

**ECM-MIMETIC HYDROGEL-BASED DELIVERY OF SMALL
MOLECULE DRUG AND BIOMIMETIC POLYMER FABRICATION TO
ENHANCE REGENERATION AFTER VOLUMETRIC MUSCLE LOSS
INJURY**

A Thesis Proposal

by

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To friends and family, for your unerring encouragement.

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LIST OF ABBREVIATIONS

ECM	Extracellular matrix
HA	Hyaluronic acid
NF	Nanofiber scaffold
PEG	Polyethylene glycol
S1P	Sphingosine-1-phosphate
S1PR	Sphingosine-1-phosphate receptor
S1PR3	Sphingosine-1-phosphate receptor 3
VML	Volumetric muscle loss

CHAPTER 1. INTRODUCTION

1.1 Background

Of all U.S. service members deemed unfit for active duty due to combat-related disabilities, over half suffer from extremity trauma [1, 2]. This type injury, which results from a traumatic or surgical loss of appendage skeletal muscle, is classified as having undergone volumetric muscle loss (VML) and poses a significant risk of functional impairment in the damaged limb due to a critical loss of tissue [3-6].

The currently accepted clinical treatment for VML is autologous free flap grafting, yet this treatment's efficacy is limited by available healthy tissue, donor-site morbidity, and the need for an experienced surgical team [6, 7]. Successful muscle flap transfers are plagued by subpar functional recovery within the limb, often due to the endogenous myogenic repair mechanisms inherent to skeletal muscle being overwhelmed by VML conditions [6]. In VML the muscle microenvironment becomes ill-suited for proper regeneration due to a chronic dysregulated immune response which shifts the normal healing process into a pro-fibrotic niche prone to scar tissue formation [4, 6, 8]. In an attempt to better restore function to the damaged limb, newer VML treatments have considered numerous biomaterial applications including tissue engineered muscle grafts, inductive scaffolds made of porcine small intestinal submucosa-extracellular matrix, synthetic nanofiber scaffolds, and bulk hydrogels [9, 10]. These biomaterials vary greatly in their fabrication, morphology, and interaction with the wound microenvironment [9, 11, 12].

The type of biomaterial used for VML treatment should be a topic of serious consideration, as synthetic, natural, and hybrid polymers have different inherent biological effects. Natural polymers such as hyaluronic acid (HA) exist organically within human biology

and are thus incredibly biocompatible and capable of eliciting specific responses without additional material manipulation [9, 13]. However, due to these biomaterials being derived from a biological source they are more susceptible to uncontrollable variation, a property that is not desirable in critical wound conditions [9, 14]. Alternatively, synthetic polymers such as polyethylene glycol (PEG) and poly lactic-co-glycolic acid (PLGA) have significantly more tunable physical characteristics and are easier to fabricate at the cost of less biocompatibility [9, 13, 15]. Combining both natural and synthetic components results in a hybrid polymer with a mix of customizability and biocompatibility that is better suited for complex biological environments [16]. This makes hybrid polymers of particular interest towards the *in vivo* treatment of VML, since the damaged microenvironment combined with the need to mimic healthy skeletal muscle extracellular matrix (ECM) results in the need for a complex structure to aid in better healing. Additionally, while the natural fatty acid-derived polyesters have not been extensively researched with regards to wound healing, their capacity to act as a biomaterial alongside high availability makes them a material of interest [17, 18]. Omega hydroxy fatty acids as a biodegradable biomaterial component that could assist in the local delivery of drugs is something yet to be thoroughly researched in regards to VML treatment.

Besides the nature of biomaterial components, its physical structure also plays a large role in how the biomaterial interacts with its environment. The two structures of interest are bulk hydrogels and nanofiber scaffolds. Recent studies indicate that bulk hydrogel and nanofibrous structures have potential viability concerning wound healing and that the ease of fabricating these structures makes them suitable for widespread use [9]. Bulk hydrogels made through polymerization display homogeneous physical properties in a nanoporous structure that swells with the addition when in aqueous environments [19, 20]. Nanofiber scaffolds are given a more

specific fibrous form through electrospinning, self-assembly, or phase separation [21, 22, 23]. Electrospun nanofiber scaffolds are of particular interest concerning VML treatment as the fibrous structure mimics muscle fascicles. Briefly the electrospinning process involves secreting solution through a syringe in an electric field to guide elongated fiber placement resulting in a fibrous structure that is retained post-crosslinking [22, 24, 25].

Biomaterial implantation into the VML wound site alone would change the injury's microenvironment, however, this impact could be made greater when coupled with controlled drug release. Sphingosine-1-phosphate (S1P) is a bioactive lipid critical to immune cell trafficking [26, 27]. S1P signals through five G-coupled receptors (S1PR1-5) to elicit a range of cellular functions critical to the regulation of inflammatory processes, such as the migration of macrophages. Research has shown that S1P receptor 3 (S1PR3) has a pro-inflammatory effect while retaining macrophages in the wound microenvironment, thus making it a prime target for antagonism [28, 29]. This study is interested in how an S1PR3 antagonistic drug could positively impact wound healing, and will determine the drug's impact through histological assessments including Gomori Trichrome (collagen deposition) and immunohistochemistry (immunostaining for regenerative metrics in muscle).

The complications of current VML treatments and incomplete understanding of the impact of biomaterial-based drug delivery on the severe wound microenvironment has led this study to strive to improve VML patient healing by leveraging novel biomaterials, structures and drug combinations.

1.2 Literature Review

VML injuries often rob a damaged limb of its full function due to improper healing [3-6]. This disability-provoking injury can be acquired by military and civilian populations through

scenarios that range from explosion proximity to car accidents [6]. VML injuries are especially rampant within the military, with functional deficits caused by VML one of the top 10 reasons why wounded service members are medically retired. These functional deficits occur as a consequence of VML conditions creating a microenvironment that opposes proper wound healing [8]. The need to better understand the dysregulated immune microenvironment and its contribution to restoration impairment is reflected in recent literature [8, 11, 13]. Specifically, the innate immune system's macrophages play a critical role in the impaired healing process, with literature focusing on how VML impacts M2-like macrophage interaction with the wound [30, 31]. New studies suggest that M2-like macrophage retention in the wound site has a pathological effect, with M2-like macrophages unusually elevated 7 days post-injury [30, 32]. Another area of relevant research involves studying how the structure of bioscaffolds influences healing once integrated into the wound site [11, 12]. The large deficit region that defines VML makes the plethora of implantable bio-structure fabrication methods of significant interest.

Considering implantable biomaterials, nanofiber scaffolds and bulk hydrogels have been considered as viable options to treat VML injuries [33, 34]. Nanofiber scaffolds are fabricated through the process of electrospinning, where a solution is slowly excreted from a syringe to form elongated fibers that are deposited onto a collection mat [22, 24, 25]. The arrangement of these fibers depends on the electrospinning technique, which can range from Magnetic Field Assisted Electrospinning to using a rotating collector [22, 33]. Bulk hydrogels do not undergo any fabrication procedure to influence their structure and as such lack any scaffolding qualities.

Biomaterial structure determines their practical applications. A 2011 study from Dr. Bayer emphasizes that nanofiber scaffolds provide more available surface area for localized drug delivery, while bulk hydrogels display greater flexibility inherent to native tissue [35]. These

differences differentiate how biomaterials interact with the wound microenvironment both physically and immunologically, as well as their capacity for drug diffusion. A publication from Dr. Armato demonstrates that electrospinning offers enhanced tensile strength while simultaneously reducing drastic scar development, concluding that nanofiber scaffolds are practical for VML treatment [36]. Meanwhile, Dr. Basurto's research argues that bulk hydrogel stiffness induces isometric forces equivalent to healthy muscle, a finding that classifies bulk hydrogel as a feasible VML treatment [37].

Besides providing structural support, biomaterials can also localize drug delivery to the wound site [38]. This allows for a more potent healing effect which is particularly relevant to VML injuries. While nanofiber scaffolds and bulk hydrogels have been considered for VML treatment, the lack of literature demonstrating their differences in terms of small molecule drug release makes it challenging to determine which has better physiological relevance. Of particular interest is the drug VPC01091, a small molecule S1PR3 antagonist. S1PR3 has signaling influence over inflammation as well as collagen and ECM deposition, which are processes that could prevent proper healing [39, 40, 41]. The extent to which antagonism of S1PR3 could promote VML regeneration alone and when delivered via biomaterials are significant gaps in current research, but hold potential worth pursuing.

Another factor to consider when synthesizing biomaterials are the components utilized. Dr. Kunduru advocates the fabrication of polyesters with the renewable resource of fatty acids because they provide desirable properties such as biodegradability and capacity for controlled drug release [17]. There is specific interest in using omega hydroxy fatty acids, as this specific fatty acid offers flexibility, heat resistance, and a lack of toxicity along with the previously-described characteristics [42, 43]. Dr. Faucher's research demonstrates that fatty-acid

derived biomaterials could serve as a promising material for local drug delivery through implantation, with its composition influencing the drug release kinetics [44]. While there have been experiments focused around using fatty acid-derived polyesters as implantable biomaterials, there is a lack of in-depth research regarding those specifically synthesized from omega hydroxy fatty acids.

By studying S1PR3 antagonism effect on VML alone and when paired with biomaterial release methods, the current project will indicate if the small molecule drug VPC01091 is feasible to treat VML injuries, as well as indicate if biomaterial treatment has potential to result in better regeneration. Additionally, new biomaterial components will be explored through fatty acid-derived polyesters, which could later prove useful to VML treatment if biomaterial drug delivery proves itself viable. As current treatments fail to restore limb function and gaps persist concerning biomaterial contribution to wound healing, this study will overall contribute to the better treatment of VML injuries.

CHAPTER 2. METHODS AND MATERIALS

2.1 Assessment of Bioactive Lipid VPC01091 Hydrogel Delivery Impact on Volumetric Muscle Loss

The impact of S1PR3 antagonism will be assessed through the use of the bioactive lipid VPC01091. This drug will be integrated into both biomaterial structures of interest to assess impact on VML wound healing.

2.1.1 *Synthesis of Nanofiber Scaffold and Bulk Hydrogel for Local VPC01091 Delivery*

Four-arm polyethylene glycol (PEG) norbornene terminated macromers (10kDa, Sigma Aldrich) at a final density of 10% (w/v), hyaluronic acid (HA) (10kDa, HA Works) at a final density of 5% (w/v), thiol-containing RGD adhesive peptide (1 mM, GRGDSPC), polyethylene oxide (PEO) (400kDa, Sigma Aldrich) at a final density of 5% (w/v), PEG-dithiol (1000 Da, Sigma Aldrich), and deionized water were combined to make the nanofiber scaffold precursor solution. The concentration of PEG-dithiol crosslinker was determined based on the amount of norbornenes on the PEG macromers not reacting with HA. The precursor solution was incubated overnight at 37°C after which the photoinitiator LAP (1 mM, Sigma Aldrich) was added to the solution and then shaken for one hour. If the nanofiber scaffold needed to be loaded with the VPC01091, the drug was added to the solution immediately before electrospinning at a drug:polymer ratio of 1:200. To get the final structure of the nanofiber scaffold, the solution was electrospun at a flow rate of 0.8 mL/hr with an applied voltage of 18 kV with a working distance of 20 cm between the syringe needle and aluminum collection plate. After electrospinning, the

nanofiber mat was extracted from the collection plate with tweezers and placed beneath a UV lamp at a wavelength of 320 nm for 30 minutes per side in inert conditions to cause thiol-ene click chemistry to crosslink the mat.

The bulk hydrogel solution was prepared in the same manner but with the exclusion of the addition of PEO, since its addition was to solely ensure better electrospinning flow. The solution was inserted via a syringe between two microscope slides with two coverslips on either side to serve as spacers. To form the crosslinked mat the slides were placed beneath a UV lamp at a 320 nm wavelength for 5 minutes.

To prepare for *in vivo* experimentation, 8mm diameter biopsy punches were used on the nanofiber mat and bulk hydrogel. Equal weights between nanofiber and bulk hydrogel punches were ensured by measuring them post-lyophilization.

2.1.2 Volumetric Muscle Loss Quadricep Injury Model

The surgery performed for this experiment adhered to a previously published procedure [45]. The left hindlimb of the mice were prepped and sterilized after anesthesia was induced with through short exposure to 2% isoflurane. After an incision above the quadricep, a VML injury was caused through use of a 3 mm biopsy punch (VWR supplier, 21909-132, 21909-136). After injury, the skin was either closed or the wound was treated with unloaded or loaded hydrogels depending on the desired experiment. The mice were then left to recover without intervention for 1, 3, 7, 14, or 28 days before they were euthanized by CO₂ inhalation.

2.1.3 Systemic VPC01091 Delivery

VPC01091 (Avanti Polar Lipids) with a working solution of 1 mg/mL was prepared in 5% ethanol and sterile PBS. 5 mg/kg of VPC01091 was injected intraperitoneally at day 0 and day 6.

2.1.4 Local VPC01091 Delivery

After injury as detailed in the Volumetric Muscle Loss Injury Quadriceps Model, mice were then treated with an unloaded bulk hydrogel, VPC01091-loaded bulk hydrogel, unloaded nanofiber scaffold, or VPC01091-loaded nanofiber scaffold.

2.1.5 Tissue Histology and Immunostaining

The tissue histology and immunostaining completed for this experiment adhered to a previously published procedure [45]. After euthanization of the mice, the muscle of interest was dissected, weighed, and snap frozen in liquid nitrogen. Cryosections with dimensions of 10 μ m blocked and permeabilized with blocking buffer for 30 minutes before stained with Gomori's Trichrome (Polysciences, 24205-1) and DAPI immunostaining (Abcam, ab104139) according to the manufacturer's instructions.

2.1.6 Imaging and Quantification of Percent Fibrosis

A Zeiss Z1 microscope was used to obtain images at a magnification of 10x. These images were then stitched together with Zen software. 1.5 x 1.5 regions of interest centered on the defect were imported into ImageJ with the Bio-Formats plugin and converted to a .jpeg for better thresholding analysis. The defect region was then color thresholded for blue collagen

staining, which was measured to identify collagen area and was then normalized to the area of the region.

2.1.7 Imaging and Quantification of Centrally Located Nuclei

A Nikon W1 Spinning Disk Confocal microscope was used to obtain immunofluorescence images at a magnification of 20x. These images were then stitched together with Nikon Elements AR for analysis. Centrally located nuclei were analyzed by taking five 500 um x 500 um regions of interest images from each section, with three replicates per animal. The centrally located nuclei were counted using the ImageJ Multi-Point Tool. The average centrally located nuclei count was obtained for each section and normalized to the area of the section to obtain final values.

2.2 Development of Fatty-Acid Derived Polyester

2.2.1 Synthesis of Fatty Acid-Derived Polyester

Two solutions were developed in an attempt to synthesize a thin film of fatty-acid derived polyester. The first solution was made with a C2 omega hydroxy fatty acid, while the second was made with a C6 omega hydroxy fatty acid. Prior to making the solution, strips of overlapping teflon tape were placed on a hot plate. To make the first solution, 100 uL of glycolic acid (99%, thermo scientific), 100 uL of deionized water, and 10 uL of sulfuric acid (thermo scientific) were combined and briefly vortexed. To make the second solution, 100 uL of 6-Hydroxyhexanoic acid (95%, thermo scientific), 100 uL of deionized water, and 10 uL of sulfuric acid (thermo scientific) were combined and briefly vortexed.

Each solution was independently pipetted onto the teflon tape so that they did not mix. The hot plate was warmed to a temperature of 105°C for 3 hours, until all of the deionized water was removed from the solution and a film remained. The film was extracted from the teflon tape and stored at room temperature.

2.2.2 Degradation Study of Long Fatty Acid Chain-Derived Polymers

Polymer degradation was conducted by placing each sample within one well of a six-well plate and submerged in 4 mL of dPBS. The initial weight of the C2 and C6 polyester were measured before beginning the experiment. Over the course of 26 hours, the samples were extracted from the fluid, dried, and weighed.

2.2.3 Drug Loading and Release from Fatty Acid-Derived Polymers

Once the C2 and C6 polymers were formed, they were melted and 10 uL of the drug Eliglustat (5 mM, Cayman Chemical Company) was pipetted into each polymer. Once the polymers had re-solidified, they were each placed within one well of a six-well plate and submerged in 1 mL of dPBS. Over the course of 20 hours, the dPBS was collected and stored at specified time points for analysis of drug release from the samples via plate reading by a SpectraMax M2.

2.3 Statistical Analysis

All data values are reported as mean +/- standard error of the mean unless otherwise noted. Graphpad Prism 8.0 software was utilized to perform general statistical analysis.

CHAPTER 3. RESULTS

3.1 Histological Analysis of Systemic VPC01091 Delivery

Systemic delivery of the S1PR3 antagonist VPC01091 to VML quadriceps injury was assessed for myofiber regeneration through cross-sectional analysis of centrally located nuclei. Centrally-positioned nuclei are a healing metric that indicates in-progress regeneration of the muscle's myofibers [46]. Figure 3.1 shows that the muscle treated with VPC01091 has a significantly higher count of centrally located myonuclei compared to the untreated VML injury, suggesting that the treatment resulted in more efficient wound healing. Additionally, visual analysis of the images show that the newly-developed myofibers have closed the defect region more when treated with VPC01091 as compared to when untreated. This defect region can be clearly seen in the top right section of Figure 3.1A.

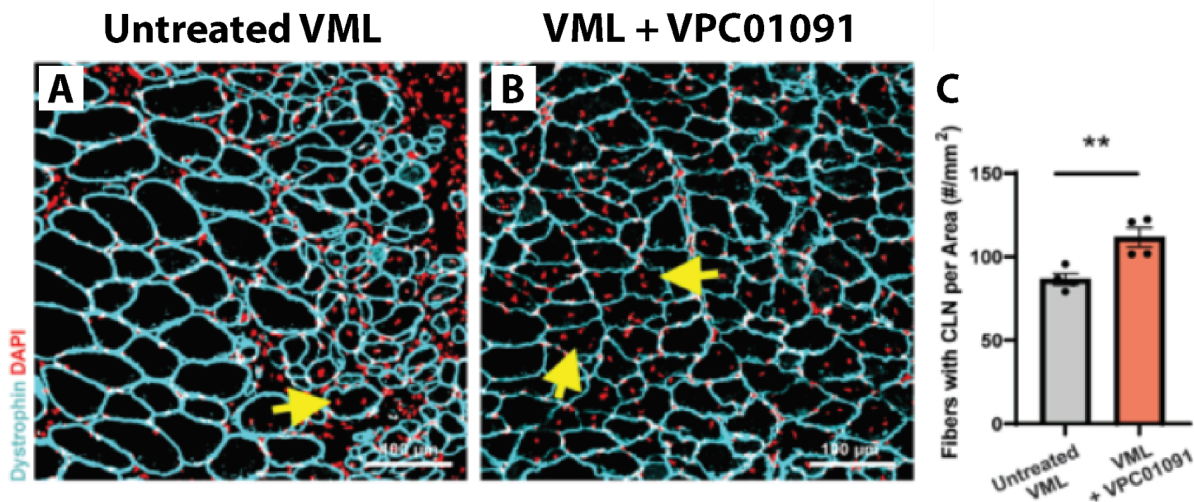


Figure 3.1 – Small molecule S1PR3 antagonism treatment demonstrates more effective VML regeneration through significant increase in centrally located myonuclei. (A, B) Representative images of 14 days post-VML quadricep injury cross sections stained for dystrophin (cyan) and DAPI (red), with scale bars representing 100 μ m. Yellow arrows indicate fibers with centrally located myonuclei. (C) Quantification of fibers with centrally

located myonuclei normalized to the region of interest. Unpaired t-test was used for statistical analysis with * $p < 0.05$, ** $p < 0.01$ and $n = 4$ animals per group.

3.2 Histological Analysis of Local VPC01091 Delivery

Since systemic delivery of VPC01091 was confirmed to have a positive healing effect, its impact through a change in delivery methods was analyzed through local delivery via biomaterials. Figure 3.2 shows the assessment of nanofiber scaffolds and bulk hydrogels impact on regeneration both loaded with VPC01091 and without drug. The pink arrows indicate centrally located myonuclei, while yellow arrows indicate eMHC⁺ myofibers, whose expression visually indicates myofibers undergoing regeneration [47]. Nanofiber scaffold delivery of VPC01091 has a significant increase in the centrally located myonuclei compared to both blank and VPC01091-loaded bulk hydrogels. Additionally, visual assessment of both biomaterials show some defect closure, with the nanofiber scaffolds outperforming the bulk hydrogels by having a smaller defect size.

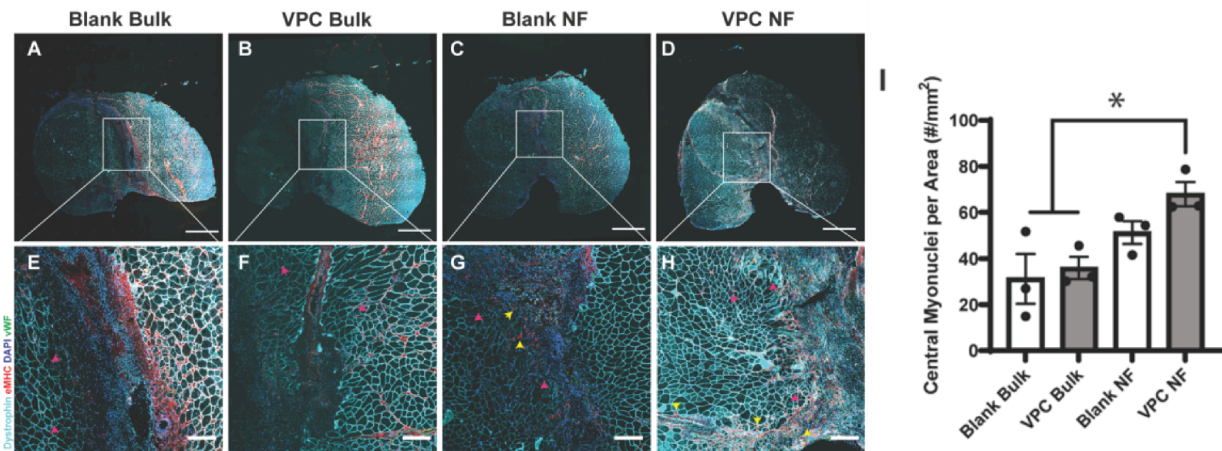


Figure 3.2 – VPC01091 delivery via nanofiber scaffold (NF) shows better VML regeneration through significant increase in centrally located myonuclei. (A-D) Representative stitched images of 14 days post-VML quadriceps injury cross sections receiving a blank bulk hydrogel (A), a VPC01091-loaded bulk hydrogel (B), a blank NF (C), and a VPC01091-loaded NF (D) with scale bars representing 1000 μm . (E-H) A zoomed-in image focused at random on a portion of the defect region for the blank bulk

hydrogel (E), VPC01091-loaded bulk hydrogel (F), blank NF (G), and VPC01091-loaded NF (H) treatments with scale bars representing 200 μm . Pink arrows indicate fibers with centrally located myonuclei and yellow arrows indicate eMHC⁺ myofibers. (I) Quantification of fibers with centrally located myonuclei. One-way ANOVA with Tukey's *post hoc* test was used for statistical analysis with * $p < 0.05$ and $n = 3$ animals per group.

Collagen deposition is an indicator of fibrosis, and as such the reduction of excessive collagen within the wound region would be beneficial for more effective healing. Figure 3.3 shows the collagen deposition in blue, in which percent of fibrosis with respect to the muscle section's area was taken to determine whether nanofiber scaffolds or bulk hydrogels either loaded with VPC01091 or unloaded were more effective at reducing fibrosis. VPC01091-loaded bulk hydrogel demonstrated less collagen deposition compared to the plain bulk hydrogel, however, both bulk treatments have larger defect regions and more collagen deposition compared to both nanofiber scaffold treatments. It can also be qualitatively observed that the VPC01091-loaded nanofiber scaffold has the smallest defect region.

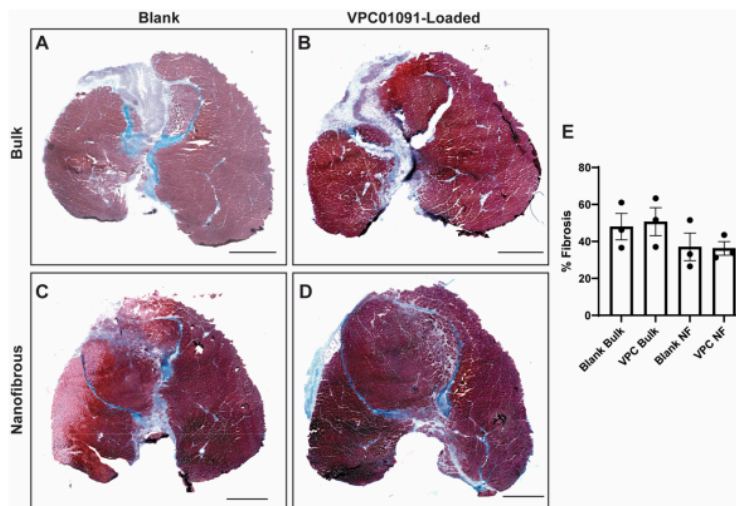


Figure 3.3 – VPC01091 delivery via NF reduces collagen deposition in VML injury. (A-D) Representative stitched images of 14 days post-VML quadriceps injury cross sections stained for Gomori's Trichrome and receiving a blank bulk hydrogel (A), a VPC01091-loaded bulk hydrogel (B), a blank NF (C), and a VPC01091-loaded NF (D) with scale bars representing 1000 μm . (E) Quantification for percentage of fibrosis through

measurement of area stained for collagen as a percentage of total area measured. Mean +/- standard error of the mean was used for statistical analysis.

3.3 Feasibility of Fatty Acid-Derived Polyester for Drug Delivery

Proving that hydroxy fatty acid-derived polymers can perform functions necessary for implantable biomaterials, such as natural degradation and localized drug release, is critical to proving its potential viability in VML treatment. Figure 3.4 shows the degradation of C2 and C6 fatty acid-derived polymers, with a steep initial decline of C2's weight compared to the C6 polymer. Overall, the results indicate that the longer chain of C6 degraded at a lower rate as compared to the C2 polymer.

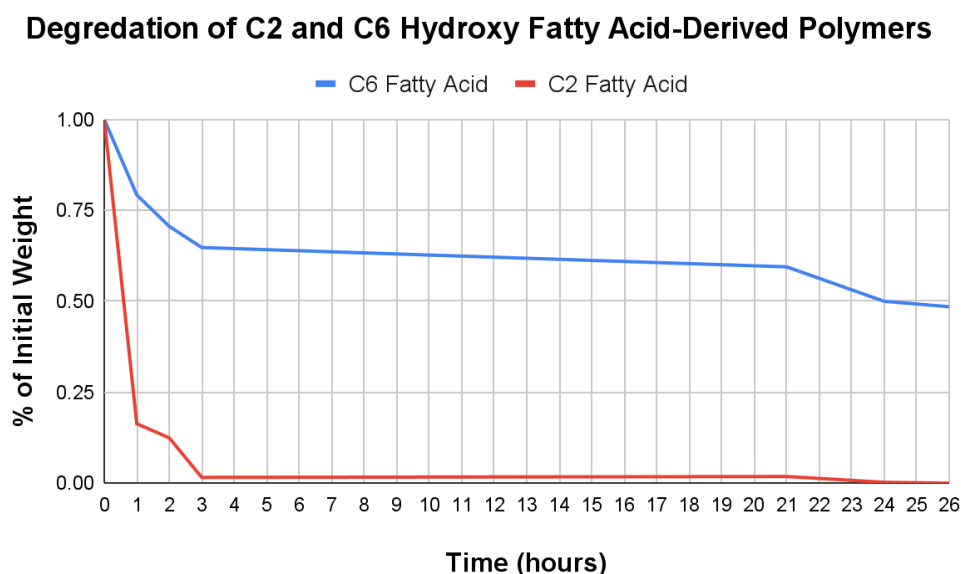


Figure 3.4 – Current weight of C2 and C6 fatty acid-derived polymers after 26 hours submerged in dPBS as compared to initial polymer weight.

Sustained, localized drug delivery is a crucial facet of implantable biomaterial function, and as such is something that was assessed considering the fatty acid-derived polymers [49]. Figure 3.5 demonstrates the drug loading and release capabilities of both C2 and C6 polymers.

The C6 fatty acid-derived polymer released more of the drug as compared to the C2 fatty acid-derived polymer, despite the same amount of drug being added to both.

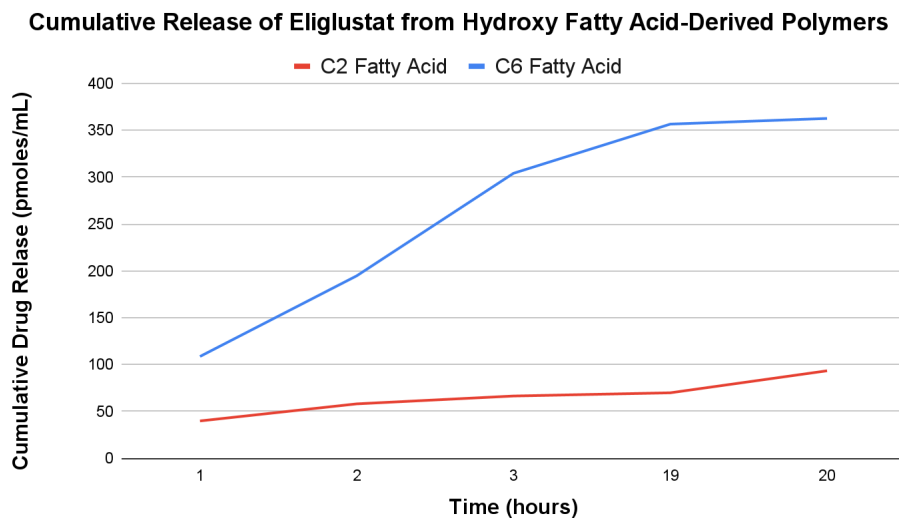


Figure 3.5 – Cumulative release of Eliglustat from Eliglustat-loaded C2 and C6 fatty acid-derived polymers after 20 hours submerged in dPBS.

CHAPTER 4. DISCUSSION

The main challenge towards treating volumetric muscle loss injuries is preventing the functional impairment that commonly occurs even after treatment [1]. Whether it is fibrosis and inflammation persisting within the defect region despite treatment or absence of an advanced surgical team, current methods of healing the substantial wound remain ineffective [7, 48]. Biomaterial delivery of drugs provides an exciting new method to combat this nonfunctional healing in volumetric muscle loss injuries. Specifically, antagonistically targeting the sphingosine-1-phosphate receptor that contributes to inflammation and harmful macrophage retention is assessed for feasibility through the delivery of small molecule S1PR3 antagonist VPC01091. This drug is delivered systemically and locally through biomaterials, and with both has shown a capacity for promoting regenerative factors responsible for myofiber regeneration within the defect region.

In a wound whose defect region size results in an inability of the skeletal muscle to regain proper function, it is important to ensure that new, healthy muscle fiber can fill that vacancy instead of fibrotic collagen. The results gathered from systemic delivery of VPC01091 shows significantly higher counts of centrally located myonuclei, indicating that the drug causes better skeletal muscle regeneration in VML injuries. When paired with biomaterials for localized VPC01091 delivery, drug-loaded nanofiber scaffolds demonstrated better regenerative metrics, with a significantly higher count of centrally located myonuclei and less collagen deposition. While there was no identified significance in the percentage of fibrosis for either biomaterial, there was less empty space observed when the wound was treated with a nanofiber scaffold as

compared to a bulk hydrogel. The histological analysis suggests that small molecule S1PR3 antagonism is a viable path to reduce risk of nonfunctional volumetric muscle loss recovery, especially when paired with a localized release through a nanofiber scaffold. Moving forward with this information could help to reduce disabilities caused by such injuries in both military and civilian populations.

To combat limitations presented by these experiments concerning a lack of significance of fibrosis between biomaterial treatments, an additional experiment for biomaterial-based delivery of VPC01091 with analysis of the muscle after day 14 could yield beneficial supporting data. The end goal of this treatment would be to replace the defect area with regenerating myofibers, and additional time points could help determine which biomaterial resulted in regeneration being overwhelmingly favored over fibrosis. The research conducted for this thesis points towards nanofiber scaffolds as the better structure, however, more information would be desirable, especially since fibrosis is a large factor in causing nonfunctional healing for volumetric muscle loss injuries. Additionally, assessing different nanofiber scaffold fabrication methods such as Magnetic Assisted Electrospinning to yield different fiber alignments and how this impacts volumetric muscle loss healing could be an interesting point of study, as random vs. aligned fibers may influence healing efficiency differently through drug release and physiological impact [49, 33].

While PEG norbornene-derived biomaterials have shown potential in serving as a pathway for VML treatment, fatty acid-derived polyesters have been of growing interest in the biomaterial field. As fatty acids are a more available material, it has the potential to encourage widespread use of the fatty acid-derived biomaterial [17]. Additionally, as fatty acid-derived polyesters are lipophilic, they should ideally interact better with lipid-based drugs such as the

S1PR3 antagonist explored in this research. The research done for this thesis points towards C6 omega hydroxy acid as the material better suited for localized drug delivery through sustained drug delivery and biomaterial degradation. Exploring longer chain fatty acids to prolong drug delivery and reduce degradation rate would be of interest for future research to help decide which fatty acid would be ideal for VML wound treatment. This research has overall proven that ECM-mimetic biomaterial delivery of an S1PR3 antagonistic drug contributes towards enhanced VML healing, and this effect can be improved with further research into different biomaterials.

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