Diabetic Complications Consortium

Application Title: Muscle-derived mesenchymal stem cells (mMSCs) as a novel regenerative therapy to repair and reverse diabetic vascular complications

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Project Accomplishments:

Peripheral arterial disease is a debilitating complication of type-1 diabetes mellitus (DM-1). Use of mesenchymal stem cells (MSCs) represents an important direction for the field of regenerative medicine. Our group has previously developed a novel αvβ3-integrin targeted PET-CT tracer (^{64}Cu-PEG$_4$-cRGD$_2$) for PET imaging of peripheral angiogenesis. In this study we applied a comprehensive approach to investigate the potential for transplanted muscle-derived MSC's to stimulate angiogenesis (assessed with PET-CT imaging) and improve muscle function in diabetic mice with hindlimb ischemia.

Our results strongly indicate that muscle-derived MSC's do enhance angiogenesis, but the concomitant increase in blood flow and improvement of muscle function remain ambiguous. Larger sample sizes and a more refined measurement of muscle function will enable us to better resolve these effects. Based on our results we hypothesized that MSCs could affect arteriogenesis and current studies are focused on development of quantitative strategy to assess maturation of angiogenic vessels. Future work will also focus on studying stem cell viability after transplantation, and the mechanisms by which they promote angiogenesis and arteriogenesis.

Specific Aims:

Aim 1: To serially assess peripheral angiogenesis (with PET-CT imaging of $\alpha_V\beta_3$ integrin activation) in the ischemic muscle of diabetic mice following the implantation of MSCs.

Methods:

Isolation of mesenchymal stem cells (mMSCs) from skeletal muscle:

Five week old C57BL/6 mice gastrocnemius-soleus complexes were excised, mechanically and enzymatically digested, filtered, and incubated with anti-mouse CD16/CD32 in order to block non-specific Fc-mediated interactions. Cells were stained with monoclonal anti-mouse Sca-1-PE and CD45-APC antibodies and isolated with FACS. Sca-1+CD45- cells were collected in medium for culture (**Figure 1**).

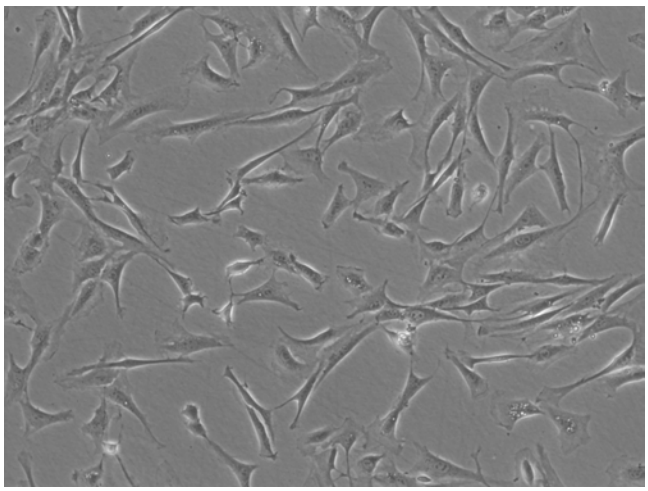

Figure 1: mMSCs at 10x with phase contrast. Stem cells were derived from skeletal muscle and treated to prevent non-specific Fc-mediated interactions, stained with monoclonal anti-mouse Sca-1-PE and CD45-APC antibodies, isolated with FACS, then cultured in medium.

Animal Experimental Model:

Type-1 DM was induced by streptozotocin (STZ) administration (50mg/kg, i.p. daily for 5 consecutive days) in 2-month old wild-type mice (C57BL/6). Control (n=10) and DM mice (n=10) were anesthetized with isofluorane and unilateral hindlimb ischemia performed (**Figure 2**). All animals were divided into two groups: untreated DM (n=10), and DM receiving mMSCs injections (n=10).

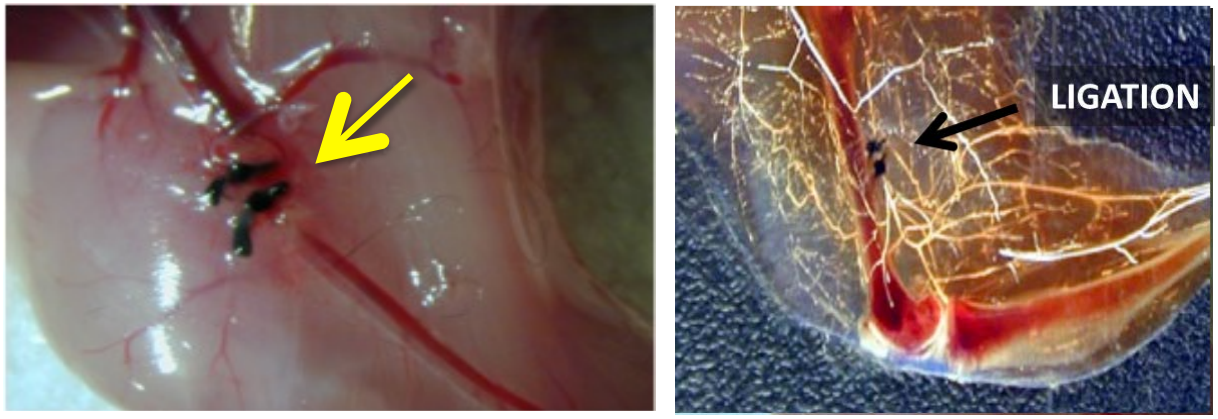

Figure 2: Surgical ligation of right femoral artery induced unilateral hindlimb ischemia and stimulated peripheral angiogenesis in mice. mMSCs were injected immediately after the surgery into muscle segments distal to the ligation (yellow arrows).

Results:

Peripheral angiogenesis was stimulated by muscle-derived MSCs via activation of $\alpha v\beta_3$ integrins. PET-CT images were acquired using Siemens Inveon scanner (**Figure 3)**. MicroPET images were fused with microCT images and quantified using a semi-automated approach. Briefly, complex irregular volumes of interest (VOIs) were generated from the microCT images and applied on the co-registered microPET images to calculate absolute ^{64}Cu activities. These complex VOIs included only soft tissue (muscle) structures after the removal of bone structures during the image segmentation process.

PET-CT imaging demonstrated significantly ($P<0.05$) increased uptake of ^{64}Cu-PEG$_4$-cRGD$_2$ (targeted at the activated $\alpha v\beta_3$ receptor as a marker of angiogenesis, **Figure 4**) in stem cell-treated diabetic mice at 1 week after the onset of ischemia vs. non-treated controls. This observation was confirmed by immunohistochemical analysis of tissue sections (**Figure 5**).

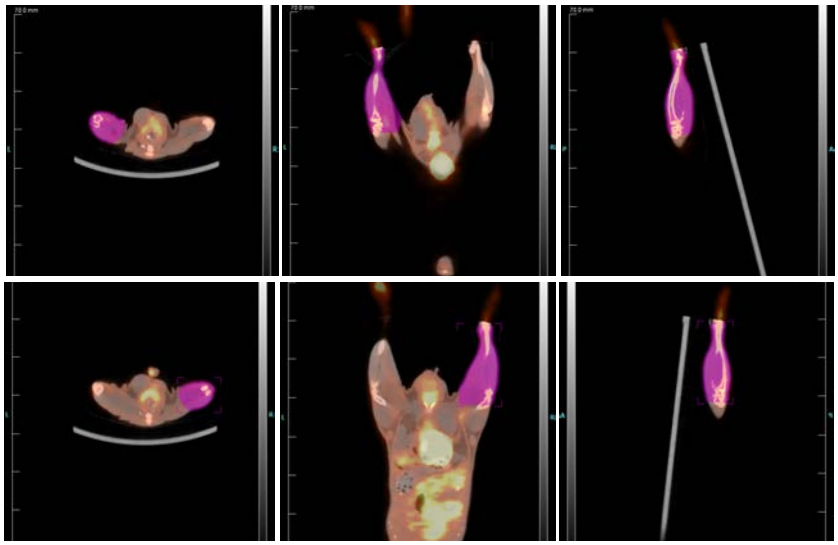

Figure 3: Representative microPET-CT images of peripheral angiogenesis with defined VOIs for image analysis (red).

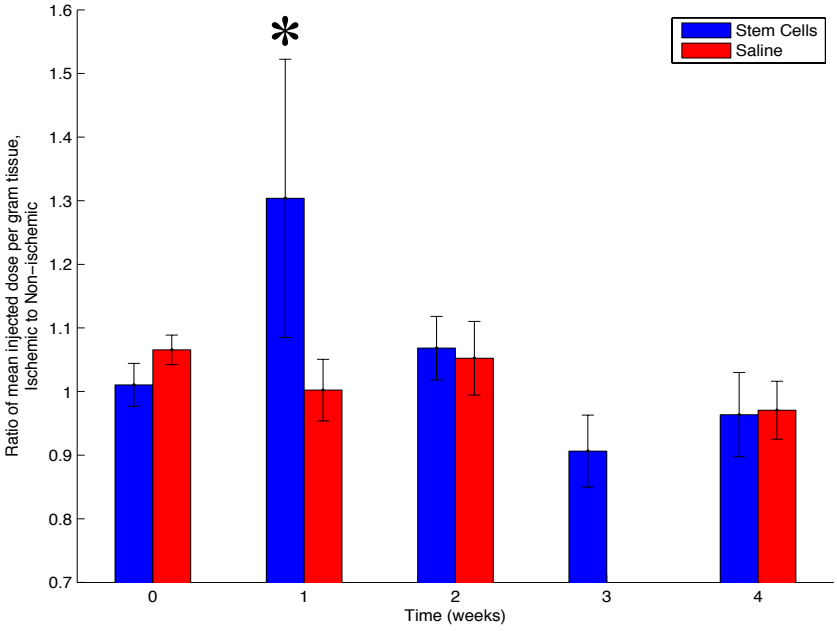

Figure 4: Bar plot showing the ratio (ischemic leg to non-ischemic leg) of mean injected dose per gram tissue of the ^{64}Cu-PEG$_4$-cRGD$_2$ tracer. One week after dosing, the stem cell-treated mice show significantly enhanced angiogenesis in their ischemic leg relative to saline-treated control mice.

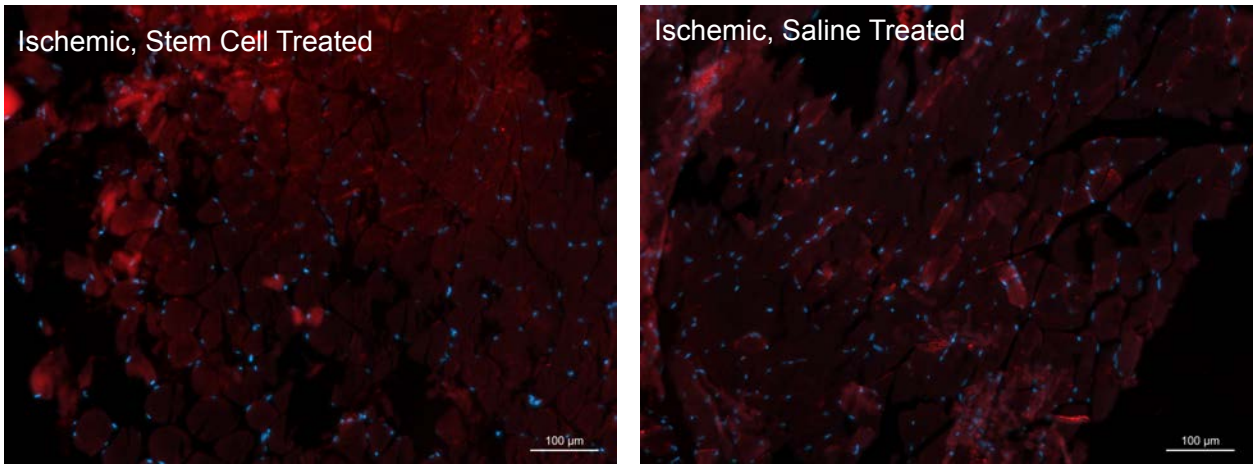

Figure 5: Immunohistochemistry (IHS) staining revealed enhanced CD31 staining in the stem cells treated ischemic muscles of diabetic mice at 1 week after the mMSCs transplantation. This is indicative of stem cell-induced angiogenesis, further supporting the PET-CT results.

Aim 2: To evaluate whether the MSCs-related changes in angiogenesis, arteriogenesis, and flow will result in the repair and functional recovery of diabetic muscle. The recovery of muscle function in mice was assessed with the measurement of grip strength before and at 1, 2 and 3 weeks after the surgery. The recovery of blood perfusion was evaluated at 1 and 2 weeks after surgery with a needle-type laser Doppler probe.

Methods:

Grip Strength:

The potential of MSCs to recover the muscle function in mice was assessed with the measurement of grip strength before and at 1, 2 and 3 weeks after the surgery (**Figure 6**).

Figure 6: Current grip-strength assay.

Blood Flow:

The recovery of blood perfusion was evaluated at 1 and 2 weeks after surgery with a needle-type laser Doppler probe (**Figure 7**).

Figure 7: Laser Doppler probe to measure blood perfusion in mouse hindlimb.

Results:

Despite increased angiogenesis (as assessed with PET-CT imaging using targeted agent), diabetic mice did not show statistically significant increase in blood flow (**Figure 8**) or grip strength (**Figure 9**) after the therapy with mMSCs. Current studies are focused at increasing number of animals in each group.

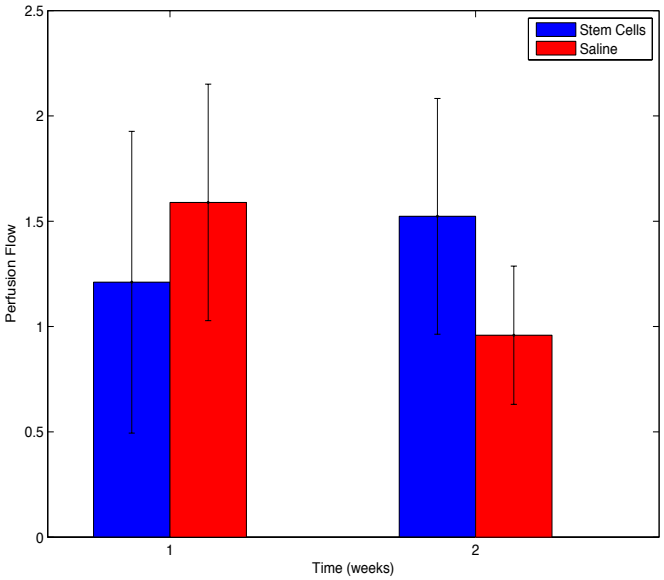

Figure 8: Blood flow recovery assessed in mice at 1 and 2 weeks after surgical ligation of right femoral artery with needle-like laser Doppler probe in DM mice treated with MSCs or saline (control).

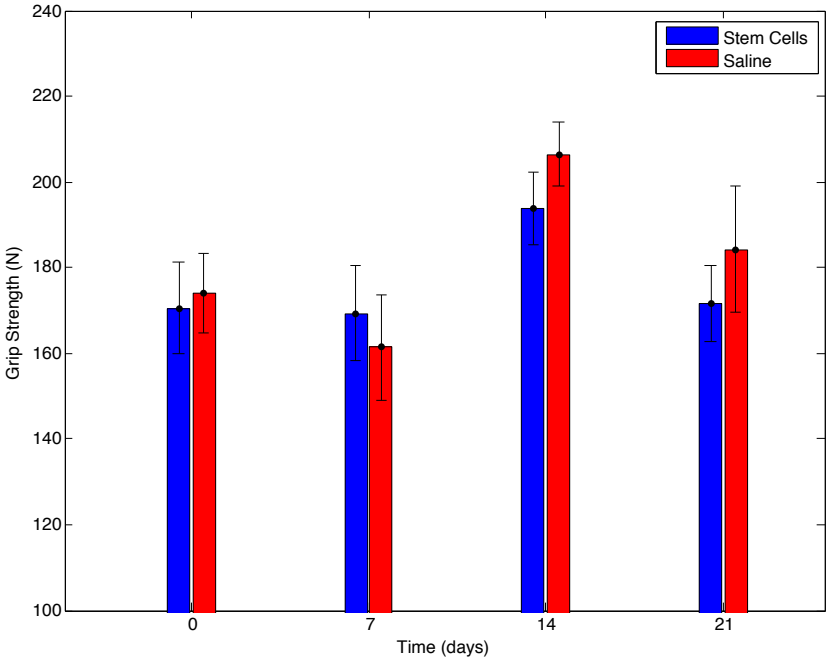

Figure 9: There were no statistically significant changes in grip strength observed between mMSCs treated and control groups.

Publications:

1. Hedhli et al, “Muscle-derived mesenchymal stem cells (mMSCs) as a novel regenerative therapy to repair and reverse diabetic vascular complications” (abstract submitted for the annual meeting of SNMMI)
2. Hedhli et al, “Muscle-derived mesenchymal stem cells (mMSCs) as a novel regenerative therapy to repair and reverse diabetic vascular complications” (manuscript in preparation)
3. Slania et al. “Evaluation of novel dimeric-cRGD peptide for targeted PET-CT imaging of peripheral angiogenesis in diabetic mice” (manuscript submitted to Journal of Nuclear Medicine)