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Optimization of human CD34+ cells for reprogramming using tailored media and alternate timelines

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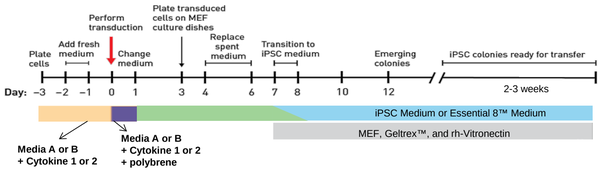
ABSTRACT

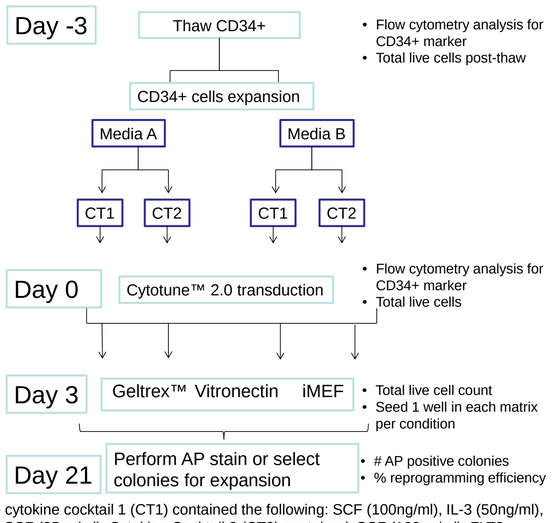
The focus on human fibroblasts as the somatic cell source for induced pluripotent stem cells (iPSC) is transitioning towards more easily reprogrammable cell types and/or methodologies. Somatic cells derived from blood are easier to obtain, and are more suited for a clinical grade workflow. In addition, these blood cells allow for minimal upfront manipulation of cells prior to reprogramming, thus reducing the potential for somatic mutations, while reducing the associated time and cost.

The aim of this study was to optimize the media, cytokines, and timelines for reprogramming blood-derived cells with Sendai virus, while maintaining high reprogramming efficiency. Using CD34+ cells, two different media known to maintain CD34+ cells were used in combination with two known combinations of cytokine cocktails. Flow cytometry analysis indicated the novel media with cytokine cocktail 1 (CT1: SCF, GM-CSF, IL-3), showed a relatively higher percentage of CD34+ cells with 2-3 days of culture. The cells reprogrammed with tailored media and CT1 also resulted in a 3-fold increase in reprogrammed alkaline phosphatase-positive iPSC colonies. Additionally, this combination facilitated reduction of the reprogramming timeline from the standard three week timeline to a fourteen day protocol. Microscopic evaluation of the resulting colonies confirmed that the iPSCs were mature with ESC-like morphology and ready for downstream clonal selection and expansion. The combination of tailored media with the optimal cytokine cocktail, coupled with an improved protocol can enable reprogramming of other blood-derived cells, such as PBMCs, and small volumes of blood. Streamlining the process of iPSC generation by minimizing manipulation and timelines reduces the costs and effort involved with large-scale iPSC generation.

MATERIALS AND METHODS

Figure 1. CD34+ cell reprogramming flowchart.





The cytokine cocktail 1 (CT1) contained the following: SCF (100ng/ml), IL-3 (50ng/ml), GM-SCF (25ng/ml). Cytokine Cocktail 2 (CT2) contained: SCF (100ng/ml), FLT3 (100ng/ml), IL-3 (20 ng/ml), IL-6 (20ng/ml). Total cells seeded in feeder free condition was $5x10^4$ cells per well and $2.5x10^4$ cells per well in feeder dependent condition.

RESULTS

Figure 2. Schematic representation of reprogramming

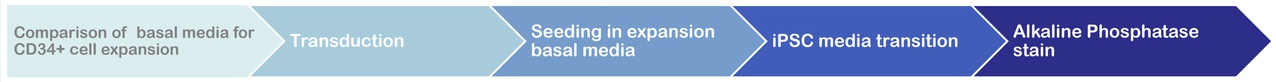
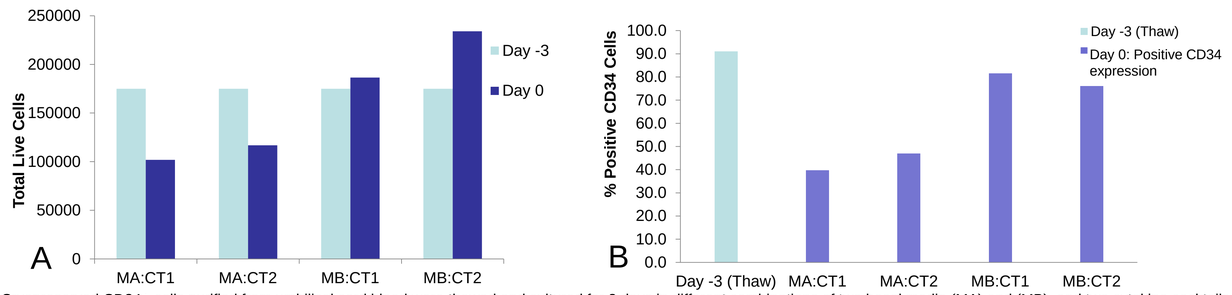
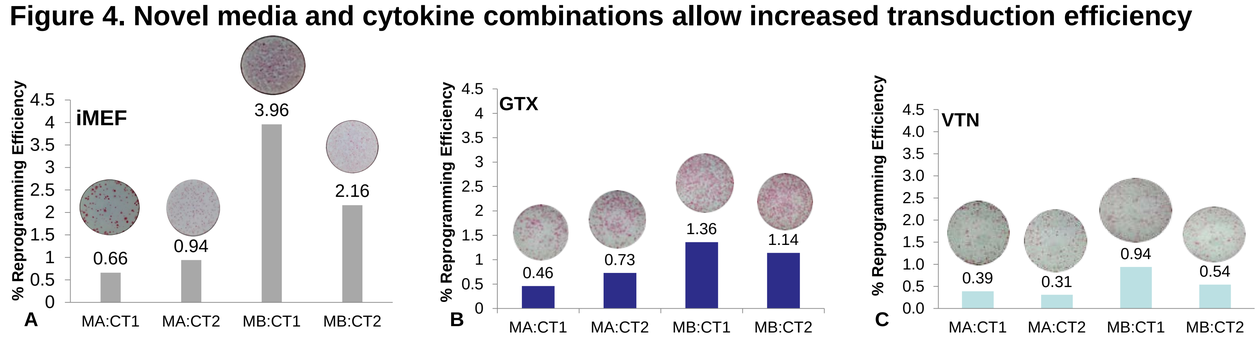


Figure 3. Novel media and cytokine combinations allow for better maintenance and expansion of CD34+ cells



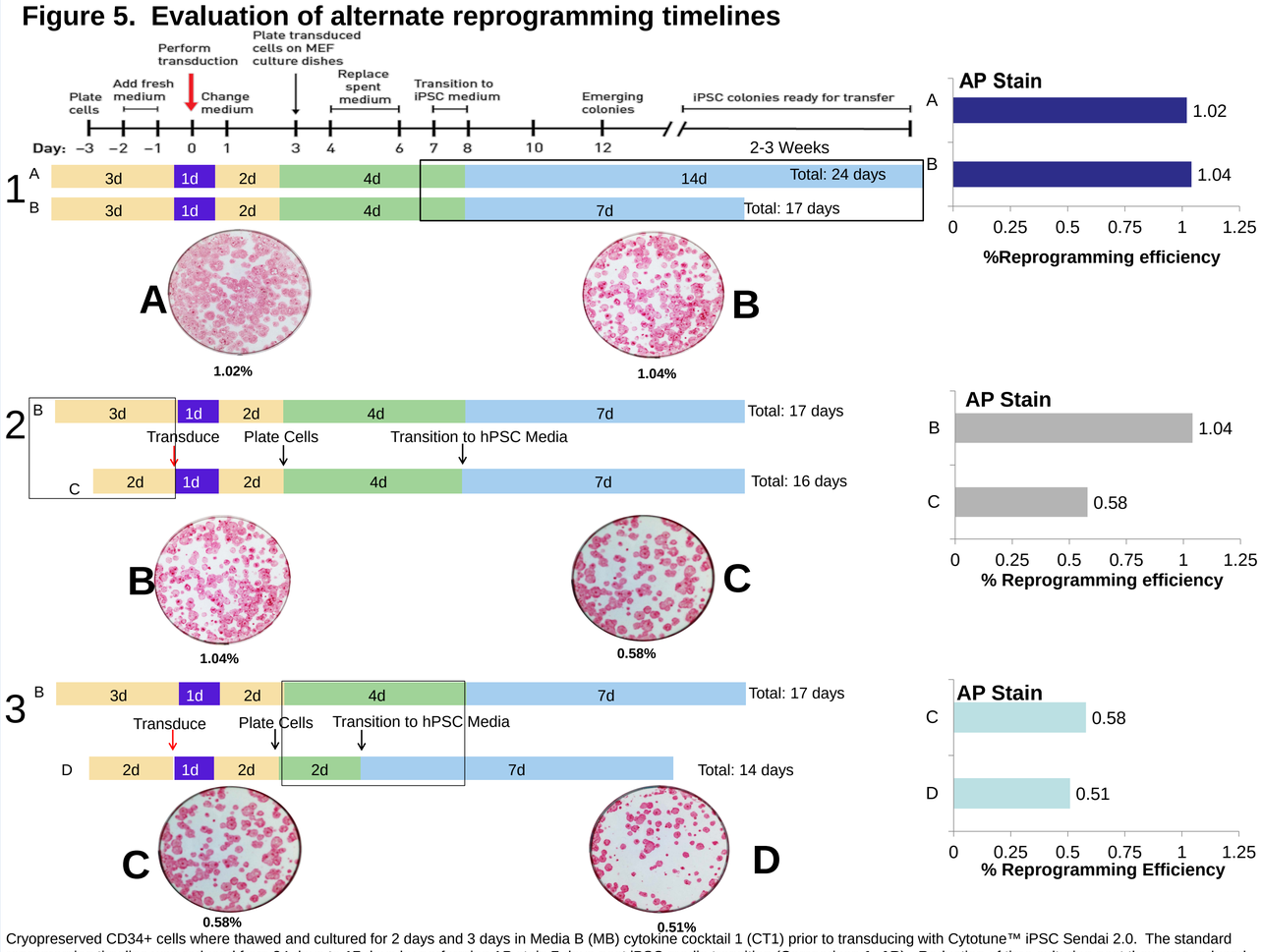
Cryopreserved CD34+ cells purified from umbilical cord blood were thawed and cultured for 3 days in different combinations of two basal media (MA) and (MB), and two cytokine cocktails (CT1) and (CT2). Cells were counted for total cell number and viability via trypan blue on the Countess™ II automated cell counter at thaw (Day -3) and 3 days later (Day 0) **(A)**. Cells were also stained with CD34 antibody and analyzed by flow cytometry analysis of CD34+ expression from live cells at day -3 and day 0 **(B)** .

Figure 4. Novel media and cytokine combinations allow increased transduction efficiency



Cells from each basal media and cytokine combination were counted using trypan blue on the Countess™ II automated cell counter. From each condition $7.5x10^4$ live cells were transduced using Sedai virus containing Klf4, Oct3/4, Sox2, C-Myc (Cytotune™-iPS Sendai 2.0). Three days post-transduction (day 3) cells were seeded on the following matrixes iMEF (**A**), Geltrex™ (**B**), and Vitronectin (**C**). At 21 days post transduction iPSC colonies were stained using Vector® Red Alkaline Phosphatase (AP) stain . Percent reprogramming efficiency is calculated by counting total number of positive AP colonies divided by the total number of cells seeded.

Figure 5. Evaluation of alternate reprogramming timelines



Cryopreserved CD34+ cells where thawed and cultured for 2 days and 3 days in Media B (MB) cytokine cocktail 1 (CT1) prior to transducing with Cytotune™ iPSC Sendai 2.0. The standard reprograming timeline was reduced from 24 days to 17 days by performing AP stain 7 days post iPSC media transition (Comparison **1: AB**). Reduction of time culturing post thaw was reduced from 3 days to 2 days (Comparison **2: BC**), the remaining subsequent events remained the same. The third modified timeline reduced the iPSC media transition by two days in addition to 1 day post-thaw (comparison **3: CD**).

CONCLUSIONS

Evaluation of CD34+ cell expansion media

- Media B allowed for the greatest expansion and recovery post thaw, and MB:CT2 was the best combination, with an observed 33% increase in cell population.
- At thaw (day -3), cells displayed 91% CD34+ expression measured using flow cytometry. CD34 expression was retained best with the combination MB:CT1, which resulted in 81.5% CD34+ cells after three days in culture.
- MB:CT1 yielded the highest reprograming efficiency , with approximately 3-fold higher reprogramming efficiency in feeder free and feeder-dependent conditions, relative the MA:CT1.
- iPSC colonies were selected and expanded from MA:CT1 and MB:CT2. These iPSC clones displayed expression of pluripotent markers, normal karyotype, and functional pluripotency as measured by embryoid body formation and expression of markers for the three major lineages (data not shown).

Evaluation of alternate reprogramming timelines

- MB:CT1 was used for alternate reprogramming timeline analysis.
- Reduction of 7 days from the end of the reprogramming timeline yielded no significant changes in reprogramming efficiency.
- Reduction of 1 day from thaw to transduction yielded approximately 50% reduction in reprogramming.
- Reduction of 2 days from the time between transduction and transition to iPSC medium yielded no significant change in reprogramming efficiency.
- A 14 day reprogramming is achievable, yielding a 0.51% reprogramming efficiency. Colonies were defined and spaced out for easy selection.
- The 24 day reprogramming timeline is optimal to acquire the highest reprogramming efficiency. However, reducing the total length in reprogramming may be essential to reduce somatic mutations post transduction.

ACKNOWLEDGEMENTS

CSUSM/CIRM Bridges to Stem Cells Program Grant.

TRADEMARKS/LICENSING

ThermoFisher SCIENTIFIC

California State University SAN MARCOS

CIRM CALIFORNIA INSTITUTE FOR REGENERATIVE MEDICINE