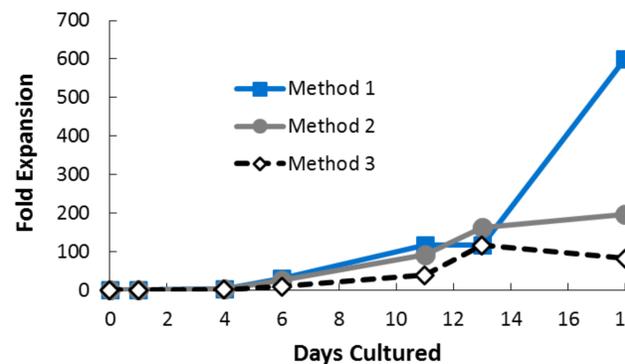
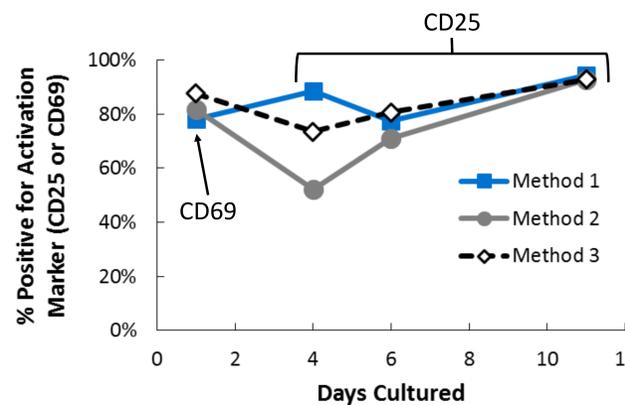


\* mAb = mouse anti-human CD3; FF = rat anti-mouse IgG1 FF

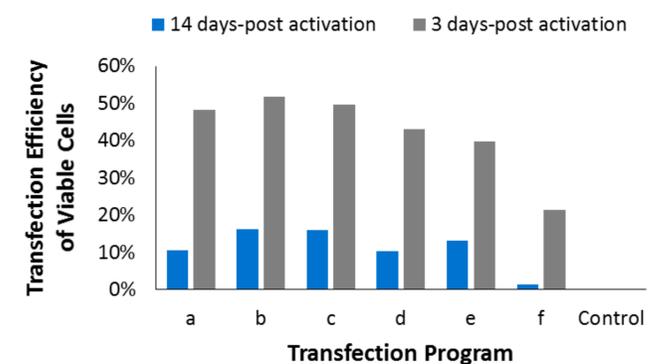
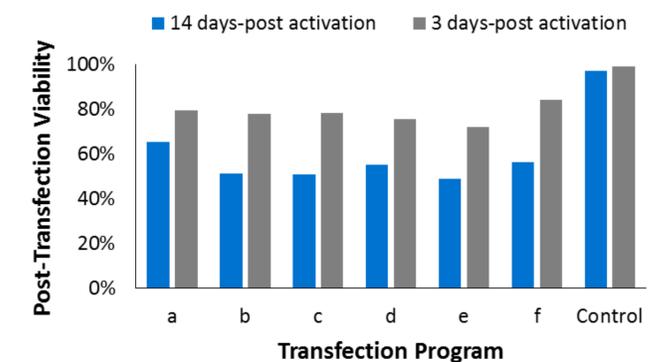
- Magnetically separate, wash, & re-suspend CD3<sup>+</sup> PBMC at 1x10<sup>6</sup> cells/mL in expansion medium + 100 IU/mL IL-2
- Incubate overnight at 37°C, then add 0.5 µg/mL @CD28
- Activation markers (CD69 & CD25) measured by flow cytometry and expansion monitored over 18-d period
- Transfection with GFP plasmid performed using 4D-Nucleofector X unit 3 d and 14 d following activation
- Viability and transfection efficiency quantified for each transfection program by flow cytometry

## Results

### Activation/Expansion Data



### Transfection Data



## Conclusions

- After 4 days in culture, **pre-formed conjugate** method displays **highest activation level** (88.4% CD25+)
- **600-fold expansion** observed at 18 days for best case
- Transfection with **high viability** and **efficiency** achieved **3 days-post activation**
- **In summary, CC-FFs can be used to activate T cells for *in vitro* expansion and transfection/transduction**

## Acknowledgements

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