area burn (C57BL6 mice; n=4 per time point). Imaging of the injured limb and skin incision only contra-lateral limb (control) was performed weekly (1–9 weeks post-injury) in a longitudinal fashion. Acoustic concentration (10*log(mm-3)) was calculated for each ultrasound frame. Hounsfield units were used to calculate HO volume on microCT imaging. Histology was used to confirm the presence of HO and to correlate with imaging findings at each time point.

RESULTS: Using SUSI, the acoustic concentration of bone was significantly different than that of muscle, cartilage, and tendon (61.3±7.3 vs. 36.5±8.6 vs. 49.5±5.4 vs. 39.8±0.67; p<0.05). HO was visualized on SUSI as early as 1 week after injury and 5 weeks prior to detection by MicroCT. The acoustic concentration of HO was significantly greater than that of the control limb (56.9±10.9 vs. 29.0±10.7; p<0.05) at all time points. The surrounding edema also had a significantly lower acoustic concentration than the foci of HO (28.9±5.6 vs. 56.9±10.9; p<0.05), allowing for clear anatomic and structural delineation within this region. Spectroscopic foci of HO present at 1 week within the left limb correlated with the HO present at 9 weeks on microCT and histology.

CONCLUSION: Spectroscopic ultrasound visualizes HO as early as one week after injury. As prophylaxis must be initiated within 1–3 weeks of initial HO formation to prevent the need for surgery, SUSI represents the ideal imaging modality to guide treatment. Additionally, SUSI can be used to monitor the progression of HO to measure when growth has halted in preparation for surgical excision.

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Osseointegrated Neural Interface (ONI): Rethinking a Conventional Surgical Treatment for Amputation Neuromas in the Digital Age.

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PURPOSE: Modern prosthetic limbs have reaped the benefits of the Digital Age, with improvements in materials, degrees of freedom and computational power. What has lagged behind these advances, is the ability of the recipient to control these devices. Neural interfaces are devices that aim to bridge the gap between the biological tissues and the robotic prosthesis. In most cases, the neural interface is placed on the skin, actuated by myoelectric signals highly susceptible to motion artifact and muscle signal crosstalk, ultimately preventing widespread clinical application. In 1943 Edwin Boldrey first published the transposition of nerve in bone to treat amputation neuromas. This method is still in use today, under the fundamental principal that placing the nerve in bone protects the neuroma from the mechanical and electrical stimuli that causes neuropathic pain. By re-directing transected nerves into the medullary cavity of long bones, the terminal end of the nerve is protected from external stimuli, whilst also providing direct access to the highly vascular stem cell niche. This already established surgical model presents the perfect in vivo bioreactor for the potential interfacing of transected nerves and electronic prosthetic devices. The research objectives of this pilot study were to create an animal model -termed the Osseointegrated Neural Interface (ONI), utilizing histology to demonstrate the stability and health of the nerve and surrounding tissues and electrophysiology to demonstrate nerve conductivity.

METHODS: Transfemoral amputation was performed in New Zealand white rabbits. Briefly, the sciatic nerve was isolated and severed above the point of bifurcation. The femur was amputated at the midpoint and the nerve passed through a corticotomy. The terminal end of the nerve was sutured into a PDMS nerve sleeve, representing a mock electrode, which was pressed back into the opening of the medullary cavity forming a tight seal. The muscle and skin were closed over the femur. Animals were explored at 5 weeks via histology and electrophysiology.

RESULTS: Gross examination of the ONI limb demonstrates that the nerve is stable at 5 weeks. Healthy nerve morphology can be identified by Schwann cells (S100+) along the length of the transected nerve. Cross sections of proximal portions of the nerve demonstrate the ONI nerve contains smaller myelinated axons when compared to the contralateral healthy sciatic nerve. Electrophysiology demonstrates that the nerve is alive within the bone,
as demonstrated by compound action potentials. The transected nerve demonstrated action potentials equivalent to half that of the contralateral healthy nerve, which correlates with the smaller diameter of the myelinated axons in the ONI nerve.

**CONCLUSION:** Terminal ends of amputated nerves are functional following being re-directed into the medullary cavity of the femur at 5 weeks. This result provides proof of principle for the ONI model and its ability to house functional prosthetic interfaces. Work is currently underway to test various electrodes in this model.

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**Local Delivery of Supplemental Agrin at the time of Injury Prevents Motor Endplate Degradation**

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**PURPOSE:** Traumatic nerve injuries often result in loss of function despite optimal surgical management. One reason for poor recovery is the degradation of the neuromuscular junction (NMJ) after injury. Our lab has previously shown the novel finding that preservation of the NMJ after nerve injury actually improves functional recovery following reinnervation. Developmental neurobiology literature suggest that Agrin, a proteoglycan involved in synaptogenesis, may play a critical role in NMJ preservation. Our experiments with a murine model demonstrated that genetic deletion of MMP-3, a matrix-metalloproteinase that degrades agrin on a homeostatic basis, allowed sufficient agrin levels to remain in denervated motor endplates. Moreover, our lab further demonstrated that denervated motor endplates with sufficient agrin levels have superior morphometric qualities and are highly conducive to promoting successful reinnervation. Here, we sought to determine if direct local delivery of agrin after a traumatic nerve injury might preserve the motor endplates.

**METHODS:** As previously described, a denervation model was created in 6 week old WT and agrin deficient C57BL/6 murine strains by excising 10 mm right sciatic nerve segment from the mid-thigh of the mice and suturing the proximal nerve stump to the gluteal muscle with 9-0 suture so as to prevent regeneration. Agrin deficient mice were either injected with supplemental agrin or PBS as a control at the site of injury. The downstream denervated and contralateral control soleus, plantaris and gastrocnemius muscles were harvested for immunohistochemistry, cryo-sections with H&E staining, and quantitative western blots at the 1, 2, 4, 8 and 16 week timepoints.

**RESULTS:** Fluorescence confocal imaging of harvested soleus muscles revealed that agrin supplemented animals retained superior motor endplate morphology over control animals up to the 16 week timepoint. The average surface area of agrin supplemented denervated endplates were significantly greater than control endplates in all analyzed timepoints.

**CONCLUSION:** These experiments demonstrated that supplemental agrin delivered locally at the site of injury is effective in preserving motor endplates in denervated mice hindlimbs. Consistent with our work that showed the knockdown of MMP3 allowed agrin to remain at the NMJ and thereby preserved the motor endplates so as to improve function recovery, these current experiments support this strategy and role of agrin in maintaining the motor endplates after traumatic nerve injury. These data support our strategy of developing therapeutic targets that will delay or prevent NMJ degradation so as to prolong the window of opportunity for surgical intervention and maximize the opportunity for functional recovery.

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**Expression Of Markers For Pericytes And Myofibroblasts In Bleomycin-induced Dermal Fibrosis: Potential Role Of Neuropeptide Receptors In A Mouse Model For Scleroderma**

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