Graft Vascularization for Skeletal Muscle Regeneration

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STATEMENT OF PURPOSE:

A key component of tissue engineered constructs is the presence of a vasculature capable of sustaining cell viability[1]. Scaffolds containing predeveloped networks have the distinct benefit of being able to circumvent the need for vessel infiltration from the host, thereby accelerating tissue perfusion. This is an especially useful concept for the treatment of volumetric muscle loss, where without adequate perfusion myogenic cell transplantation is limited and fibrosis ensues. Fibrin has been previously investigated for skeletal muscle grafts, but limited data exists on its utility when both vascular cells and myogenic cells are included. In this study, we evaluate vascular density and co-culture conditions of rat myoblasts and human microvascular cells (MVCs) as well as rat primary microvascular fragment-derived stem cells (MVF-SCs) engrafted in fibrin gels for the development of a vascularized construct to accelerate skeletal muscle regeneration.

METHODS:

Fibrin using previously reported methods[2] was fabricated at a density of 5.6 mg/mL. In the first study, 3D gels were seeded with MVCs (2.5×10^5, 5×10^5, 1×10^6) or with L6 rat myoblasts (10^6 L6/ 10^5 MVC, $5x10^5$ L6/ $5x10^5$ MVC or 10^5 L6/10^6 MVC per ml) for 7 days to evaluate the optimal seeding conditions for VEGF production. In the second study, MVF-SCs were evaluated as strategy means to achieve vascularization by seeding at 20,000 and 40,000/ml on matrigel and their capillary-like tube formation visualized. The 20,000 MVF-SC group was then cultured with 10^5 L6 myoblasts and cultured for 7 days and VEGF was measured. In both studies VEGF production was measured in supernatants and normalized to cell number using a dsDNA kit, and the groups were compared using 2-way ANOVA across time & materials with Tukey's post hoc test ($p<0.05$).

RESULTS:

VEGF producton was compared across different co-culture experiments in this study. When examining day 3 results, there is a decrease when L6 cells are cultured with either MVCs or MVF SCs.

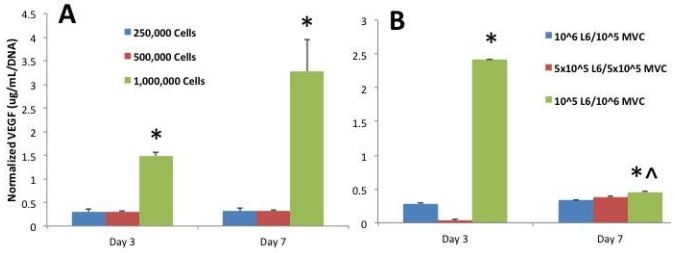
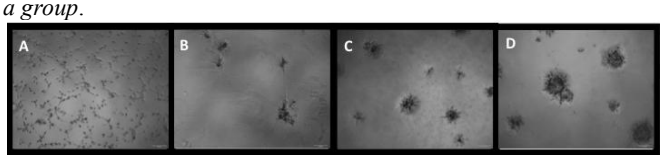


Figure 1. (A) *VEGF production in MVCs at different densities and* **(B)** *for co-culture of L6 cells and MVCs after 3 and 7 days of in vitro culture. * $p<0.05$ within day; ^ $p<0.05$ between days within a group.*



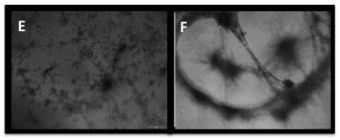


Figure 2. *Matrigel Angiogenesis Assays: Development of MVF SCs in spheroids at 4hr (A), 24hr (B), 7 days (C) and 14 days (D) without induction media show prolonged angiogenic activity. When induced, a seeding density of 20k MVF SCs (E) shows greater capillary tube formation at 24hr than aggregation seen in 40k MVF SCs (F) (10x mag).*

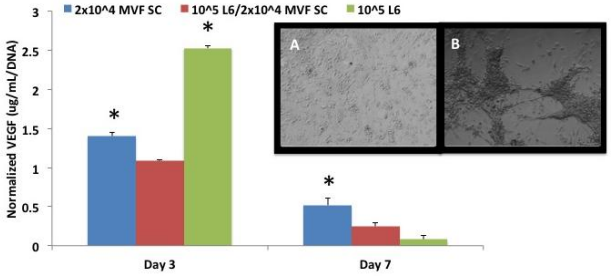


Figure 3. *When MVF SCs and L6s are co-cultured together, the two cell types move from proliferation in day 3 (A) to having the MVF SCs appear to lay on top of the L6s and use them as a platform for vascular networking by day 7 (B) (10x mag).*

DISCUSSION:

Both L6 myoblasts and MVCs (endothelial cells) secrete VEGF (Fig. 1A and 3A), however, when co-cultured for 3 days the effects are not additive (Fig. 1B) but are equivalent to L6 cells alone (Fig. 3A), and decrease by day 7 (Fig. 1B). The co-culture of L6 cells and MVF-SCs (a heterogeneous mixture of vascular cells and mesenchymal stem cells) results in a level of VEGF less than L6 cells alone. Collectively this is indicative of a feedback mechanism whereby VEGF is inhibited when L6 cells are cultured with MVF-SCs, but not MVCs. Despite these growth factor changes, the MVF SCs and L6s increased their interaction over time as the MVF SCs appear to associate with L6 cells. MVF SCs are ideal for pre-vascularization of skeletal muscle grafts as they have natural vascular differentiation properties observed in the matrigel assays. Spheriod development of the cells may allow a concentrated number of vascular cells to be transported *in vivo* for early vascular development.

CONCLUSIONS:

- Fibrin was shown to support both muscle, microvascular cell and primary stem cell growth over the study duration.
- Microvessel-derived stem cells demonstrate the sustained potential to form vascular networks without induction.

REFERENCES:

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