# Diabetic Complications Consortium

**Application Title:** Comparing therapeutic potency of healthy and disease-derived human mesenchymal stromal cells in experimental diabetic nephropathy

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## 1. Project Accomplishments:

Provide broad overview of the accomplishments of this project

Our long-term goal is to develop a novel cell-based therapy for diabetic kidney disease (DKD). Our hypothesis underlying the current studies is that MSC harvested from individuals with DKD (DKD-MSC) have comparable kidney repair effects *in vivo* to age-matched controls (Control-MSC). DiaComp funding has allowed us to pursue studies testing of this hypothesis.

Project Progress:

- IACUC protocol creation, approvals, and modifications
- New staff training in animal care and experimentation
- Development of the accelerated nephropathy model in db/db mice with superimposed Angiotensin II-induced hypertension (dbAngII). Most effort has been spent working to successfully treat diabetic mice with osmotic minipumps containing Angiotensin II. We encountered several challenges due to 1) minipump placement complicated by wound ulcerations and 2) mouse morbidity. We have worked closely with our veterinarians, Jackson Laboratories, and Alzet pump manufacturer to troubleshoot potential contributing factors. db/db mice require special care in our hands for optimal outcomes and significant expenses have been encountered. Fortunately, the accelerated nephropathy model shows promise (see below).
- Our goal is to compare our dbAngII model with a well-characterized accelerated nephropathy model of DKD. However, study of eNOS db/db mice is also complicated by several barriers. Due to significant breeding difficulties, cell lines are cryopreserved and breeding for dedicated supply projects is necessary. Our lab worked with Jackson Laboratory and recently received breeder pairs to minimize costs. However, we are also revising agreements to pursue Jackson Lab breeding plans to provide genotype mice of interest, albeit at substantial cost.
- Transplantation of human adipose-derived MSC in Type 2 diabetes mouse model. We successfully initiated experiments infusing human MSC via tail vein to our dbAngII mice. Despite small study groups, early findings show evidence of MSC-induced repair. We will continue to assess MSC kidney repair effect differences between DKD-MSC and Control-MSC for Aim 1 and Aim 2.

The DiaComp grant has provided essential support to allow a new lab the opportunity to translate *in vitro* findings to experiments *in vivo*. Substantial progress was made in this study. However, we have also encountered several unanticipated challenges including PI and lab team transitioning to a new institution and the COVID-19 pandemic. Despite

the numerous pauses in activity, we are on the trajectory to complete the proposed studies. We are indebted to DiaComp for funding our project which has helped advance our overall mission to contribute to science, improve opportunities for intramural and extramural funding, and enhance academic advancement towards an independent research program.

## 2. Specific Aims:

For each specific aim provide the data (includes figures and tables) and progress in each aim.

**Aim 1:** Test the hypothesis that DKD-MSC exhibit similar potency vs. Control-MSC to repair mouse kidneys *in vivo* by reducing albuminuria, kidney function, inflammation, and fibrosis in advanced experimental DKD.

- As noted above, that majority of effort has been put into optimizing the dbAngII model. Implantation of angiotensin II (1,000 ng/kg/min) minipump resulted in increased fibrosis (trichrome staining) and higher protein excretion rate (24 hour urine protein) at 4 weeks compared to controls (C57BL6 and saline pump db/db) as shown in Figure 1.
- Our preliminary work transplanting human MSC (DKD-MSC or age-matched Control-MSC[n=1]) or vehicle is also encouraging. At 2 weeks following a single tail vein infusion in dbAngII mice, creatinine was lower, and a trend was observed for lower proteinuria in DKD-MSC compared to vehicle-treated groups. Only 1 animal was treated with Control-MSC therein limiting comparisons. Assessment of kidney injury markers was further undertaken by examining gene expression (Figure 2). Markers of fibrosis (Collagen I), kidney injury (Kidney Injury Marker-1; KIM-1) and inflammation (tumor necrosis factor-α; TNF-α) were assessed. AngII infusions resulted in increased Collagen I and KIM-1, but surprisingly TNF-α was lower. Following MSC infusion, no statistically significant differences were found in adjusted analyses.
- These investigations represent a first in which human DKD-MSC are compared to age-matched Control-MSC to compare therapeutic efficacy *in vivo*. Initial results are encouraging, and we will continue to work diligently to complete planned studies over the next 1-2 years.

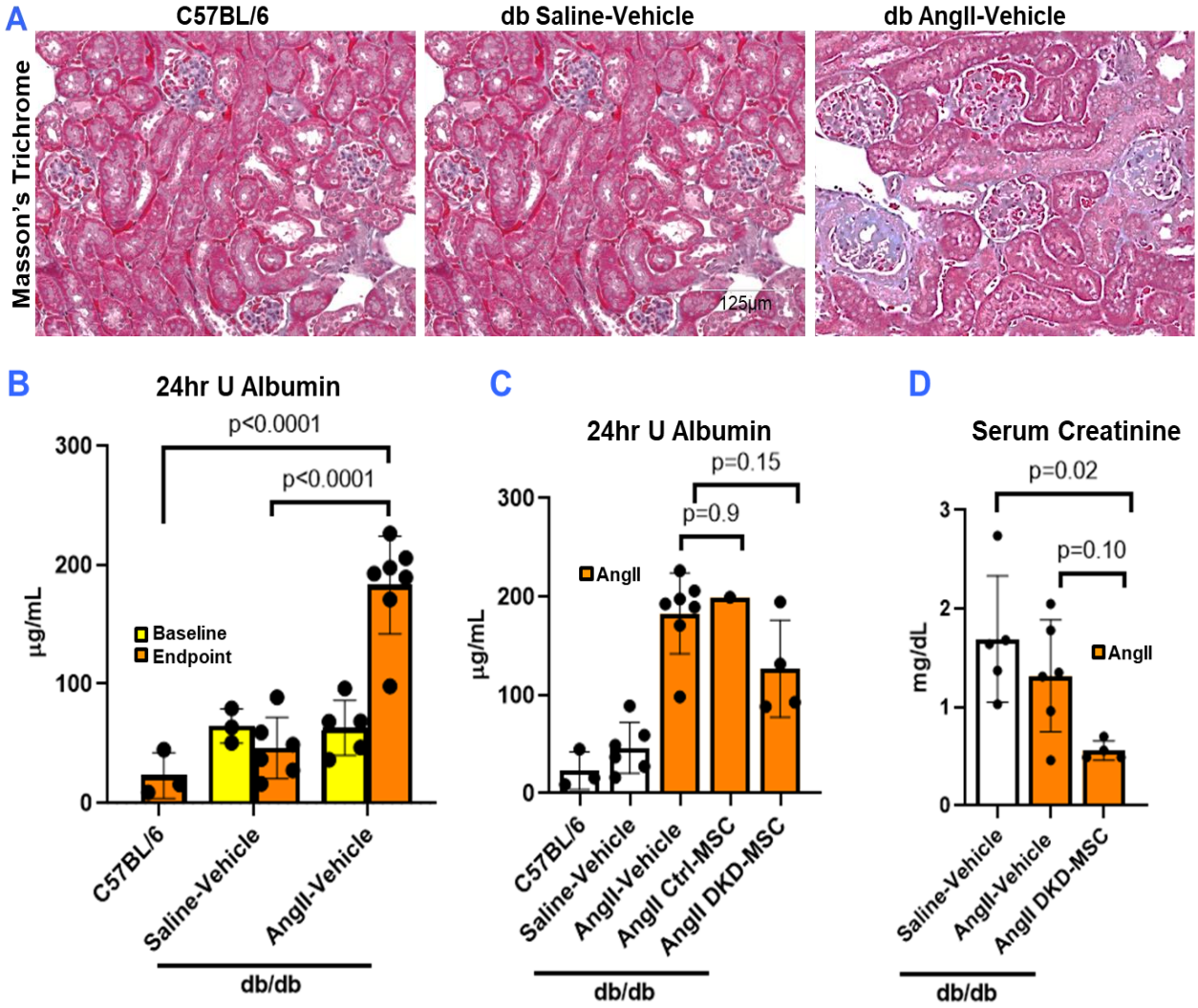


**Figure 1. Type 2 Diabetes accelerated nephropathy mouse model**. In db/db mice, angiotensin II (AngII) infusions via osmotic mini-pump (1000 ng/kg/min; 4-week duration) increased markers of kidney injury including fibrosis (A) in trichrome-stained kidney sections compared to C57BL/6 and sham (Saline) infusion treated mice. Following 4-week infusion of AngII, db/db mice also exhibited progressive albuminuria compared to control groups (B). A single injection of MSC ($5x10^5$ cells in 200µL PBS via tail vein) led to attenuated albuminuria (C) and (D) improved kidney function (serum creatinine) in DKD-MSC treated vs. AngII Vehicle treated mice. Comparisons were limited in the Ctrl-MSC group (n=1). Saline-Vehicle: PBS mini-pump and vehicle injection; AngII-Vehicle: angiotensin II mini-pump and vehicle infusion; AngIICtrl-MSC: angiotensin II mini-pump and vehicle infusion. Adjusted p-values are shown.

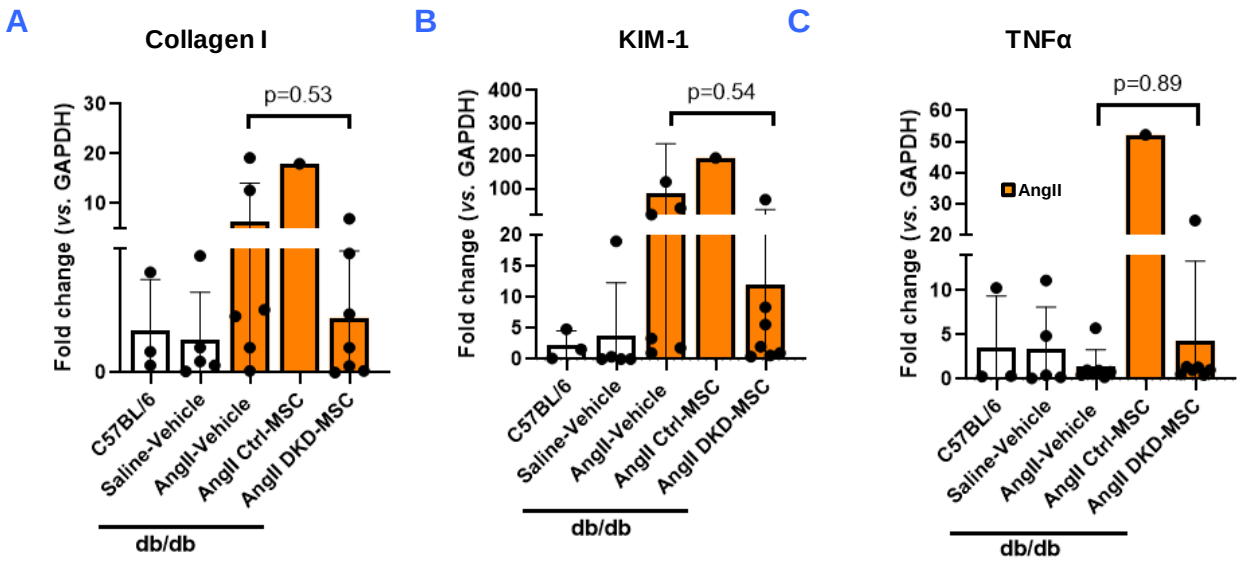


**Figure 2.** Type 2 Diabetes accelerated nephropathy mouse model. In db/db mice, angiotensin II (AngII) infusions via osmotic mini-pump (1000 ng/kg/min; 4-week duration) increased kidney gene expression of: (A) Type I collagen and (B) kidney injury marker-1 (KIM-1). Surprisingly, tumor necrosis factor-α (TNF-α) was lower in treated mice compared to db/db mice with sham (PBS) infusions (C). A single injection of DKD-MSC ($5x10^5$ cells in 200µL PBS via tail vein) did not significantly reduce AngII injury effects at 2 weeks compared to the vehicle injection group. Saline-Vehicle: PBS mini-pump and vehicle injection; AngII-Vehicle: angiotensin II mini-pump and vehicle infusion; AngIICtrl-MSC: angiotensin II mini-pump and vehicle infusion. Adjusted p-values are shown.

**Aim 2:** Test the hypothesis that DKD-MSC exhibit similar potency vs. Control-MSC to repair mouse kidneys *in vivo* and that this is associated with restored microvascular density and a reduction in kidney hypoxia

- We are working to obtain necessary data for this Aim after optimizing studies in Aim 1.

## 3. Publications:

We are still gathering needed data and are optimistic that these studies will be considered of high scientific relevance leading to publication in strong diabetes or kidney focused journal within the next 1-2 years.

Given our enthusiasm and background review for the DiaComp project, we conducted a systematic review and meta-analysis of 40 original investigations testing cell-based therapies in DKD animals. This manuscript is undergoing minor revisions for *Stem Cell Therapeutics and Medicine Journal* and we are hopeful that it will be accepted for publication in 2021. Going forward, we hope to update this systematic review and meta-analysis to include our future DiaComp-funded and published investigations as well.

We recently submitted a revised manuscript to *Diabetes Journal* which assessed MSC-DKD *in vitro* function compared to age-matched controls. The *in vivo* function of these MSC are now being studied in accelerated mouse models of the DiaComp project.

Finally, we highlighted the promise of MSC for treatment of DKD in our invited review entitled, “Progress toward the clinical application of mesenchymal stromal cells and other disease-modulating regenerative therapies: Examples from the field of nephrology” for *Kidney360 Journal* (forthcoming).

**NIDDK Pilot Award**


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