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Developing Genetically and Chemically Converted Stem Cells for Cell Therapy

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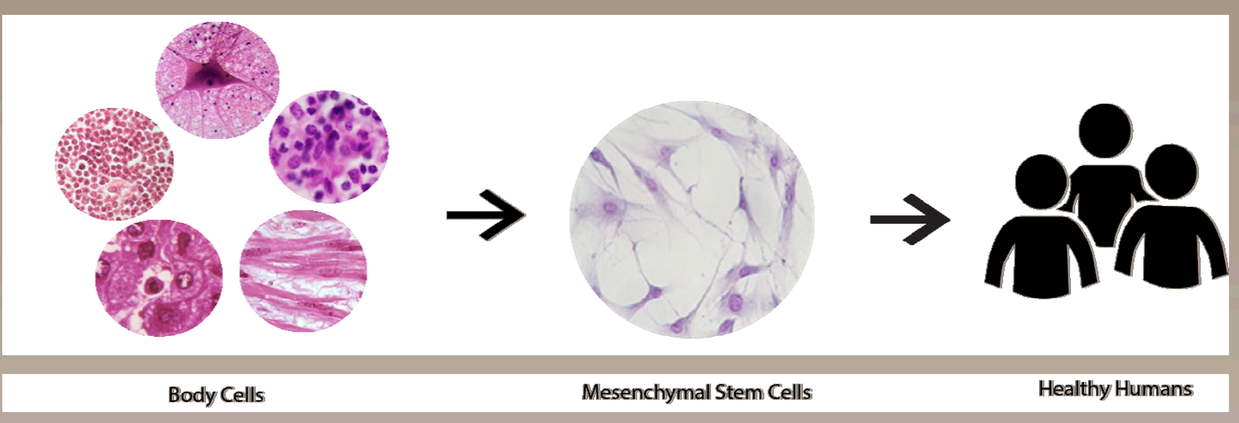
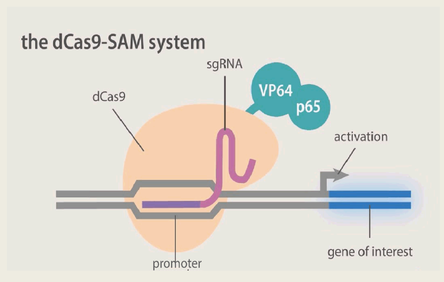
PROJECT BACKGROUND

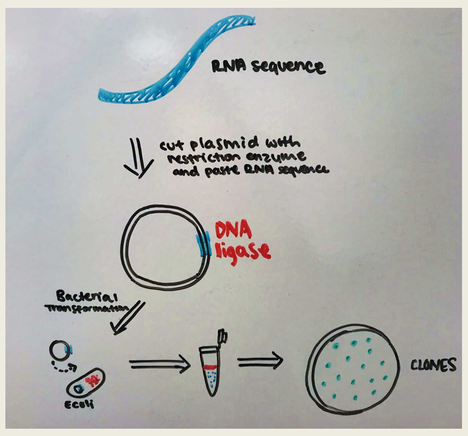


Studies have shown that exogenously administered mesenchymal stem cells (MSCs) have promising therapeutic potential in fostering tissue repair and increasing bacterial clearance in various preclinical models of infection [1]. While cell replacement via MSC differentiation plays some role in these effects, MSCs are believed to have the ability to respond to and modulate the activity of the body's innate and adaptive immune cells [2]. MSCs secrete a variety of immune effectors such as chemokines, cytokines, and antimicrobial peptides– a class of molecules of great therapeutic interest as antibiotic resistance remains an imminent threat to public health. Through genetic and chemical manipulation, we are developing a protocol for engineering readily available cells such as fibroblasts into MSCs for therapeutic use.

GENETIC APPROACH



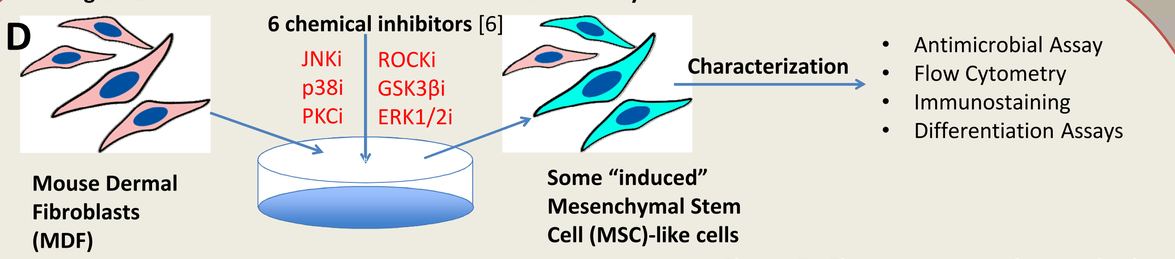
The CRISPR/dCas9 Synergistic Activation Mediators (SAM) system is an engineered protein complex that is used for transcriptional activation of a gene of interest (**Figure A**)[4,5]. In this work, we are using CRISPR activation to turn on a master transcriptional regulator of stem cell pluripotency called Oct4. It is unclear what locus of the Oct4 promoter will be most effective for gene activation (**Figure B**). Here, we targeted 8 locations in the Oct4 proximal promoter using 8 unique sgRNAs.

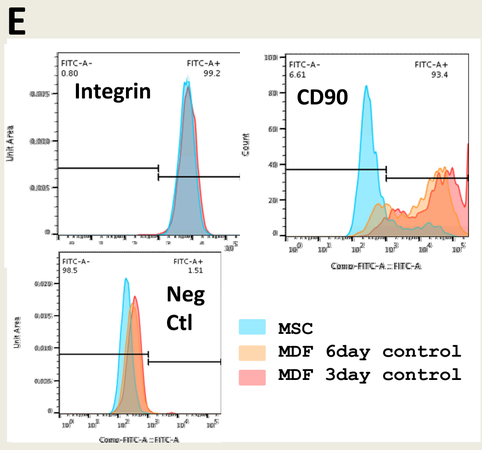


Each sgRNA was cloned into a mammalian expression vector using the Golden Gate cloning strategy and transformed into competent *E. coli* (**Figure C**). The resulting colonies, or clones, were then analyzed by DNA sequencing to check correct integration of the sgRNA sequence. Correct cloning constructs were then amplified in larger scale and maxiprepped for transfection of mammalian cell lines.

CHEMICAL APPROACH

Figure D: Method for chemical fibroblast conversion study





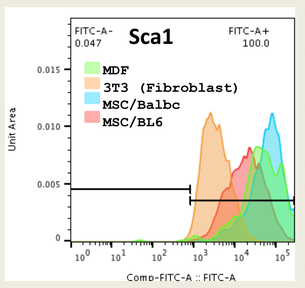


Figure E: Flow cytometry characterization of MSC and MDF surface markers. In order to sort for and assess the conversion efficiency of treated MDFs, we stained MSCs and MDFs with standard MSC markers– Integrin, CD90, and Sca1 [7]. While MDFs seemed to have comparable Sca1 and Integrin expression to MSCs, this set of MSCs were largely CD90-negative while subsets of MDFs had either negative or positive expression. However, previous data (not shown) have yielded similar double populations of MSC CD90 expression, so different markers will need to be investigated.

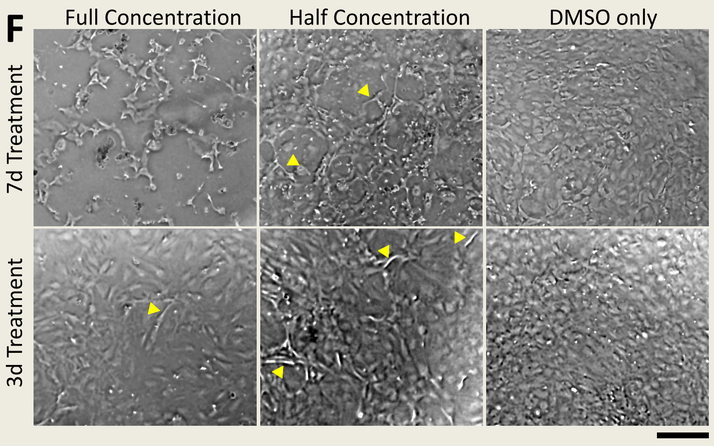


Figure F: Length of treatment and concentration of inhibitors affects cell viability and morphology. MDF cells were treated for 3 days or 7 days with chemical inhibitor cocktail. After 7 days, we observed extensive cell death from treatment with fully concentrated media. We next tested a range of chemical concentrations, and found that a 2-fold reduction of inhibitor concentration allowed for increased cell viability (middle panels). Treated cultures included cells with more extended processes and spindle-like morphology (arrowheads), possibly indicating conversion to a more MSC-like state [2] . Scale bar=250 um.

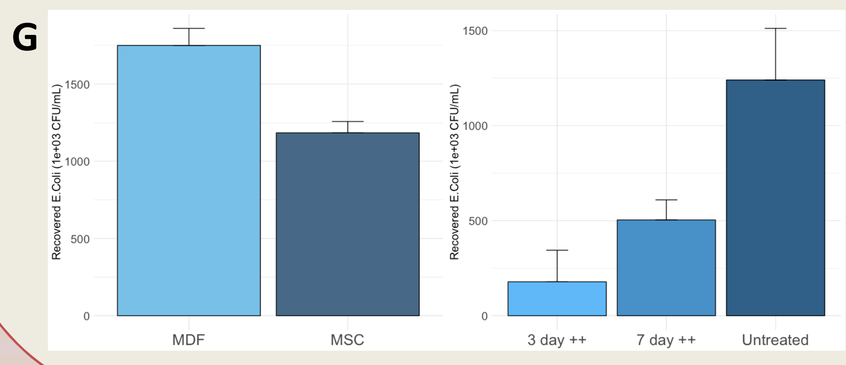
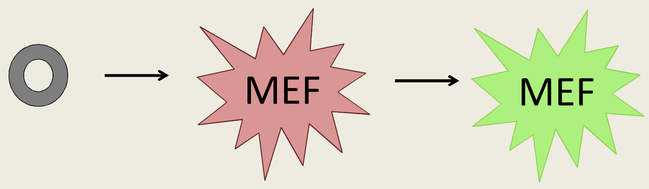


Figure G: Chemically treated MDFs may decrease bacterial viability. MDFs did not decrease bacterial viability as much as MSCs, which are believed to have anti-microbial properties [3]. MDF treatment appears to have an effect on antimicrobial activity, in which 3 day and 7 day treated cells appear to have caused decreased bacterial viability compared to the untreated controls.
Data expressed as mean + SD.

FUTURE DIRECTIONS

- Transfect the plasmids into MEF's. If the Oct 4 gene turns on, we should see the MEF's glow



This can then help us identify which guide RNA best activates the Oct-4 gene

- Refine chemical conversion protocol to address viability effects of treatment media and select for MSC-like, antimicrobial cell populations
- Identify better markers for assessing cell conversion efficacy-- sequence transcriptome, identify target genes/markers.
- Explore combination genetic and chemical treatments for cell conversion or antimicrobial enhancement

REFERENCES & ACKNOWLEDGEMENTS

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