# Repairing Volumetric Muscle Loss with Commercially Available Hydrogels in the Ovine Peroneus Tertius Muscle

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**INTRODUCTION:** Traumatic injuries such as Volumetric Muscle Loss (VML) can cause irrecoverable damage and severe loss of skeletal muscle. VML is defined as a 30% or more loss of skeletal muscle tissue that exceeds the muscle's self-repair mechanism and results in acute inflammation, excessive fibrosis, and decreased muscle regeneration. Current surgical treatments for VML consisting of autologous muscle grafts are limited by tissue availability and donor site morbidity. Novel biomaterials such as hydrogels (HG) may have significant potential for decreasing acute inflammation and subsequent fibrosis, as well as enhance skeletal muscle regeneration. This study assessed the biocompatibility of commercially available poly(ethylene glycol) (PEG), methacrylated gelatin (GelMA), and hyaluronic acid (HA) hydrogels and the efficacy of combining HA with our skeletal muscle units (SMUs) to treat VML following a 6-week recovery from a 30% injury in the peroneus tertius (PT) muscle of an clinically relevant large animal model.

**METHODS:** 3 to 4-month-old male Polypay wether sheep weighing 19-32kg were randomly divided into six groups: VML-Only (n=4), PEG (n=5), GelMA (n=5), HA (n=4), SMU-Only (n=5), and SMU+HA (n=5). All animal procedures were conducted in accordance with The Guide for the Care and Use of Laboratory Animals (Public Health Service 2011 NIH Publication No. 83-23). In all groups, an incision was made along the midline of the lower left leg to expose the PT muscle and peroneal nerve, and a v-cut longitudinal portion of the PT constituting 30% total muscle volume was dissected, avoiding the nerve and major vasculature. The distal branch of the peroneal nerve with its vasculature was isolated and sutured into the VML site to aid with reinnervation. The fascia was closed and the HG was injected behind the fascia and into the VML site. The skin was closed with suture and staples. Following a 6-week recovery, the animals were euthanized, and both the contralateral (right) and surgical (left) PTs were dissected, weighed, and prepared for histological analyses. Statistical differences between groups were evaluated with a one-way ANOVA and Tukey's multiple comparison test. Significance was indicated by a $P \leq 0.05$. Bars on graphs indicate mean ± standard deviation.

**RESULTS:** At the 6-week recovery period, there was a significant muscle mass deficit between the contralateral and VML repaired PTs in the VML-Only, PEG, GelMA, and HA groups (P=0.0074, P=0.0135, P=0.0433, P=0.0421, respectively). In contrast, the SMU-Only and SMU+HA groups were statistically indistinguishable from the uninjured contralateral PT muscle (P=0.3021, P=0.0603). Specifically, in just 6 weeks, the SMU-Only group recovered approximately 15% of the VML injury, reaching a muscle mass of 85% in comparison to its contralateral muscle (Fig. 1). In accordance with these observations, the MF20 and Laminin analysis revealed an increase in the number of regenerating muscle fibers in the SMU-Only and SMU+HA repair sites when compared to the hydrogel groups. Furthermore, enhanced migration into the VML-injury site and more regenerated fibers were observed in the PEG and HA group (Fig. 2). In addition, the M1 and M2 macrophage staining revealed a smaller number of M1 macrophages, pro-inflammatory markers, in the PEG and HA hydrogels (Fig. 3A). Similarly, the M2 macrophage analysis revealed an increase in anti-inflammatory markers in the HA group, as well as the SMU-Only and SMU+HA groups (Fig. 3B). However, injury sites repaired with PEG were observed to be sealed off by fluid filled sacs and exhibited a smaller number of regenerating fibers compared to the HA group.

**DISCUSSION**: Overall, the results from the 6-week recovery period showed fibrotic infiltration into all VML sites, regardless of repair method. However, the PEG, HA, SMU-Only, and SMU+HA groups exhibited decreased inflammation as well as enhanced migration of satellite cells and more regenerated muscle fibers in the repair site. We summarize that the mass recovery in the SMU-Only group cannot solely be attributed to connective tissue deposition due to these observations, and that the contribution of the SMU led to a more effective muscle mass regeneration when compared to the hydrogels alone. Thus, these results demonstrate the efficacy of the HA hydrogel in controlling acute inflammation and fibrotic response in the repair site during muscle regeneration following a 30% VML injury. Moreover, these finding collectively underscore the synergistic potential of our SMUs in conjunction with HA hydrogel in mitigating inflammatory responses and increasing muscle regeneration in the repair site following VML injury.

**CLINICAL RELEVANCE:** This study constitutes a significant step towards the development of biomaterials for a VML treatment that decreases acute inflammation and fibrosis on a scale that is clinically relevant to humans. Furthermore, sufficient quantities of hydrogels can be readily obtained commercially, providing researchers the ability to develop treatments for clinical use in VML injuries.

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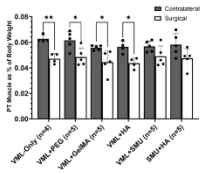


**Figure 1.** There was a significant muscle mass deficit in the VML-Only, PEG, GelMA, and HA groups (P=0.0074, P=0.0135, P=0.0433, P=0.0421). In contrast, the SMU-Only and SMU+HA groups did not exhibit a significant difference in muscle mass when compared to the contralateral muscle. Significant values are identified by * and **.

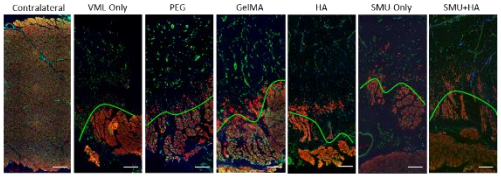


**Figure 2. MF-20/Laminin Staining.** Stained muscle (MF20) and extracellular matrix (Laminin) of PT mid-belly cross-sections following the 6-week recovery from VML injury and repair. MF20 staining of the injury sites suggest muscle regeneration in all groups, and enhanced cell migration in PEG, HA, SMU-Only, and SMU+HA groups. Area above the green line represents the VML injury site. Scale Bar = 500μm.

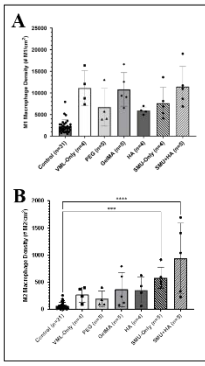


**Figure 3. (A)** M1 and M2 Macrophage stain of mid-belly cross-sections of PT muscle revealed no significant difference in the density of M1 macrophages between all surgical groups and the contralateral muscle. However, the data exhibited a smaller number of M1 macrophages in the PEG and HA hydrogels. **(B)** The density of M2 macrophages in the SMU-Only and SMU+HA groups were significantly higher than the control (P<0.001, P=0.004). Significant values are identified by *** and ****.