

MODIFIED SKELETAL MYOBLAST THERAPY FOR CARDIAC FAILURE USING AAV SDF1

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Introduction: Myoblast therapy for ischemic cardiac failure has been successfully tried in many animal models and also extended to human clinical trials. This pre-clinical study was designed to evaluate a safe and efficient method of gene delivery by Adeno-associated virus (AAV) to human myoblasts. SDF-1 α (Stromal Derived Factor 1 α) and its receptor, CXCR4, are essential for cardiogenesis and vasculogenesis during embryonic development. SDF-1 α levels are elevated post-ischemic injury and has a role in the repair and regeneration of the damaged myocardium. This study also involved the transplantation of superparamagnetic iron oxide (SPIO) labeled human myoblasts to track the progress of the transplant. Post-transplant cardiac MRI was done to evaluate cardiac functional recovery and the changes in cardiac wall dimensions following transplant. We analyzed the outcomes of AAV1 SDF1 transduced myoblasts expressing SDF1 α to media injected controls. This study also evaluated the effect of delivering myoblasts in scaffolds (hydrogel) and nutrient hydrogel(enriched with hepatocyte growth factor-HGF), which provides a temporary foundation that helps increase retention of cells at the site of delivery.

Materials and Methods: Cell labeling and SDF-1 transduction of human myoblasts: Human myoblasts (Bioheart Inc. Sunrise, FL) were labeled using poly-L-lysine (375ng/mL) and Feridex(50 μ g/mL) in this study though our later trials found more efficient labeling

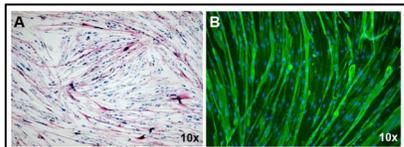


Fig 1. Protamine sulphate/feridex labeled myoblasts stained with Prussian blue(A)with good Feridex uptake and desmin expression(B) at 2weeks.

with Protamine sulphate (5 μ g/mL) and Feridex (50 μ g/mL)(Fig1). Labeled cells were spun down at 200g for 10minutes and re-suspended to the desired concentration in HTc (Hypothermosol ,BioLife Solutions, Inc., Owego, NY) to deliver 4million cells per animal in 150 μ l of HTc. AAV SDF1 infection or cells was carried out at an MOI of 10⁴ viral particles per cell. **Myocardial Infarction and myoblast transplantation:** Myocardial infarction was created in 8 weeks old

nude rats. Echocardiography was done to screen the animals for significant infarcts. Baseline cardiac MRI was performed 3 weeks post-infarction. SPIO labeled myoblasts were injected with or without AAV-SDF-1 transduction into the infarcted myocardium. **MRI:** Cardiac function was assessed by MRI at 3 weeks post-infarction and at 1,4 and 8 weeks post-transplant. Cardiac MRI was performed on a 4.7 T Bruker Avance spectrometer using acquisition triggering at the peak of the R wave (SA instruments). Celltransplants were imaged using a Fast Low Angle Shot (FLASH) sequence (matrix = 256 · 128, TE = 4 ms, slices = 12, thickness = 1 mm, FOV = 5 · 5 cm²). Pulse repetition time (TR) was dependent on the R-R interval (~200 ms). Control animals receiving non-transduced myoblasts transplants were also followed by the same MRI protocol. The images were analyzed using CAAS MRV software (Pie Medical Imaging, Netherlands). The endocardium and epicardium were traced through 8 slices in end-diastolic and end-systolic phases to determine the regional wall motion and ejection fraction, stroke volume, and cardiac output. **Immunohistochemistry:** The transplanted tissues were analyzed with antibodies specific to human skeletal and cardiac muscle lineage.

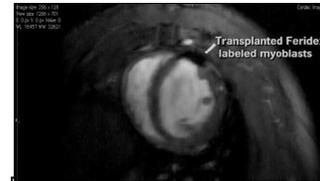


Figure 2. Short-axis MRI images from an infarcted nude rat heart transplanted with SPIO labeled human myoblasts 1 week post-transplant.

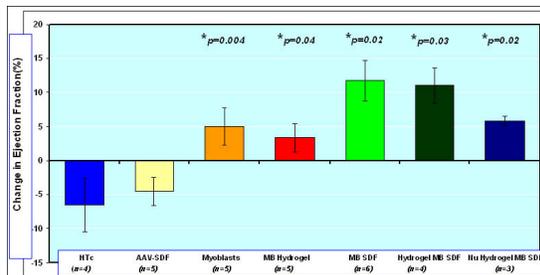


Figure 3. Changes in the ejection fraction (EF) between the different treatment groups. AAV1 SDF1 transduced myoblasts had improved ejection fraction at 8 weeks with an increased EF of 11.7 \pm 2.9% to the 3 week baseline.

are as shown in Figure 3. Control (HTc) injected hearts show a consistent deterioration of function. The AAV SDF1 only injected animals also did not show any improvement in cardiac function. The AAV1 SDF1 transduced myoblasts (MB SDF) had improved ejection fraction at the end of 8 weeks with an increased EF of 11.7 \pm 2.9% to the 3 week baseline. AAV1 SDF1 transduced myoblasts in hydrogel (HydrogelMB SDF) showed an early improvement of cardiac function and had an increased mean ejection fraction of 11.0 \pm 2.5%. The Nutrient hydrogel MBSDF (Nu Hydrogel) and myoblasts without any SDF1 had a moderate improvement of the ejection fraction with mean values of 5.8 \pm 0.8% and 5.1 \pm 2.6% respectively to baseline at the end of 8weeks.

Conclusion: This study demonstrates the efficacy of combining viral induced gene transfer with cell therapy in significantly improving post-ischemic cardiac function. AAV transduced myoblasts with SDF1 demonstrates a definite improvement of cardiac function compared to using isolated myoblasts in athymic nude rats. The improvements could be attributed to the improved contractility due to the transplanted myoblasts with the added benefit of cells recruited to the myocardium by SDF1. Immunohistochemistry revealed enhanced transplant tissue and angiogenesis in the infarcted myocardium which may also contribute to the improvement of indices of left ventricular function.

Acknowledgement: Bioheart Inc. Sunrise Florida for partially funding the project.

Results: We were able to successfully transplant human myoblasts intramyocardially in the post-infarction of nude rats.

The SPIO labeled cells were tracked till the end of the study (8 weeks). The changes in ejection fraction

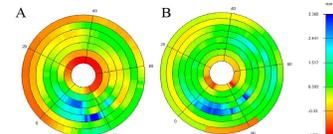


Figure 4. Bullseye plot of regional wall motion (A) 3 weeks post-infarction with an Ejection Fraction of 67.98% and (B) 8 weeks post-transplantation with an Ejection Fraction of 77.87%.

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