Growth Factor Influence on Myoblastic Cells Attached to a Novel Polymer

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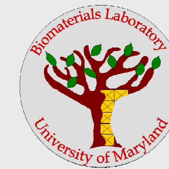


Abstract

Incisional hernias are a common clinical problem occurring in up to ten percent of all patients undergoing abdominal incisions. Current repair techniques involve the placement of a prosthetic biomaterial, xenografts, or allografts. 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. The development of this biomaterial scaffold will be advantageous in hernia repair strategies. To this end, myoblastic cell populations that are attached to our novel biodegradable polymer network of 5-ethyl-5-(hydroxymethyl)-β,β-dimethyl-1,3-dioxane-2-ethanol diacrylate (EHD), have been studied. Also, by adding growth factors such as insulin-like growth factor 1 (IGF-1) to our system, we can improve the proliferation of the myoblastic cells attached to our network.

Objective

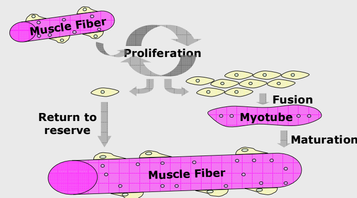
Determine the percent attachment of myoblastic cells to EHD networks over time. Investigate the effect of adding IGF-1 to the culture media on cell attachment to the EHD networks.

Introduction

The abdominal wall is composed of six layers: the skin, subcutaneous fascia, musculature, transversalis fascia, preperitoneal tissue and peritoneum. Incisional hernias create a defect in the musculature which is made up of the transverus adbominis, internal oblique, external oblique and rectus abdominis. Therefore, to effectively treat defects in the abdominal wall, the strength and flexibility of the musculature must be restored. To do this we investigate skeletal muscle regeneration via myoblastic precursor cells or satellite cells.

Skeletal Muscle Repair

When trauma to the musculature occurs, the satellite cells become activated and migrate to the site of the defect and proliferate. Once this occurs, the newly produced cells have two options. The first option is to replenish the satellite cells reserve and return to quiescence. Otherwise, they can remain activated and begin to migrate to the site of the defect to regenerate the muscle. In the defect they align parallel to the remaining myofibers, as well as to each other, similar to muscle development. They then fuse into new myotubes and undergo the maturation process to become functioning myofibers.



Current Treatment Methods

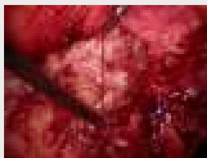


Figure 1: An example of a flank hernia which is being repaired through laparoscopic surgery.

Figure 2: The flank hernia from Figure 1 has been repaired using a DualMesh of polytetrafluoroethylene (PTFE).

EHD Biomaterials

Most degradable polymer scaffolds that are utilized in clinical applications today, have one major drawback; their degradation products are acidic. These acidic byproducts raise the pH of the local tissue, which in turn, can cause further tissue damage, as well as, it can accelerate the scaffold degradation. EHD however is a polymer that is unique in the fact that it degrades at the cyclic acetal group, producing a diol and an aldehyde as the byproducts. Crosslinking this polymer to form a network results in a potentially biodegradable and biocompatible cellular scaffold.

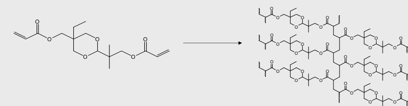


Figure 3: The EHD monomer crosslinks via radical polymerization to form an EHD network

Methods

EHD Network Production

EHD sheets were fabricated by combining a radical initiator, benzoyl peroxide (BP), dissolved in acetone with EHD. The accelerant *N,N*-Dimethyl-*p*-toluidine (DMT) was added to decrease the gelation time. The solution was poured between two glass plates while gelation occurred and a sheet, approximately 0.5 mm thick, was formed. This sheet was then cut into disks with a diameter of 2 cm. The disks were subsequently washed and sterilized in four stages. First they were rinsed with phosphate buffer saline solution (PBS), then acetone, and again in PBS for 15 minutes each and then placed under UV light for sterilization overnight.

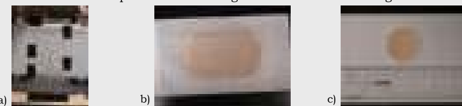


Figure 4: a) EHD network gelling in our glass plate system b) Sheet of EHD network c) EHD network cut into disks.

Cell Attachment Studies

Skeletal muscle was harvested and isolated from the hind legs of rats. The muscle was digested in collagenase P for 2 hours. The cell suspension was passed in succession through three cell strainers with a mesh size of 100 µm, 70 µm, and 40 µm respectively. The cells were spun down and the pellet was resuspended in F-10 Ham Media containing 10% Fetal Bovine Serum (FBS) and 1% Penicillin/Streptomycin (growth media). The resulting suspension was plated on a T-25 culture flask for 7 days. After this time, the myoblastic cell population was lifted off the T-25 culture flask and seeded onto the EHD disks weighted down by stainless steel inserts. After 4, 6 and 8 hours the disks were washed twice with PBS and the remaining cells were lifted and counted using a hemacytometer.

Figure 5: Skeletal Myoblastic Cells attached to an EHD network

IGF-1 Studies

Skeletal muscle was harvested as in the cell attachment studies. After being pre-cultured for 7 days the myoblastic cell population was lifted off the T-25 culture flask and seeded onto the EHD disks weighted down by stainless steel inserts. These cells were cultured on the disks for 48 hours. After 48 hours, the growth media was changed and IGF-1 was added at concentrations of 0 ng/mL (control group), 10 ng/mL and 15 ng/mL of IGF-1. All media was supplemented with FBS to provide proteins so the cell could remain attached to the networks. After 3 days the cells were lifted and counted using a hemacytometer. Values are reported in total number of cells. At Day 0, cells were seeded at $0.1\times10^6 \pm 0.07\times10^6$ cells/disk.

Results

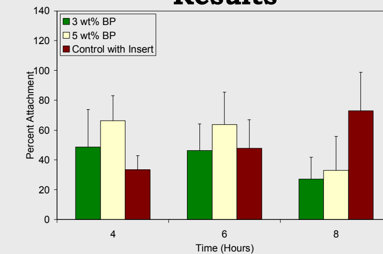


Figure 6: **Effect of Initiator Concentration on Myoblastic Cell Attachment to EHD Networks**. Different concentrations of BP solution were used, as an increase in initiator concentration should correlate to a increase in crosslink density. Result indicate that all groups are significantly similar, therefore showing that EHD networks can support myoblastic cell growth.

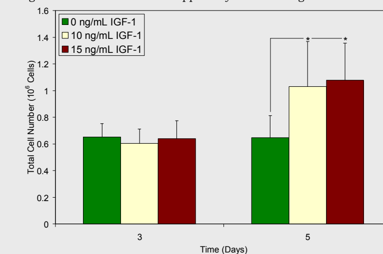


Figure 7: **The Effect of IGF-1 on Myoblastic Cell Proliferation Attached to EHD Networks.** Cells were cultured in IGF-1 and FBS supplemented media. At Day 0 there were $0.1\times10^6 \pm 0.07\times10^6$ cells per disk. Results indicate that at Day 3 all groups are statistically similar, whereas at Day 5 the groups containing IGF-1 are significantly higher than the control which contains 0 ng/mL of IGF-1. Cells remained greater than 94% viable during the study. * indicates significance

Discussion

Results indicate that there are no significant differences in BP initiator concentration and cell attachment between all groups and over all time points. For the control group in this study, myoblastic cells were seeded onto tissue culture polystyrene (TCPS). These results support the hypothesis that these EHD networks can support myoblastic cell growth.

Using the EHD network formed using 3 wt% of BP, the IGF-1 studies were performed. This formulation minimizes the use of solvent, in this case acetone, as acetone can affect the cells. When looking at the proliferation of these studies, and more specifically Day 3 individually, IGF-1 had no significant effect over the control group. The control for this study was cells cultured on the network in growth media and 0 ng/mL of IGF-1. At Day 5 groups containing IGF-1 at a concentration of 10 ng/mL and 15 ng/mL are significantly high than the control. This supports the hypothesis that IGF-1 supplemented media can enhance the cell proliferation on the EHD networks.

In conclusion, we found that our novel biodegradable EHD networks support cell growth and proliferation of the myoblastic cell population as well as on TCPS. These key concepts are fundamental to the regeneration of skeletal muscle.

Acknowledgements

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