Growth Factor Influence on Myoblastic Cells Attached to a Novel Polymer

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Statement of Purpose: Incisional hernias are a common clinical problem occurring in up to ten percent of all patients undergoing surgeries involving abdominal incisions. Current repair techniques involve the placement of prosthetic biomaterials, xenografts, or allografts at the defect site. Despite these techniques, the incidence of hernia recurrence is in excess of ten percent. A functional biomaterial will incorporate myoblastic cells for skeletal muscle regeneration and will consequently be advantageous in hernia repair strategies. To this end, there are two growth factors that play an important role in the support of skeletal muscle: insulin-like growth factor 1 (IGF-1) and vascular endothelial growth factor (VEGF). In the following studies the focus was on IGF-1 and it's effect on the proliferation of myoblastic cells attached to a 5-ethyl-5-(hydroxymethyl)-β,β-dimethyl-1,3-dioxane-2-ethanol diacrylate (EHD) polymer network.

Methods: Skeletal muscle was harvested and isolated from the hind legs of rats. The muscle was digested in collagenase P for 2 hours. Growth media containing F-10 Ham media, 10% FBS and 1% Penicillin/Streptomycin (Pen/Strep) was added and this solution was then passed through a 100μm, 70 μm and 40μm filter respectively. The cells were centrifuged into a pellet and then resuspended in fresh growth media. The resulting suspension was plated on a T-75 culture flask for up to 1 week. The growth media in these flasks was replaced every 2-3 days.

During this pre-culture period, EHD sheets were fabricated by combining benzoyl peroxide (BP) dissolved in acetone and EHD. The accelerant *N,N*-Dimethyl-*p*-toluidine (DMT) was added to decrease the gelation time. The solution was poured between two glass plates and allowed to gel, forming a sheet with a thickness of approximately 0.5mm. This sheet was then cut into disks with a diameter of 2 cm. The disks were subsequently washed in three stages: first in phosphate buffer solution (PBS), then acetone, and again in PBS for 10 minutes each. Finally they were placed under UV light for sterilization overnight.

The myoblastic cell population was seeded onto the EHD disks using F-10 Ham media containing 1% Pen/Strep and IGF-1 at concentrations of (0, 5, 10, 15 ng/mL). After 24 hours, the media was replaced with fresh F-10 Ham media containing the same amounts of Pen/Strep and IGF-1. The total number of cells, as well as, the viability of the cells was determined 48 hours after the initial seeding time by means of a trypan blue stain and a hemocytometer. Both living and dead cells were counted to determine total cell count as well as the percent viability. Statistical analysis of the data containing 5 samples groups was performed using ANOVA.

Results / Discussion: Preliminary data shows that the myoblastic cells attach and proliferate on the EHD networks.

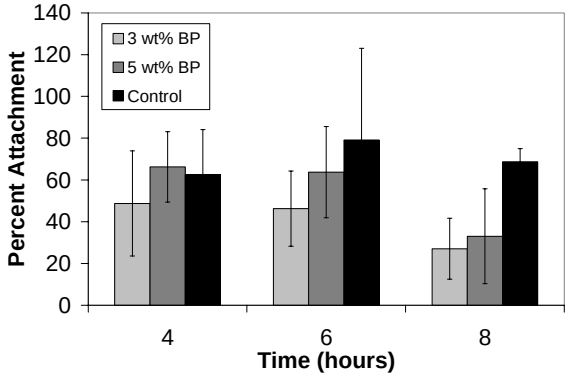

Figure 1: Percent Myoblastic Cell Attachment on EHD Disks

In this study we varied the weight percent of BP added when making the EHD disks. There was no significant difference found between the control group of polystyrene and the disks formed with 3 wt% BP and 5 wt% BP at all time points. Next, when looking at the IGF-1 effect directly, the following results were obtained.

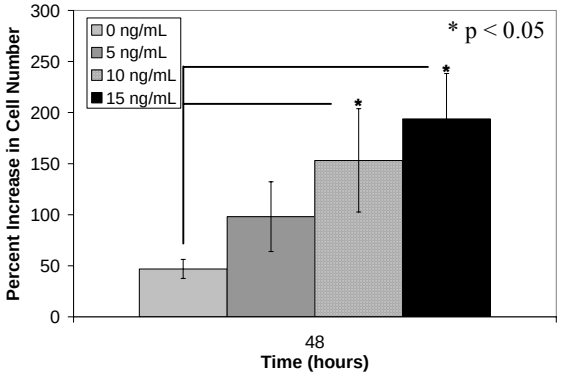

Figure 2: Percent Increase in Average Cell Number after 48 hours in IGF-1 Supplemented Media

There is a significant difference in proliferation of the myoblastic cells between the IGF-1 concentrations of 10ng/mL and 15 ng/mL and the control group of 0 ng/mL of IGF-1.

The results indicate that EHD networks support the attachment and growth of the mypblastic cell population, therefore indicating that the EHD polymer is a viable option as a biodegradable scaffold for tissue engineering of soft tissue. Future studies may involve mechanical testing of the EHD network as well as the characterization of fusion and maturation of myotubes and myofibers on our network.

References:

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2. Johnson, J, et al., Curr Surg, **61**(1), 2004, 53