

Progress Report

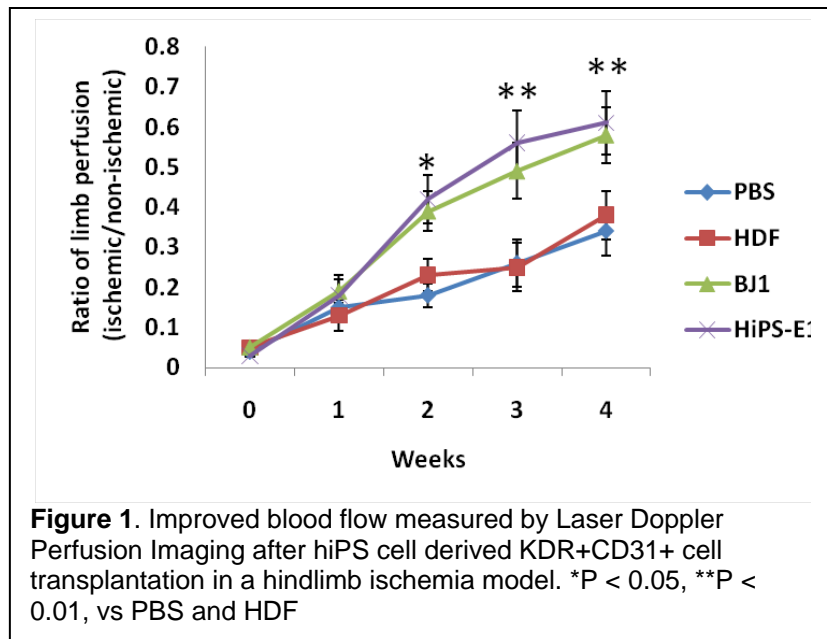
Title: Treatment of diabetic limb Ischemia with human induced pluripotent stem cells

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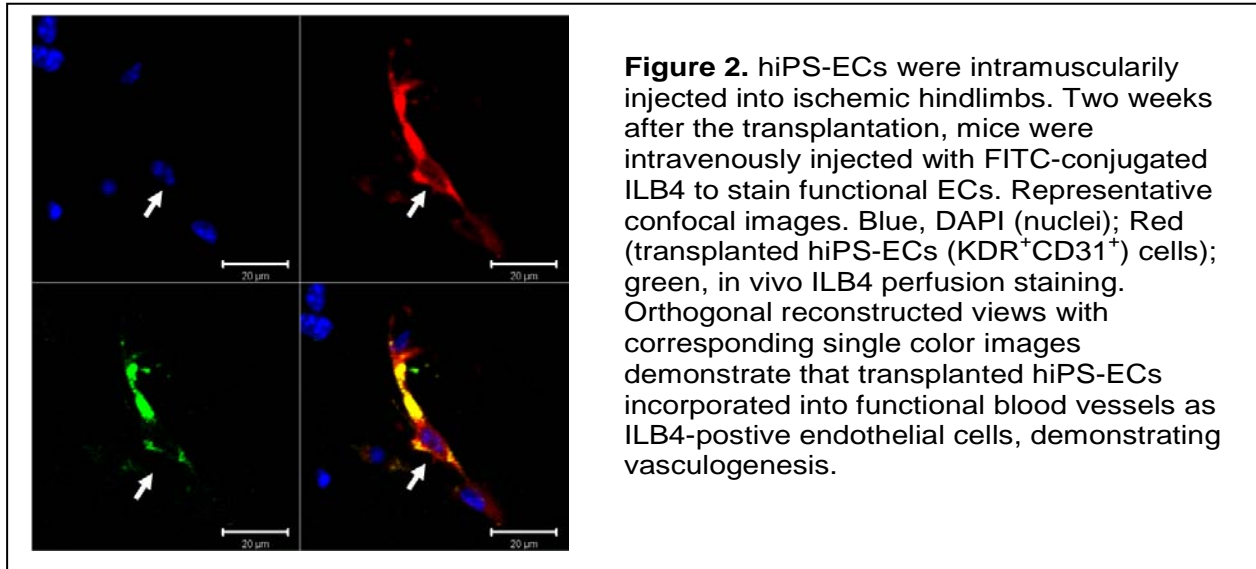
In this pilot grant, we evaluated human iPS cell (hiPSC)-derived endothelial cells for treating diabetic hindlimb ischemia. We induced diabetes in 8 week-old male athymic nude mice by intraperitoneal injection of streptozotocin (150 mg/kg) per day for 3 days. We checked serum glucose one week later and mice showing glucose ≥ 250 mg/dl were included in this study. Four weeks later, we created hindlimb ischemia, in which the femoral artery and vein in the left leg are ligated and excised while the right leg is left undisturbed as a control reference. hiPSCs were spontaneously differentiated through EB formation for 7 days and were sorted for KDR using FACS. These KDR⁺ cells were further cultivated in complete EBM-2 medium for another 21 days and were sorted for both CD31 and KDR. Such isolated KDR⁺CD31⁺ cells (hiPS-derived endothelial cells, hiPS-ECs) were directly injected into the thigh muscles of hindlimb immediately after induction of limb ischemia. Five hundred thousand cells were injected in 500 μ l of PBS. We used two lines of hiPSCs, BJ1 and HiPS-E1. Before cell injection, cells were labeled with CM-Dil (red fluorescence) for cell tracking. As controls, phosphate buffered saline (PBS) and the same number of human dermal fibroblasts (HDF) were used.

We performed Laser Doppler perfusion imaging (LDPI) to evaluate the recovery of hindlimb blood flow before cell injection and every wk for 4 wks thereafter. As seen in **Figure 1**, hiPS-EC injected groups showed significantly higher blood flow compared to the control groups (*P < 0.05 at wk 2 and **P < 0.01 at wk 3 and 4).

We next examined the vasculogenic potential of injected hiPS-ECs in ischemic hindlimb. For doing that, we injected isolectin B4 (FITC-labeled), sacrificed mice and harvested muscles. After freezing and sectioning the

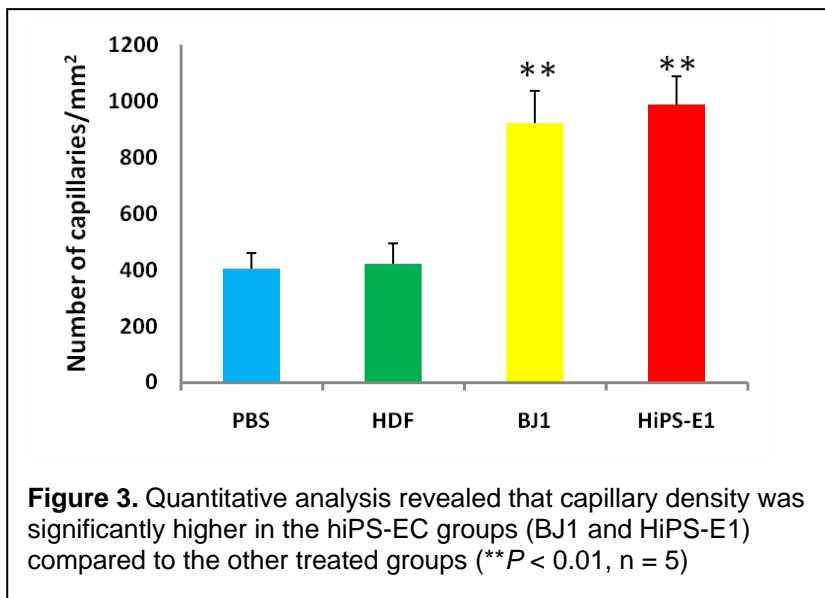


samples, the tissues were examined under the confocal microscopy. As seen in **Figure 2**, a portion of Dil labeled hiPSC-ECs were colocalized with ILB4 stained ECs and orthogonal images and three dimensional reconstruction confirmed the colocalization of injected cells with the EC marker, ILB4. Together these finding clearly suggest that intramuscularly injected hiPS-ECs (KDR^+CD31^+ cells) contribute to new vessel



formation.

We also compared the capillary density in the hiPS-EC injected and control groups. Functional capillary density was counted from histologic sections of the muscles perfused with ILB4. Total 6 sections were averaged for calculating capillary density. As shown in **Figure 3**, capillary density was significantly greater in the hiPS-EC injected groups compared to the control groups at 4 weeks (** $P < 0.01$ vs PBS and HDF). In addition, gross and microscopic examined of the harvested muscle samples demonstrated no teratoma formation or adverse effects during this follow-up period. Currently we are conducting real-time RT-PCR to investigate the paracrine and angiogenic effects of hiPS-ECs on hindlimb ischemia and detailed



histologic studies using short-term and long-term samples to explore serial angiogenic and vasculogenic changes.

Together these results suggest that hiPSC-derived KDR⁺CD31⁺ cells are effective for treating diabetic hindlimb ischemia by augmenting both angiogenesis and vasculogenesis and do not induce adverse effects during 4-week follow-up.