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GENERATION OF INDUCED PLURIPOTENT STEM CELLS FROM MESENCHYMAL STEM CELLS OF OSTEOPETROSIS PATIENTS BY TWO DIFFERENT INTEGRATION-FREE METHODS UNDER FEEDER-FREE CONDITIONS

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Aim of this study is to generate induced pluripotent stem cells (iPSC) from bone marrow-derived mesenchymal stem cells (BM-MSC) of children with malignant infantile osteopetrosis (MIOP), a rare autosomal recessive disorder caused by defects in osteoclast formation/function, and establish an institutional human iPSC bank by using MIOP as a prototype disease. Osteopetrotic and healthy donor BM-MSCs which were used as control were reprogrammed by using CytoTune-IPS 2.0 Sendai (SeV) and Epi5 Episomal reprogramming kits according to the given instructions. iPSC colonies which started to appear around day 18 for SeV and day 21 for episomal vector were picked, manually passaged and expanded in matrigel-coated plates. Assesment of colony morphology, immunofluorescence (IF) staining and flow cytometry besides live staining with alkaline phosphatase were performed for characterization (≥3 clones/line). Expression of pluripotency genes, detection of SeV and episomal vectors were evaluated with PCR. *In vitro* and *in vivo* pluripotency were assessed by embryoid body and teratoma assays. Karyotype analyses were performed to evaluate genetic stability. iPSC lines exhibiting typical ESC-like colony morphology were shown to express pluripotency markers (OCT4, SSEA-4, SOX2,TRA-1- 60) by IF staining. 85–95% of the cells were found positive for SSEA-4 and Oct3/4 and negative for CD29 with flow cytometry. Immunohistochemical staining of teratoma sections confirmed the trilineage differentiation potential. All iPSC lines expressed pluripotency related genes (Nanog, DNMT3B, CDH1, UTF1, REX1, TERT). Loss of SeV genome was observed at late passages. Karyotype analyses were found normal. Analysis of DNA methylation profile is in progress. In conclusion, iPSC were succesfully derived from osteopetrotic BM-MSC using two different methods. Generation of iPSC using non-integrating SeV and episomal vectors have several advantages over other methods such as ease of production, reliability, high efficiency and safety profile, which will be important in translation into clinic. Furthermore patient-spesific iPSC because of pluripotency and self renewal capacity seem to be ideal for disease modeling and investigate new therapeutic targets which will pave the way for the development of cellular replacement therapies.

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INCUBATION TIME OF HYDROXYETHYL STARCH SEDIMENTATION (HES) INCREASES CELL RECOVERY IN UMBILICAL CORD BLOOD (UCB) PROCESSING BY AUTOMATED SYSTEM

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Introduction: Umbilical cord blood (UCB) has been increasingly used as an alternative sources to treat hematopoietic malignant diseases, especially for pediatric and low-weight adult patients in terms of concerns about reaching a sufficient cell dose for engraftment. Many studies have been performed to establish techniques for volume reduction of CB units, but it can be influence to cell recovery. UCB processing was performed in the presence of additives like hidroxyethyl starch (HES).[1–4] To improve cell recovery and standardize the process, other technique of incubation time of HES have also been developed.
Materials and Method: 156 UCB samples were collected from both vaginal and caesarean deliveries. Informed consent was taken prior to UCB collection. Patients with known history of infectious diseases (HIV, Hepatitis B, Hepatitis C, Cytomegalovirus and Syphilis infection) were excluded from the study.

UCB processing was done within 48 hours after UCB collection using automated cell processing system with HES (Tetraspan 6%). A solution of HES corresponding 40% the UCB input volume was in injected into the input bag at rate of approximately 0.3 ml/sec than incubate 15 minutes (n = 81) and 30 minutes (n = 75). TNC counts, cell recovery and viability data were collected.
Result: To analyze TNC post-processing, viability and cell recovery of 156 processed UCB, we classify the data into three groups, < 50 ml, 50–80 ml and > 80 ml (Table 1). TNC number of 30' HES incubation processed UCB were not significantly different in 50–80 ml group. Whereas, cell recovery and % viability were higher in UCB processed with 30' than 15' HES incubation time in all groups.
Discussion: Based on our UCB processing results, the addition of HES with 30 minutes an incubation period can further increase the recovery cell compared than to 15 minutes HES incubation. Increasing HES incubation time improves the RBC aggregation process, which can be seen by increase of the polymer size and can increase the RBC reduction in UCB products.

In conclusion, the incubation period of HES up to 30 minutes better in binding of RBC than 15 minutes, so that the value of recovery cell is higher.

Volume	< 50 ml					50-80 ml					> 80 ml				
Time incubation	n	x Vol UCB (ml)	TNC (10^6/ml)	TNC Recovery (%)	Viability (%)	n	x Vol UCB (ml)	TNC (10^6/ml)	TNC Recovery (%)	Viability (%)	n	x Vol UCB (ml)	TNC (10^6/ml)	TNC Recovery (%)	Viability (%)
15'	29	39.69	332.54	82.72	99.52	34	66.71	628.49	80.03	99.34	18	94.55	705.84	67.49	98.66
30'	11	41.86	397.96	83.47	99.77	31	66.30	537.30	80.20	99.57	33	97.58	842.74	80.67	99.52

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GENERATION OF A LANDING PAD T CELL LINE USEFUL FOR T CELL RECEPTOR CUSTOMIZATION

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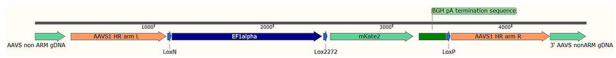
T cell biology is integral to the study of normal immune regulation as well as cancer biology, Car-T cells, epitope specificity and antigen presentation. However, primary T cells can be difficult to propagate in culture for the length of time necessary for functional assays. In addition, primary T cells express variant T cell receptor (TCR) heterodimers that can be challenging to identify and may not be optimal for downstream studies. We sought to simplify this system using transformed T cells which can be grown in culture for extended periods of time. We engineered a floxed landing pad sequence into the safe harbor AAVS1 genetic locus using CompoZr® zinc finger nucleases. Both the promoter and landing pad expression cassette are flanked by unique lox sites, allowing swapping of either the promoter and/or expression cassette as needed. We ensured that only one copy of this sequence was found within the genome to avoid any complications associated with random insertion events. We also generated a landing pad cell line null for the endogenous TCR using Cas9/CRISPR ribonucleotide complexes. Both the TCR alpha and beta loci were rendered null due to non-homologous end joining and the presence of insertions and deletions culminating in premature stop codons were genotyped using next generation sequencing. The absence of a functional TCR was validated using flow cytometry staining for surface TCR and CD3. This cell line was then used to generate a knock-in of the desired exogenous TCR heterodimer to the landing pad locus, verified using flow cytometry staining. These lines will be useful for a multitude of studies where a researcher needs to express a gene of interest in a discrete genetic locus or wants to generate a panel of TCR expressing cell lines.



Landing pad design, integrated into the human AAVS1 genetic locus.

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RAPID, REAL TIME QUANTIFICATION OF LENTIVIRUS PARTICLES USING ANTIBODY-BASED DETECTION ON THE VIRUS COUNTER 3100

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Lentivirus particles are valuable vectors for modern gene and cell therapies. Accurate enumeration of total particle count of gene therapy vectors is critical in order to minimize the risk of adverse immune response or other negative outcomes when treating patients.

Quantification of Lentivirus particles is challenging, often relying on difficult and variable methods such as ELISA and qRT-PCR. Rapid and precise analytical methods are needed to monitor vector production and enumerate particles in final formulations. The Virus Counter 3100® and antibody-based ViroTag® VSV-G reagents allow for the rapid quantitation of Lentivirus particles by utilizing serotype-specific fluorescently labeled antibodies with high affinity to intact Lentivirus particles expressing the VSV-G epitope. In conjunction with the Combo Dye® reagent, comparisons of epitope expression and