Muscle-Bone Crosstalk: Decellularized Muscle Constructs in a Preclinical Model of Open Fracture

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INTRODUCTION: Open fractures with volumetric muscle loss result in delayed healing, nonunion, infection, and worse overall patient outcomes (1). Loss of the muscle's paracrine effect on bone in these injuries impaires fracture healing and poses a challenge for clinicians and researchers. The current gold standard of treatment, fixation of fracture and autologous muscle flap, is associated with donor site morbidity, flap failure, and decreased limb function (2). Although few papers have discussed alternative treatments, there are still no solutions that help fully restore both muscle and bone function simultaneously. We aimed to determine the effectiveness of a decellularized muscle scaffold (dECM scaffold) to a hyaluronic acid-based hydrogel containing decellularized muscle (dECM+HA) on fracture healing and muscle regeneration in an open fracture with volumetric muscle loss preclinical model. Our null hypothesis is that filling the muscle defect with decellularized muscle in a scaffold or hydrogel will not alter fracture healing, while the alternative hypothesis is that decellularized muscle scaffold/hydrogel will improve fracture healing.

METHODS: Animal surgeries were performed in the Department of Orthopaedic Surgery at UC Davis in compliance with the ARRIVE guidelines and were authorized by Institutional Animal Care and Use Committee (IACUC). A total of 36 Sprague Dawley rats were used in this study (3 male and 3 female animals per group per time point). We had three surgery groups including sham (empty muscle defect), decellularized muscle scaffold, and decellularized muscle hydrogel. Critically sized femoral defects of 6mm were created using a gigli saw, and a plate with 4 angular stable titanium screws were used to stabilize the bone. A 6mm diameter full-thickness quadriceps muscle defect was then created using a biopsy punch and the defect was filled with either the dECM scaffold or the HA+dECM. Both the scaffold and hydrogel were created from harvested quadriceps of sex matched Sprague Dawley rats that were decellularized using the decellularization procedure from Gillies *et. al*. (3). To create the scaffold we took a 6mm biopsy punch of the decellularized quadriceps and to create the hydrogel solution we minced the decellularized muscle and infused it into hydrogel. All bone defects were filled with a BMP-2 collagen sponge. To study the bone tissue regeneration, we took x-rays at 4, 8, and 12 weeks post injury. We also harvested the femurs at 4 and 12 weeks and performed micro computed tomography (µCT). We will also perform histology and immunohistochemistry (IHC) on bone tissue. To study muscle regeneration, we performed cross sectional WGA immunofluorescence (IF) staining to measure fiber size. All analyses were performed in GraphPad Prism 8 (GraphPad Software Company, San Diego, CA). Significant differences were presented as $*P < 0.05$ and $**P < 0.01$,* $***P < 0.001$ and $****P < 0.0001$.

RESULTS:
To characterize muscle healing we performed WGA IF staining for fiber size and encircled the muscle defect **(Figure 1A)**. Our quantitative fiber size data showed no significant differences between all three groups **(Figure 1B)**. The macroscopical changes in bone tissue after inducing injury in our animal models were studied by using x-ray and µCT. We were able to distinguish between old and new bone using a deep learning algorithm. The µCT image depicts new bone (pink) and old bone (gray) **(Figure 1C)**. The area of old (red) and new (blue) mineralized bone along the length of the open defect gap was also recorded **(Figure 1D)**. We found no significant differences across all groups for new bone volume, bone volume/tissue volume, and trabecular thickness **(Figure 1E-G).** Our data currently demonstrates no significant differences in bone and muscle regeneration between all three groups.

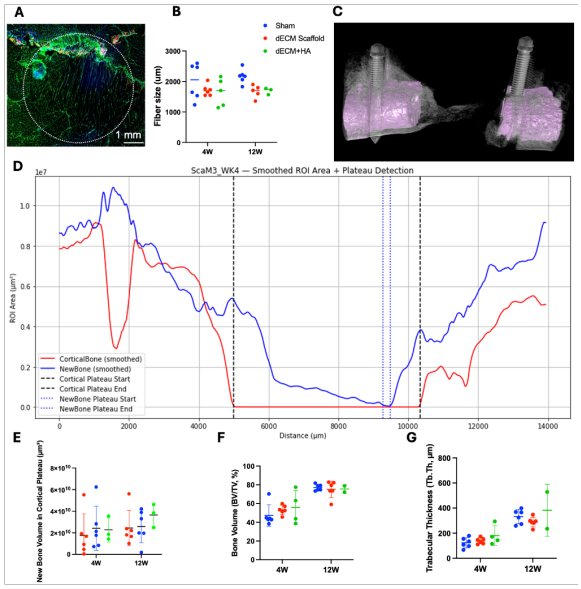

Figure 1. Muscle and bone macrostructural charactrization. (A and B) Muscle WGA immunofluoresecence (IF) staining for fiber size with defect encircled and quantitative fiber size analysis. (C) Micro CT (µCT) image of old bone (pink) and new bone (gray). (D) Area of cortical bone (red) vs new bone (blue) along the length of the femur (D). (E, F, and G) Quantitative µCT analysis of new bone volume in cortical platau, bone volume/tissue volume, and trabecular thickness.

DISCUSSION: Our data for bone regeneration thus far supports the null hypothesis, however we have yet to correlate with histology and IHC. After studying the microstructure of bone using these methods, we may have enough evidence to reject the null hypothesis. We were able to successfully distinguish between new and old bone on µCT using deep learning algorithms and will correlate these results with Movat Pentachrome and Von Kossa/Van Gieson histology and IHC. Our muscle regeneration data also supported the null hypothesis, as we found no significant differences in fiber size between the sham, scaffold, and hydrogel groups. This is likely because the muscle has already healed at these time points, resulting in similar fiber sizes.

SIGNIFICANCE/CLINICAL RELEVANCE: Open fractures with volumetric muscle loss present a significant clinical challenge due to impaired bone healing, high rates of nonunion, infection, and long term disability. Current treatment strategies, such as autologous muscle flap transfer, are limited by donor site morbidity and variable functional outcomes. There is a critical need for regenerative approaches that can restore both muscle and bone integrity in these complex injuries. This study evaluates decellularized muscle scaffolds and hydrogels as potential therapeutic strategies to promote healing in the setting of combined muscle and bone loss, addressing a major gap in clinical care and translational research.

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