Advancing Next Generation Cell and Gene Therapies to the Clinic Using Non-viral, Scalable Engineering and Genome Modification of Human Primary Cells, Stem Cells and iPSCs.

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Abstract

The use of stem cells, primary cells and human induced pluripotent stem cells (hiPSCs) has revolutionized the fields of regenerative medicine and cell and gene therapy. These cell populations often require engineering for their generation, reprogramming, differentiation or for targeted gene editing prior to their use. As more robust and scalable cell cultivation methods are developed enabling iPSC/stem/primary cell-derived therapies to advance towards the clinic, it is imperative that researchers consider whether their cell engineering method meets the stringent demands of clinical use — namely the ability to safely and reproducibly modify human primary or stem cells with high efficiency, low cytotoxicity, and at the scale required to treat patients. Flow Electroporation® Technology, a non-viral cell engineering technology designed to fulfill these demands, has an established record of rapidly advancing cell- and gene-based therapies to the clinic. In this poster we will demonstrate the high efficiency, high viability, fully scalable engineering of a variety of human primary cells, stem cells and hiPSCs as well as their real-world application in pre-clinical and IND-enabling studies.

Universal, High Performance Primary & Stem Cell Engineering

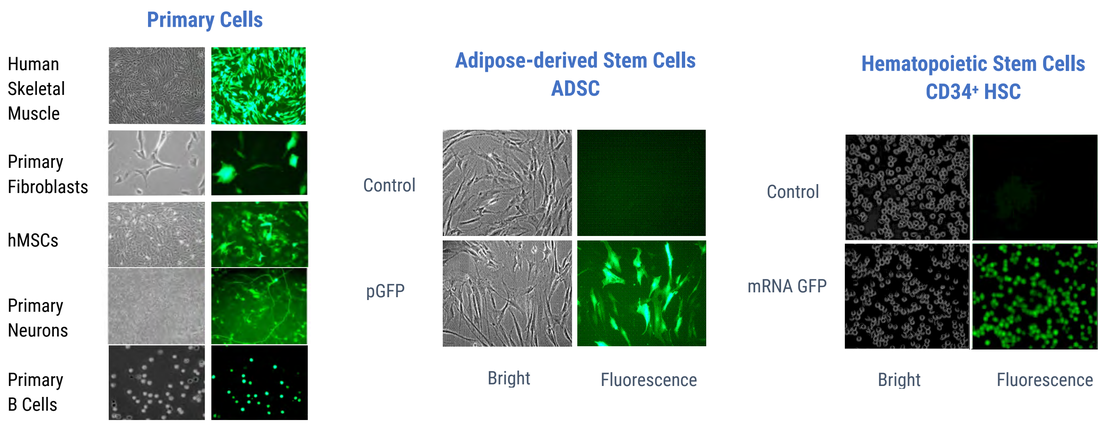
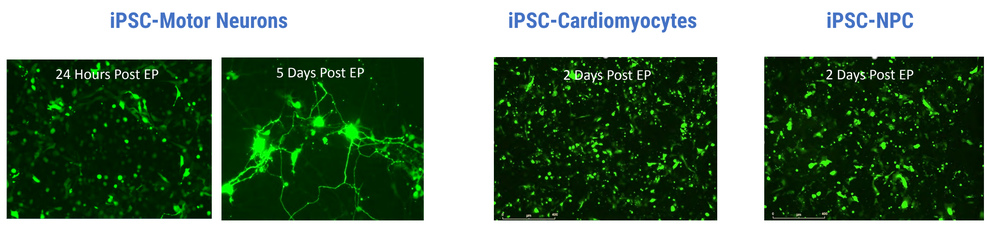


Figure 1: Human primary or stem cells were electroporated with pGFP (GFP mRNA for HSCs) using the MaxCyte STX. Transfection efficiency and viability were assessed at 24-48 hours post electroporation.

High Efficiency, High Viability iPSC Transfection

Expanding the Power of iPSCs

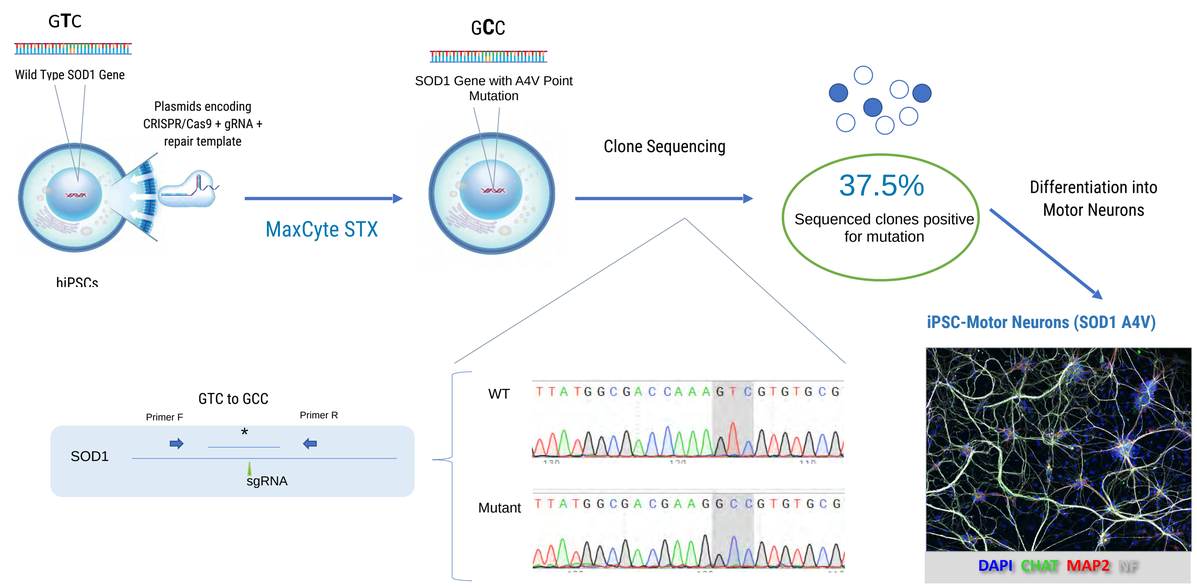


iXCells BIOTECHNOLOGIES	Lipid-based Method		MaxCyte STX	
	Efficiency	**Viability**	**Efficiency**	**Viability**
Human iPSCs	< 10%	< 70%	94%	85%
iPSC-Motor Neurons	< 10%	< 60%	87%	89%
iPSC-Cardiomyocytes	< 10%	< 60%	60-80 %	86%
iPSC-NPCs	< 10%	< 70%	60-80%	90%

Figure 2: Human iPSCs (iXCells Biotechnologies) were treated with Accutase and dissociated into single cells before electroporation. Cells were electroporated with pGFP using the MaxCyte STX and replated on Matrigel-coated plates. Images were taken 24, 48 or 120 hours post electroporation. The transfection efficiency and viability were determined using a NovoCyte flow cytometer (ACEA). These data were compared to historic results using iXCells previously optimized lipid-based transfection method.

Generating an Isogenic Model of Amyotrophic Lateral Sclerosis (ALS)

Progress Towards Understanding the Molecular Mechanisms of Disease Using Gene Editing

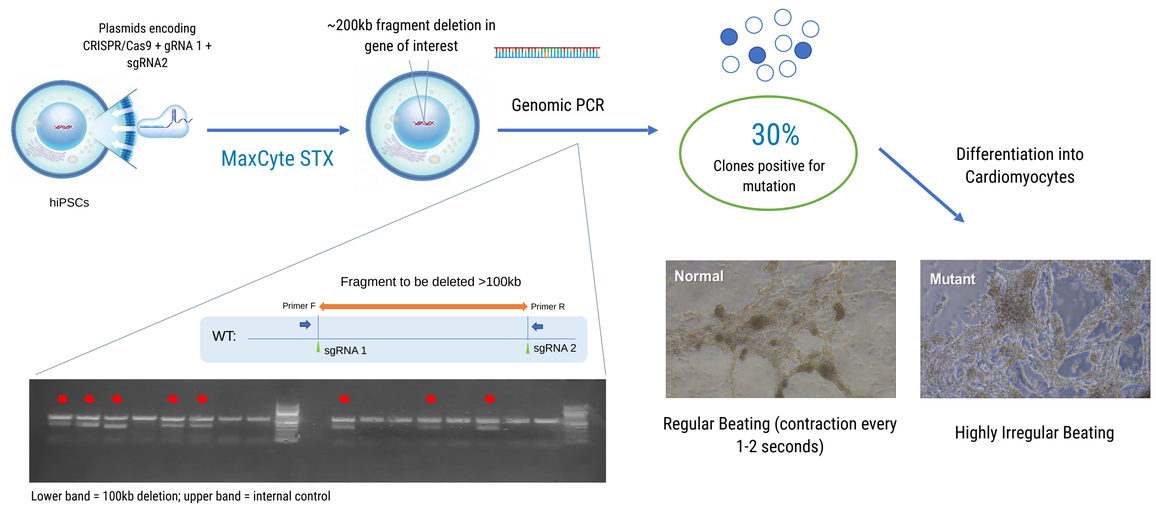


3 out of 8 hiPSC clones were positive for A4V mutation using MaxCyte, 10X higher than lipid based transfection

Figure 3: An alanine to valine mutation at codon 4 (A4V) of SOD1 causes a rapidly progressive dominant form of amyotrophic lateral sclerosis (ALS) with exclusively lower motor neuron disease and is responsible for 50% of SOD1 mutations associated with familial ALS in North America. To create a cellular disease model for ALS, a point mutation (GTC > GCC) was introduced via CRISPR in the normal human SOD gene I in hiPSCs. Cells were electroporated with gRNA and repair template and re-plated on Matrigel-coated plates. Single cell colonies were screened using Sanger Sequencing. 3 of 8 clones were positive for the intended mutation: a 37% success rate, which was >10X higher than previously established lipid-based transfection. Wild type and mutant cells were expanded and differentiated into motor neurons using iXCells proprietary methods. Motor neuron markers such as HB9, Choline acetyltransferase (CHAT), Microtubule-associated protein 2 (MAP2) and neurofilament (NF) were expressed and neuronal networks developed by Day 7 for both cell populations.

Cardiac "Disease-in-a-Dish" Using iPSCs-derived Cardiomyocytes

CRISPR-Mediated Gene Deletion Recreates Cardiac Arrhythmia

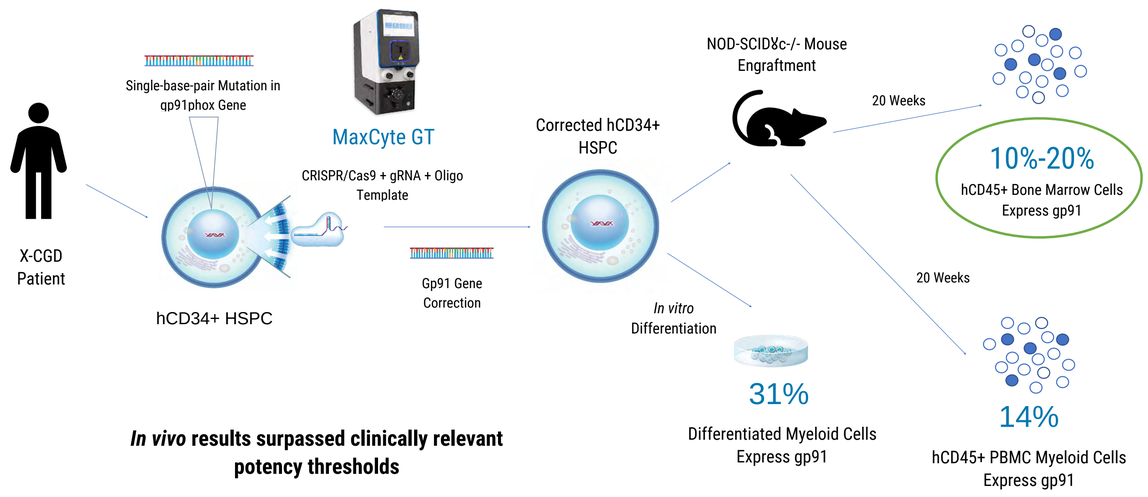


20 out of 60 hiPSC clones were positive for deletion using MaxCyte

Figure 4: A ~200kb fragment of a gene believed to be involved in cardiac arrhythmia was targeted for deletion. sgRNA 1 and 2 were sub-cloned into plasmids containing Cas9. The constructs were electroporated into hiPSCs using the MaxCyte STX. Electroporated cells were re-plated on Matrigel-coated plates and single cell colonies screened by genomic PCR (Primer F+R). 30% of the isolated clones had the desired deletion. Mutant and wild-type iPSCs were subsequently differentiated into cardiomyocytes using iXCells proprietary methods and beating monitored. Cardiomyocytes from wild-type iPSCs exhibited regular beating while those derived from mutant colonies beat irregularly mimicking cardiac arrhythmia.

Clinically Relevant Levels of Ex Vivo Gene Correction in X-linked Chronic Granulomatous Disease (X-CGD) Patient Stem Cells

Gene Editing Moves Towards the Clinic Using Non-viral, Fully Scalable Cell Engineering



***In vivo* results surpassed clinically relevant potency thresholds**

Figure 5: X-CGD is caused by a mutation in the CYBB gene which encodes a critical component (gp91-phox) of NADPH oxidase, an enzyme that is key for the anti-microbial activity of phagocytes. Correction of mutation within the faulty CYBB gene offers a new curative treatment for X-CGD patients. The patients' own cells are harvested, the mutated gene corrected using CRISPR-mediated gene editing, and the cells with the corrected gene returned to the patient. The engrafted cells multiply to create a new population of cells displaying 'normal' function and eliminating disease. In these studies, CD34+ hematopoietic stem cells (HSPCs) were isolated from X-CGD patients and electroporated with CRISPR/Cas9, guide RNA, and the gene correcting oligo template using the MaxCyte GT. A portion of the cells were differentiated *in vitro* into myeloid cells and gene correction rates determined to be 31%. The other portion of corrected HSPCs were introduced into immunodeficient mice. After 20 weeks the engrafted human cells in the mouse peripheral blood expressed the corrected gp91 gene at 14%, while the engrafted cells in the bone marrow showed a 10% - 20% gene correction rate. These correction rates are within clinically beneficial potency thresholds. *Sci. Transl. Med.*, 9(372), Jan 2017.

MaxCyte Flow Electroporation® Technology

- Ability to engineer primary and stem cells at **high efficiency** and **cell viability** enabling their use for:
  - improved, more powerful disease modeling
  - high efficiency gene editing (correction, deletion or insertion)
  - highly potent human therapeutics
- Scalability and regulatory-compliance to rapidly & seamlessly advance from R&D through patient treatment
- Enables previously unfeasible iPSC applications
- Improves cell engineering compared to lipid- or viral-based delivery methods
  - increased efficiency
  - elimination of safety and toxicity concerns
  - decreased cost and complexity
  - reduced time to clinic
- Designed to meet the stringent demands of cell and gene therapy:
  - highly efficient and reproducible transfection of difficult-to-transfect primary cells
  - non-toxic
  - clinical-scale, regulatory-compliant
  - payload flexibility
- Proven technology supported by numerous publications, clinical trials and 45+ partnered clinical development programs

References & More Information

CRISPR-Cas9 Gene Repair of Hematopoietic Stem Cells from Patients with X-linked Chronic Granulomatous Disease. Ravin S, Li L, Wu X, Choi U, Allen C, Koontz S, Lee J, Theobald-Whting N, Chu J, Garofalo M, Sweeney C, Kardava L, Moir S, Viley A, Natarajan P, Su L, Kuhns D, Zarember K, Peshwa M & Malech H. *Sci. Transl. Med., 9(372), 2017.*

On Demand Webinar: High Transfection Efficiency of Human Induced Pluripotent Stem Cells and Their Derivatives. Presenter: Dr. Nianwei Lin, Ph.D.; Chief Operating Office, VP, and Co-Founder iXCells Biotechnologies. *November 2017*
https://www.maxcyte.com/webinars/ixcells-stemcells/

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