Skeletal Muscle Tissue Engineering

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Introduction

Damage to muscle cells results in the formation of scar tissue. The scar tissue formed does not function at the same level as the original muscle tissue, and therefore innovative treatments are being sought out in order to preserve muscle function. Muscle tissue engineering allows for the implantation of differentiated muscle cells grown in vitro as a means of repairing damage while maintaining muscle function. It has been shown through previous research that growing muscle tissue from myoblasts alone does not yield optimal results, and the addition of endothelial cells further promotes the maturation process of the myoblasts and vessel networks[1]. It has also been suggested that there is a difference between adult and umbilical endothelial cells in their ability to promote muscle tissue differentiation[2]. The purpose of the experiments is to determine how adult endothelial cells differ from umbilical endothelial cells in regard to promoting muscle tissue regeneration.

Methods and Materials

Western blot was used in order to check for specific proteins in human myoblasts. Proteins were separated by size using gel electrophoresis. Gel stacking solution was added into the gel solidification rack and running gel was poured on top into the electrophorator. Samples and weight markers were added to the wells. A current was run through the gel until the tracking dye reached the bottom of the gel. The proteins were then transferred from the gel to a membrane. The first antibody was added. After washing the first antibody with PBS, the second antibody was added, and the membrane and gel were imaged.

Tri-culture containing human umbilical endothelial cell tri-culture (HUVEC) or adult endothelial cells with smooth muscle cells and myoblasts were constructed in vitro and implanted into the abdominal walls of mice. 9 days post implantation, muscle tissue were retrieved and cryosectioned for immunofluorescent staining. The muscle tissue was treated with 0.5% Tween solution to create pathways through the cell membranes. The cells were then rinsed with PBS and blocked with 5% bovine serum albumin (BSA). Desmin or myosin heavy chain (MYH) antibody solution (1:50) was added to the muscle tissue. After being washed with PBS, a secondary antibody solution was added to the tissue samples, producing fluorescence.

Results

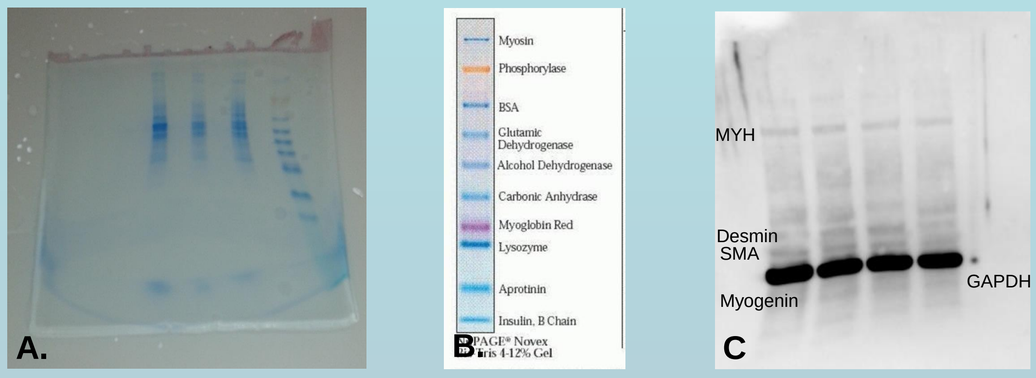

Figure 1. Results of the Western blot from the membrane and the gel. (A) The gel stained for all proteins in the samples. (B) A scale of proteins and their patterns. (C) Western blot results. Myogenin, GAPDH, SMA, Desmin, and MYH are visible. Myogenin is 25 kDa, GAPDH is 37 kDa, SMA is 42 kDa, Desmin is 53 kDa, and MYH is 220 kDa.

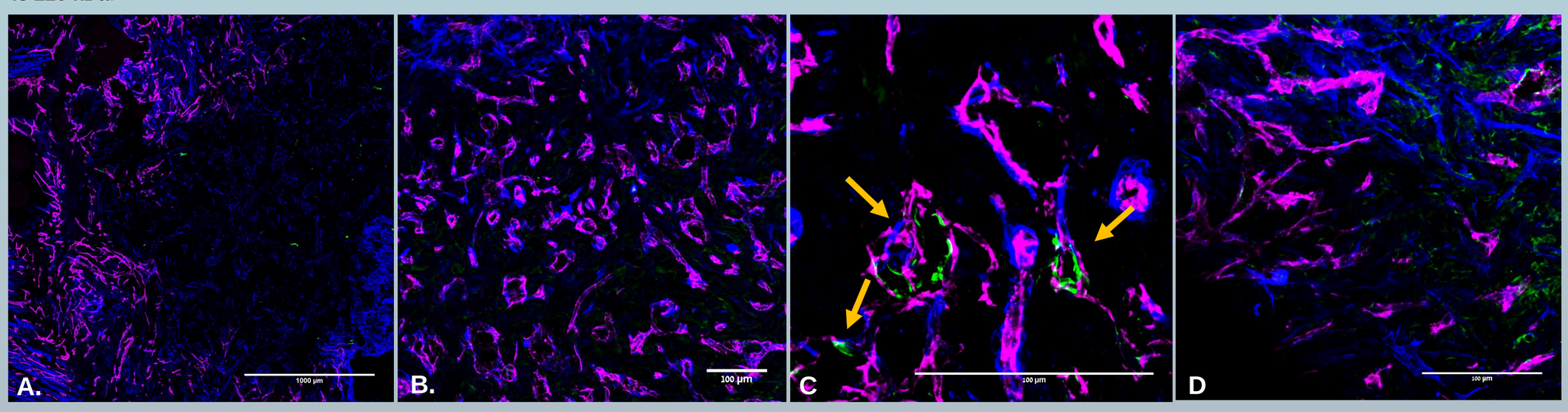

Figure 2. Tri-cultures of endothelial cells, smooth muscle cells, and myoblasts stained with Desmin (blue). Mouse endothelial cells are stained magenta, muscle cells are stained blue, and implanted human endothelial cells are stained green. (A) HUVEC3 tri-culture x10 scaffold area tile scan. (B) HUVEC5 tri-culture x10 magnification. Mouse endothelial cells are integrated into the muscle structure. (C) Adult endothelial cell tri-culture x40 magnification. Endothelial cells are attached to myoblasts and implanted human endothelial cells. (D) Adult endothelial cell tri-culture x20 magnification. Muscle and vessel networks are connected. More connections between implanted and host endothelial cells were seen in adult tri cultures than in HUVEC tri culture.

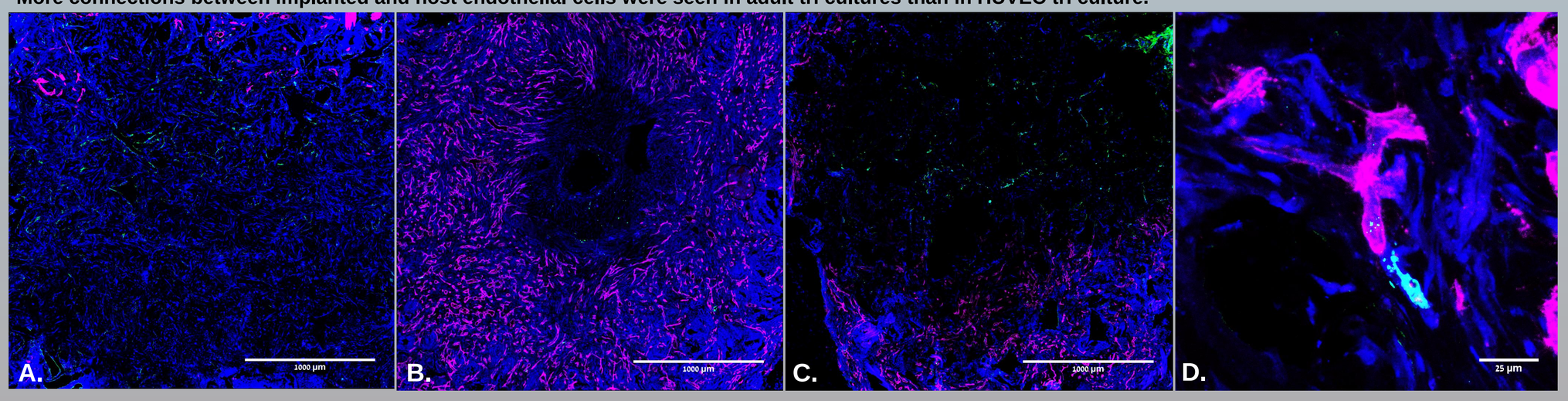

Figure 3. Tri-cultures of endothelial cells, smooth muscle cells, and myoblasts stained with Myosin Heavy Chain (MYH) (blue). Mouse endothelial cells are stained magenta, muscle cells are stained blue, and implanted human endothelial cells are stained green. (A) HUVEC1 tri-culture x10 scaffold area scan. (B) Adult endothelial cell tri-culture tile scan of scaffold area, x10 magnification. (C) Adult endothelial cell tri-culture x10 magnification tile scan. (D) HUVEC4 tri-culture x40 magnification. Mouse endothelial network is connected to the human endothelial network.

Discussion and Conclusions

The results demonstrate that all of the proteins tested for in the Western blot were present in human myoblasts, with MYH and Desmin being the most abundant. SMA and Myogenin were not present in large quantities (Figure 1), and therefore we did not include it in the immunofluorescent-staining. Tri-cultures containing adult endothelial cells contained more vessel networks than tri-cultures containing umbilical endothelial cells (Figures 2 and 3). The increase in vessel networks suggests that adult endothelial cells are better than umbilical endothelial cells at promoting vascularization of muscle tissue. There was no difference between MYH and Desmin stainings, suggesting they are both present in equal amounts (Figures 2 and 3).Using adult endothelial cells in the tri-culture as opposed to umbilical endothelial cells could be beneficial for applying muscle tissue engineering in the clinic.

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References

1.Levenberg et al., Engineering Vascularized Skeletal Muscle Tissue, Nature Biotechnology (2005)
2.Perry et al., Elderly Patient-Derived Endothelial Cells for Vascularization of Engineered Muscle, Molecular Therapy (2017)