Effect of SIRT1 Increased Expression on Myogenesis and Angiogenesis In Tissue Engineered Skeletal Muscle

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Introduction. Type 2 diabetes (T2D) is a metabolic disorder that negatively impacts angiogenesis and myogenesis in skeletal muscle tissue. Specifically, differentiation of muscle satellite cells in T2D is commonly impaired along with reduced capillary density. Therefore, there is a need to study key signals responsible for these detrimental effects to identify novel therapies. One of the possible targets is Sirtuin 1 (SIRT-1), a histone deacetylase protein responsible for the downstream regulation of many myogenic and angiogenic genes involved in the homeostasis of skeletal muscle[1]. Based on these premises, tissue-engineered skeletal muscle (TE-SkM) constructs comprised of the progeny of satellite cells, muscle precursor cells (MPCs) infected with an adenovirus to promote SIRT-1 overexpression were fabricated. A preliminary analysis was carried out to determine the effect of increased SIRT-1 on the expression of myogenic and angiogenic factors in healthy and diabetic TE-SkM.

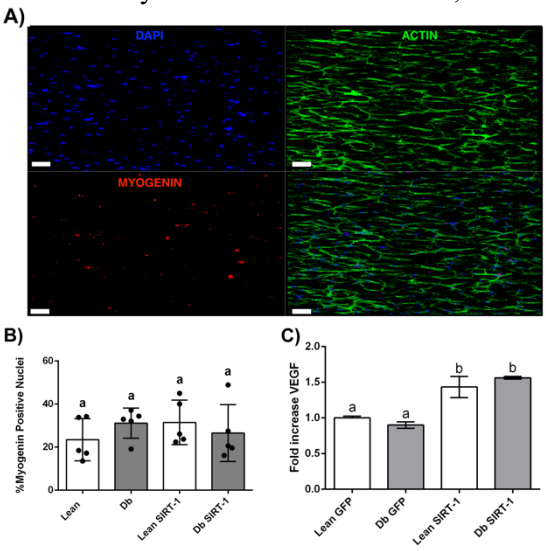

Figure 1. A) Fluorescent images of Actin, DAPI and myogenin staining of TE-SKM. Scale bar = 80 μm. B) Quantification of myogenin using Python code (5/group). C) VEGF Elisa results (4/group). Different letters signify statistical difference ($p < 0.05$).

Materials and Methods. MPCs were isolated from the skeletal muscle of the lower limbs of lean (FA/+; n=1) or obese (FA/FA; n=1) male Zucker diabetic fatty (ZDF) rats. MPCs (passage 2) were cultured in plates coated with Matrigel (0.1 mg/mL). Cells were allowed to proliferate up to 80% of confluency and then infected with an adenovirus (2000 MOI) engineered to overexpress SIRT-1 (ViraQuest, North Liberty IA). Infected and uninfected lean and diabetic MPCs were seeded within fibrin gels and cast into silicone wells ($1.0x10^6$ cells/mL) to form TE-SkM, similar to that described previously[2]. MPCs were allowed to mature for 14 days and then fixed using 4% paraformaldehyde for 2 hours. Constructs were washed with PBS and stained with Phalloidin tagged with Alexa-Fluor-488 to visualize actin filaments and DAPI for the nuclei. Additionally, cells were probed with the primary antibody against rat myogenin (5 ng/μL) diluted in goat serum at 10% and a mouse IgG secondary antibody diluted in PBS (10 μL/mL) tagged with Rhodamine Red. Confocal images were taken at a magnification of 10x (Leica TCS SP8 Confocal Microscope (Buffalo Grove, IL). Images (5/group) were analyzed using Python code with the aid of the OpenCV library (Intel). The program separated the images into three color channels, counted the number of nuclei and myogenin expressing nuclei using simple blob detection, and returned the detection figures to the user. Finally, the culture media from the TE-SkM constructs was collected every other day during the maturation, and the secreted vascular endothelial growth factor (VEGF) was quantified using an ELISA (4/group). One-way ANOVA (GraphPad Software, Inc., La Jolla, CA) was used to determine statistical differences among groups tested.

Results and Discussions. Confocal analyses of the TE-SkM constructs displays the alignment of MPCs along the axis due to the passive tension generated by the metallic pins placed at the extremity of the constructs. Cells were successfully differentiated, as displayed by the expression of myogenin (Figure 1A), which is an important regulator of myogenic differentiation. Evaluation of myogenin-positive nuclei among the different groups tested displayed no significant difference (Figure 1B), suggesting a limited impact of SIRT-1 overexpression on myogenesis in this context. In contrast, VEGF was found in a higher concentration in the culture media collected from the TE-SkM of infected MPCs, indicating SIRT-1 influences the secretion of angiogenic growth factors (Figure 1C).

Conclusions. Based on these preliminary results, SIRT-1 overexpression did not significantly influence myogenin expression, but further investigation is required to test its impact on other myogenic factors such as MyoD or myosin heavy chain. However, SIRT-1 seemed to play a more significant role in the modulation of the angiogenic response of MPCs and could potentially represent a target for therapies aimed at restoring healthy microvasculature in the skeletal muscle of diabetic patients.

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References. 1. Myers et al., Journal of Cachexia, Sarcopenia and Muscle 2019 (10): 929-949.
2. Acosta et al., Tissue Engineering Part A, 2020 (15-16): 905-914.