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# Confocal Microscopy Analysis of Human CD34+ Bone Marrow Stem Cells Intravitreal Injection on Retinal Capillary Layers in a Murine Model of Diabetic Retinopathy

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## Purpose

Diabetic retinopathy remains the leading cause of blindness among working-age adults in the United States. Vision loss occurs because diabetes damages the retinal vessels. Currently, retinal laser treatment and anti-VEGF injection therapy are available to reduce vision loss from bleeding and retinal swelling associated with diabetic retinopathy. However, no treatment reverses the retinal vascular damage associated with diabetic retinopathy.

In human bone marrow, there are CD34+ stem cells that include endothelial progenitor cells and hematopoietic stem cells. These cells are mobilized from bone marrow to sites of vascular injury and play an important role in tissue revascularization. Prior research<sup>[1-2]</sup> has shown that intravitreal injection of human CD34+ bone marrow stem cells (BMSCs) results in rapid retinal homing of stem cells with long-term integration of the cells into the vessel wall in murine eyes with transient retinal ischemic insult. The aim of this research was to determine if the intravitreal injection of human CD34+ BMSCs would preserve retinal vasculature in eyes with chronic progressive retinal vascular damage from diabetic retinopathy.

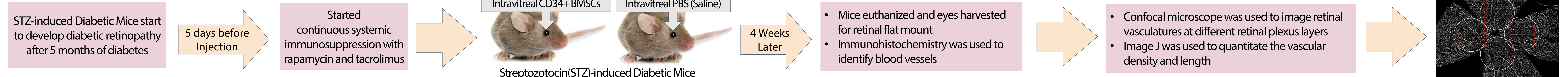
[1] Park SS, Caballero S, Bauer G, Shibata B, Roth A, Fitzgerald PG, Forward KI, Zhou P, McGee J, Telander DG, Grant MB, Nolta JA. Long-term effects of intravitreal injection of GMP-grade bone marrow-derived CD34+ cells in NOD-SCID mice with acute ischemia-reperfusion injury. *Invest Ophthalmol Vis Sci*. 53(2): 986-994. 2012;

[2] Caballero, S., Sengupta, N., Afzal, A., Chang, K.H., Li Calzi, S., Guberski, D.L., Kern, T.S., Grant, M.B. Ischemic vascular damage can be repaired by healthy, but not diabetic endothelial progenitor cells. 2007; *Diabetes* 56, 960-967.

## Methods

Streptozotocin(STZ)-induced diabetic mice were injected intravitreally with either 50,000 human CD34+ BMSCs or Phosphate Buffered Saline(PBS) into the right eye. No injections were administered on the left eye. Continuous systemic immuno-suppression with rapamycin and tacrolimus was applied 5 days before the injection and maintained for 4 more weeks after injection to prevent rejection of human cells. All mice were euthanized 4 weeks after the intravitreal injection and both eyes were enucleated for retinal flat mount immunohistochemistry. The retinal vasculature was stained with Isolectin-GS-IB4. Confocal microscopy was used to image four circular 1.0mm<sup>2</sup> areas of interest of retinas around the optic disc with a depth resolution of around 4 micrometers to observe different layers of the retinal vasculatures. Images of superficial, intermediate, and deep retinal capillary plexus layers in the areas of interest were obtained and analyzed using ImageJ software with the Vessel Analysis plugin to quantitate the vascular density and length of retinal vasculature in the three retinal vascular plexus layers.

Figure 1: Research Project Timeline



## Results

Using confocal microscopy, the three distinct retinal capillary plexus layers could be visualized and imaged in the murine retinal flat mount.

Eyes that received intravitreal injection of CD34+ BMSCs (CD34-R, N=9) had significantly higher vascular density and vascular length in all three capillary plexus layers when compared to the untreated contralateral eyes (CD34-L, N=9) or PBS treated eyes (N=13; p values < 0.05 using one-tailed t-test).

- Vessel Density: % of area covered by vessels / total area of interest
- Vessel Length: Total length of all the vessels in area of interest

Figure 2a-2c: Confocal microscopy images of the 3 retinal vascular plexus layers in a murine eye with diabetic retinopathy 4 weeks after intravitreal injection of human CD34+ BMSCs.

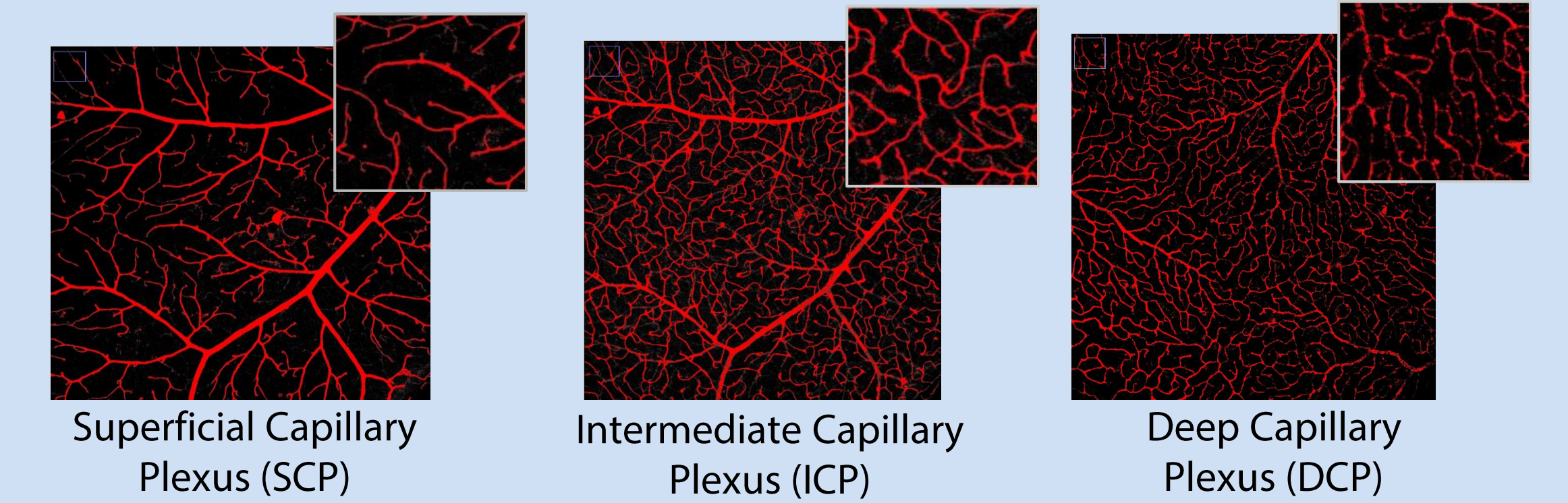


Figure 3a-3c: Vascular Densities and Vascular Lengths of various retinal plexuses.



## Conclusions

- Confocal microscopy could be used to visualize the three retinal capillary layers in a murine model of diabetic retinopathy.
- Intravitreal injection of human CD34+ BMSCs appears to have a protective effect on the retinal vasculature in all three retinal capillary plexus layers in this murine model of diabetic retinopathy.

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