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Repair of Traumatized Muscle Tissue for Improvement of Musculoskeletal Healing

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14. ABSTRACT: In this proposal we hypothesized that using a minced skeletal muscle autorgraft to fill a volumetric muscle defect in Yucatan Minipigs subjected to a segmental bone defect (SBD) combined with volumetric muscle loss (VML) would 1) improve fracture healing of the SBD; and 2) improve muscle function. We have operated on 5 pigs with isolated SBD; 7 pigs with SBD combined with VML; and 5 pigs with SBD and VML treated with minced skeletal muscle autograft (SMA). Our findings showed that SMA improved bone healing achieving union in 3 of 4 pigs and currently the 5th pig is on a healing trajectory. In contrast 5 of 6 VML pigs have developed nonunions and the 7th pig is still under evaluation. SMA has not improved muscle function at the time of sacrifice at 3 months but muscle strength was still improving in SMA pigs compared to VML pigs.

In summary, SMA improved bone healing and may improve muscle function. Detailed biochemical and histological analysis of the tissue will be conducted during a no-cost extension period from 11.1.2019 to 10.31.2020.

15. SUBJECT TERMS
None listed.

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1. INTRODUCTION:

We are investigating skeletal muscle autografting in a miniature swine model to determine how well muscle grafting improves bone healing and restores muscle function. The purpose of the investigation is to establish efficacy of muscle grafting in a pre-clinical model to translate into human use. We are using a miniature swine model that employs a 25 mm segmental bone defect that leads to delayed bone healing in tandem with a 7.0 g adjacent skeletal muscle defect in the anterior compartment.

2. KEYWORDS:

Bone defect; volumetric muscle loss; skeletal muscle autografting; nonunion; mangled limb

3. ACCOMPLISHMENTS:

Major Goals: The project involved using a porcine segmental bone defect (SBD) model of Yucatan Minipigs (YMPs) to determine how a volumetric muscle defect, termed volumetric muscle loss (VML) would affect bone healing. Subsequently, we quantified how treating the VML defect with skeletal muscle auto grafting (SMA) improved bone healing. In addition, we quantified how an isolated SBD, a SBD with an adjacent VML defect, and an SBD with a VML defect treated with SMA affected in vivo muscle function.

The final numbers of pigs that were successfully carried to sacrifice at three months included 5 YMPs with an isolated SBD, 7 YMPs with a SBD and VML defect and 6 YMPs with a SBD and VML defect that was treated with minced skeletal muscle autograft. One pig in the SBD group and three pigs in the SMA group were lost due to early catastrophic fracture of their surgical limbs in the first two weeks after surgery.

We quantified bone healing by serial radiographs at monthly intervals and calculated modified Radiographic Union Score for Tibia Fractures (mRUST) as a measure of bone healing. In addition, we are in the process of performing CT scans on all of the surgical limbs (ongoing). Muscle function was quantified by monthly in vivo strength testing of the anterior compartment of the leg using a custom-built testing apparatus. Monthly testing was carried until sacrifice. Muscle samples were collected at sacrifice for histologic and biochemical analyses (ongoing). The objectives of the project included:

1. Quantify the effects of VML on bone healing (Major Task 1)
2. Quantify the effects of a SBD on muscle healing and function (Major Task 2)
3. Quantify the effects of VML on in vivo muscle function and healing (Major Task 2)
4. Quantify the effects of skeletal muscle autografting on bone healing (Major Task 4)
5. Quantify the effects of skeletal muscle autografting on muscle function and healing (Major Task 4)

Note, Major Task 3 which was centered around collecting serial wound aspirates was discontinued when the animal model was modified early in the project.

Key Accomplishments: In summary we have accomplished the following:

1. Volumetric muscle loss (VML) impaired bone healing in the segmental bone defect (SBD)
2. Skeletal muscle autografting improved bone healing in segmental bone defects with an associated volumetric muscle loss defect
3. Isolated SBD affected in vivo muscle function
4. VML magnified strength losses in SBD specimens. SMA did not improve muscle function.

Overall Accomplishments Details

A. *VML impaired bone healing. See Figure 1 at the end of this document.* We used the modified radiographic union score for tibia fractures (mRUST) to quantify bone healing. Three observers made two randomized and independent observations of cortical healing scores on each of the four cortices (anterior; posterior; medial; lateral). Cortical scores are integers from 1 to 4 as follows: 1 = no callus observed; 2 = callus observed but no bridging callus; 3 = bridging callus; 4 = healed and remodeled bone. The overall mRUST is the sum of the four cortical scores and ranges between 4 and 16. Cortical scores of $\geq$ 3 are predictive of healing. Overall scores of $\geq$ 11 have moderate correspondence to bone healing and $\geq$ 13 are highly predictive of bone healing.

Total mRUST scores and scores for each cortex were calculated at monthly intervals. Overall mRUST scores decreased from 10.7 (+/- 1.4) in SBD specimens to 9.4 (+/- 2.2) in VML specimens although the decrease was not statistically significant ($p = 0.187$) (Figure 1 upper left panel). Individual specimen trajectories (Figure 1 upper right panel) demonstrated that 4 of 5 SBD specimens (black) were $\geq$ 10.7 indicating a likely healing trajectory. In contrast, only 2 of 7 VML specimens (green lines) had individual healing trajectories.
The majority of impaired healing secondary to VML occurred in the anterior cortex which was immediately adjacent to the location of the muscle defect (Figure 1; bottom left panel). The divergence in healing occurred between two and three months after injury with anterior cortical score decreasing from 2.7 (+/- 0.7) in SBD specimens to 1.9 (+/- 0.7; $p = 0.003$). The VML defect had minimal effect on healing on the posterior, medial or lateral cortices (Figure 1 bottom row of panels).

B. Skeletal muscle autografting improved bone healing in specimens with volumetric muscle loss. See Figure 2 at the end of this document. Skeletal muscle autografting improved overall bone healing at both two months and three months after injury. At two months, SMA improved overall mRUST scores from 8.0 (+/- 1.5) to 9.2 (+/- 1.8; $p = 0.054$). By three months SMA improve overall mRUST scores from *9.4* (+/- 2.2) to 11.1 (+/- 2.6; $p = 0.050$). Individual trajectories in the upper right panel demonstrated three of six SMA specimens clearly healed at $\geq$ 12 and two more specimens $\geq$ 10 with potential healing trajectories. Again, the majority of healing benefit was observed in the anterior cortex immediately adjacent to the muscle defect and site of autografting (Figure 2 bottom left panel.

C. Isolated Segmental Bone Defects affect in vivo muscle strength (Figure 3). While we anticipated that creating the VML defect would reduce muscle strength, we also demonstrated that

over a three-month interval, a 25.0 mm segmental bone defect also had significant effects on muscle strength to muscle groups adjacent to the defect. In vivo dorsiflexion maximal torque was reduced to less than 50% of original intact strength by two months after surgery. This improved to 58% of intact strength by three months but there were still clear residual strength deficits in SBD specimens that had no muscle injury. Formal statistical evaluation is ongoing.

D. Volumetric muscle loss defects lead to greater strength losses compared to isolated SBDs and the loss of muscle strength was not improved by skeletal muscle autografting. See attached Figure 3. At one month and two-months after injury, VML specimens demonstrated 44% and 36% of intact preoperative maximal torque strength in the affected muscle compartment in the anterior leg. This improved from 36% intact at two months to 46% intact by three months after injury (statistical analyses incomplete). The addition of skeletal muscle autografting did not improve muscle strength. At one month, two months and three months after injury, in vivo maximal torque in the affected muscle group was 34%, 31% and 43%. In all three groups, there were modest increases in strength between months two and three (See Figure 4 attached). It is unknown if the increases would have continued with longer follow up.

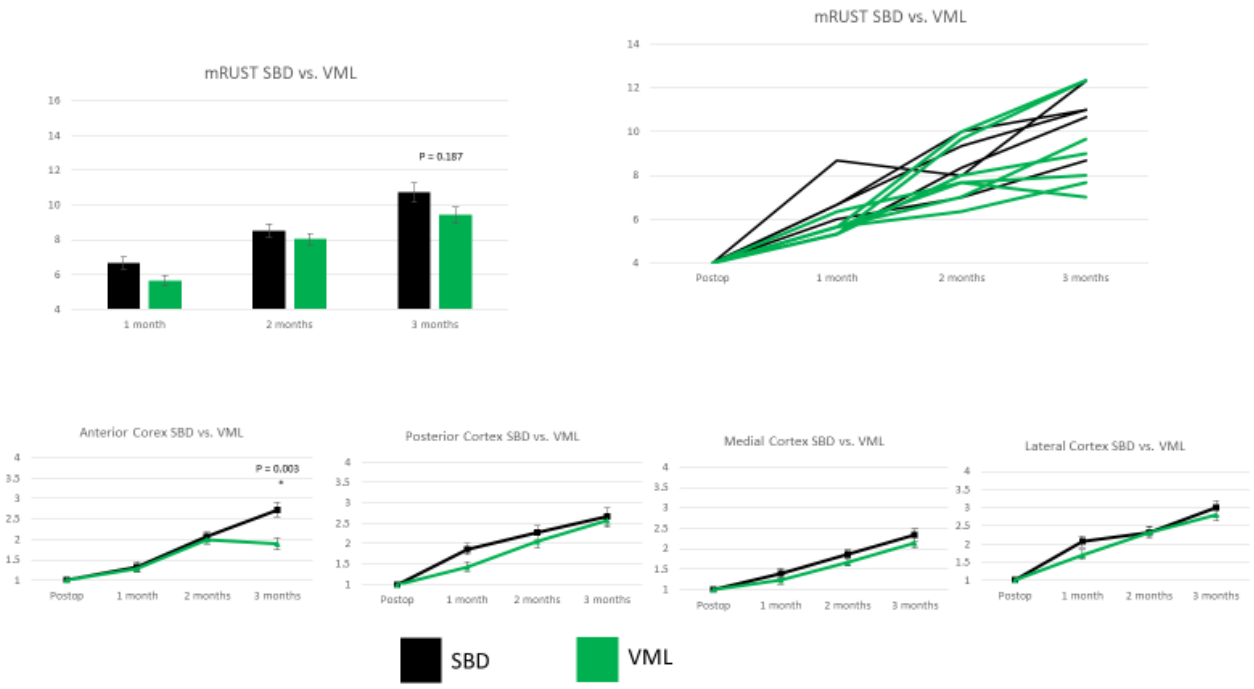

Figure 1. Volumetric muscle defects reduced overall bone healing (upper left) but the reduction in overall mRUST scores was not statistically significant. Individual healing trajectories of SBD pigs (black lines) demonstrated 4 of 5 were on healing trajectories in contrast to only 2 of 7 VML pigs (green lines) with healing trajectories. The effects of VML were seen primarily in the anterior cortex (bottom left panel) while the effects on bone healing in the posterior, medial and lateral cortices were minimal

Figure 2. Skeletal muscle autografting improved overall bone healing (upper left panel) in YMPs with VML defects at two and three months after injury. The primary effects were in the anterior cortex (bottom left panel). Individual trajectories show healing in 5 of 6 SMA pigs compared to 2 of 7 with untreated VML defects.

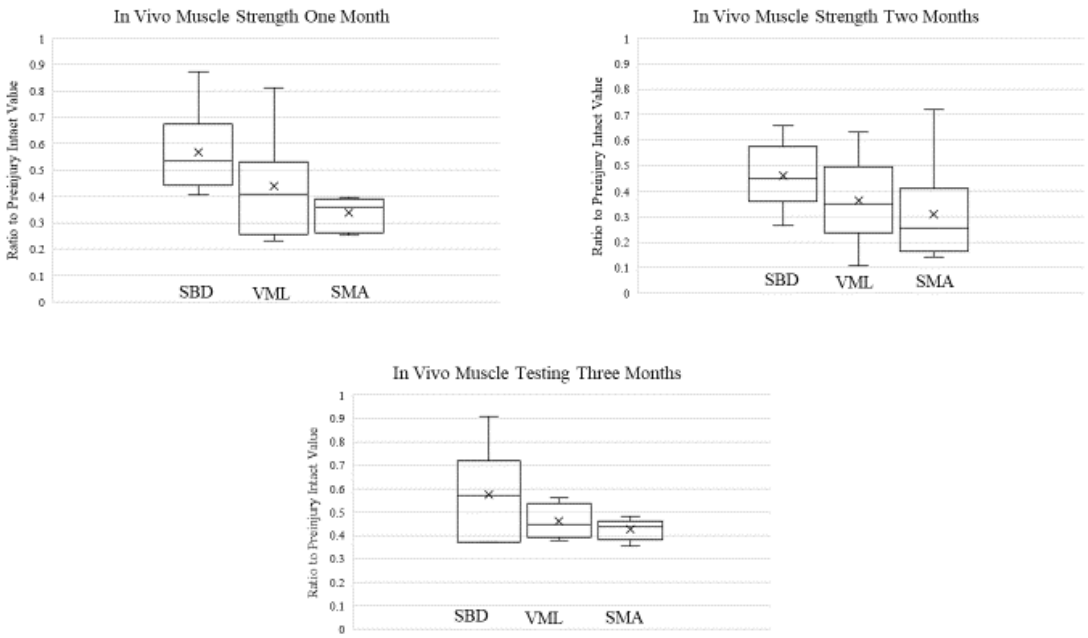

Figure 3: In vivo maximum torque of the anterior compartment muscle group demonstrates that VML defects reduced intact muscle strength by 60 to 70% at two months after injury. SMA did not improve in vivo muscle strength by 3 months after injury. In addition, an isolated SBD resulted in over 40% loss of muscle strength 3 months after injury. Statistical analyses are ongoing.

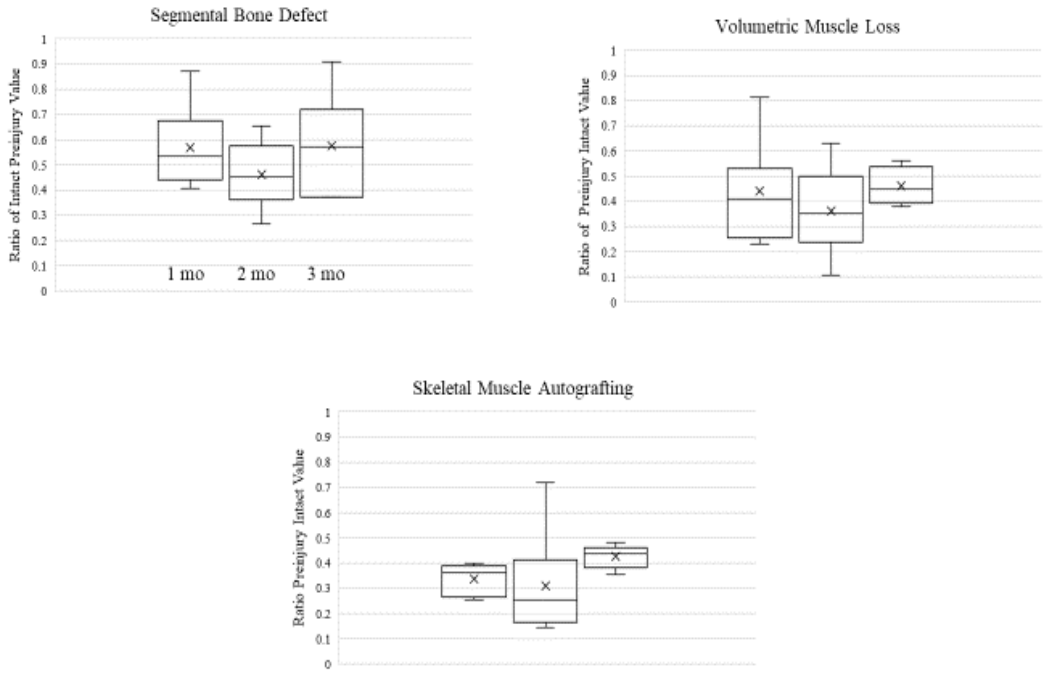

Figure 4. All three groups demonstrated improvements in muscle strength between months 2 and 3 after injury.

Training and Professional Development: We trained 8 medical students on the project including all facets of the surgery, euthanasia, tissue collection and analyses. In addition, we trained a post-doctoral (MD) student who has now matched in Internal Medicine and PGY5 resident from Neurosurgery. The two current residents have scrubbed on all of the surgeries and have taken over in vivo strength testing. The medical students have performed a variety of support tasks in the operating room and have assisted in all facets of postoperative care.

Our students will be attending the ORS and OTA meetings over the next two years with projects associated with this protocol (COVID pending). In addition, they will be preparing manuscripts.

Dissemination of Results: We had podium presentations accepted at the 2020 MHSRS and Orthopaedic Trauma Association meeting but both were canceled. Manuscripts are starting to be prepared which will disseminate our results on both bone and muscle healing in the model. The anticipated journals include the Journal of Orthopaedic Research, Journal of Orthopaedic Trauma and the Journal of Trauma and Acute Care Surgery.

Work in the Next Reporting Period: Our lab was shut down secondary to COVID but we are operational again. We have started biochemical and histologic analyses on muscle specimens and

should be completed (assuming no further lab shutdowns) in the next 3-4 months. CT scans need to be analyzed but they are all complete.

4. Impact: The results have potential to be impactful as we demonstrated that SMA does improve bone healing. In addition, the effects on longer-term muscle function remain unsolved as there was ongoing improvement at the time of sacrifice. Longer-term studies will need to be evaluated. Finally, these data were instrumental in obtaining new funding to investigate adjunctive therapies to improve muscle function.

Impact on other disciplines: nothing to report
Impact on technology transfer: nothing to report
Impact on Society: If the findings can translate into humans, they will impact civilians and warfighters who have devastating limb injuries with bone and muscle defects.

5. Changes/Problems: There were no changes to the protocol over the past year.

Actual or anticipated problems or delays and actions or plans to resolve them. Our lab was delayed secondary to COVID. We are operational again and needed a second No-Cost Extension to complete the work.
Changes that had a significant impact on expenditures: We purchased three additional pigs from our own sources.
Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents: We did have three specimens with catastrophic early (< 2 weeks) failure of fixation which we determined was secondary to animal size ($\geq$ 85 to 90 Kg) and to the design of our cages. These were rectified and we had no ongoing problems.

6. Products: Nothing to report

7. Participants & Other Collaborating Organizations

Name:	Todd McKinley
Project Role:	Principal Investigator
Person Month Worked:	2
Contribution:	In charge of the entire investigation; conducting all surgeries
Funding:	N/A

Name:	Roman Natoli
Project Role:	Investigator
Person Month Worked:	2
Contribution:	Conducting all parts of the investigation: all surgeries
Funding:	N/A

Name:	Benjamin Corona
Project Role:	Investigator

Person Month Worked 2
Contribution Muscle testing; developing muscle autograft methods; all analysis
Funding: N/A

Name: Melissa Kacena
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Person Month Worked 2
Contribution Study design; data analysis
Funding: N/A

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8. Special Reporting Requirements

9. Appendices