# Harnessing the Power of Nature: mRNA–Activated Biomimetic Hematoma Scaffold for Regeneration of Volumetric Muscle Loss

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**Question:** Volumetric Muscle Loss (VML) is a major cause of permanent disability following severe extremity injuries, often resulting from high–velocity trauma such as blast injuries, motor vehicle accidents, or muscle resections due to cancer or infection. Current treatments, including autologous muscle transplantation and rehabilitation, still frequently lead to reduced mobility and motor dysfunction, with some patients ultimately requiring limb amputation. Consequently, there is a pressing need to develop alternative approaches for VML treatment. Hematoma formation is a critical initial step in the repair of various tissue injuries, including muscle and bone, as it substantially influences tissue regeneration. Our previous studies demonstrated significant structural and biological differences between hematomas in healing and non–healing bone defects. This led to the development of a Biomimetic Hematoma (BH) scaffold, designed to mimic the fracture hematoma and serve as a delivery vehicle for growth factors. Preclinical and pilot clinical studies have shown that BH delivering rhBMP–2 effectively initiates the natural bone repair cascade, successfully regenerating large bone defects without adverse effects. Recognizing that both muscle and bone injuries initiate with hematoma formation, we hypothesized that in large muscle defects, similar to bone defects, the hematoma may become diluted, resulting in inadequate clot formation and impaired healing. Thus, this study aimed to explore the potential of the BH scaffold as a delivery vehicle for mRNA encoding Roof Plate–Specific Spondin–2 (RSPO–2), a myogenic growth factor, to promote functional regeneration of large muscle defects.

**Answer:** A full–thickness, 30% total area defect was created in the tibialis anterior (TA) muscle of male and female Fischer 344 rats (200–250 g). Rats were divided into three groups (n=6/group): 1) Empty Defect (ED); 2) Biomimetic Hematoma (BH) alone; and 3) 25 μg RSPO–2 mRNA+BH. An uninjured limb served as the control. Autologous blood was drawn and combined with calcium and thrombin to form a BH scaffold. In the mRNA+BH group, RSPO–2 mRNA was mixed with blood prior to creating the scaffold. Grip strength testing was used to assess functional recovery. After 4 weeks, rats were euthanized, and muscles were harvested for gross weight and histology analysis. Statistical analyses were conducted using paired sample t–tests.

**Results:** The ED group had increased complication rates, with one animal developing a large hematoma, four experiencing wound dehiscence, and one animal dying. In the BH and mRNA+BH groups, only one case of wound dehiscence occurred. On day 3, the mRNA+BH group showed no significant deficits in grip strength relative to the uninjured control, while BH and ED groups had notable deficits. TA muscle weights in the BH and mRNA+BH groups did not differ significantly compared to uninjured limbs; however, the ED group had significantly lower TA weights. At 4 weeks, gross inspection revealed that muscles in the BH and mRNA+BH groups closely resembled the uninjured controls. Histological analysis showed residual scar tissue in the ED group, while the regenerated muscle in BH and mRNA+BH groups demonstrated morphology and revascularization similar to the uninjured limb.

**Conclusions:** This preliminary study demonstrates that an autologous BH scaffold, alone or with RSPO–2 mRNA, effectively regenerated VML in a rat model within 4 weeks. The BH alone group showed no morphological difference from the uninjured limb at 4 weeks, while RSPO–2 mRNA restored grip strength more rapidly than BH alone. In contrast, only fibrotic tissue was observed in the ED group, indicating impaired healing. This study is the first to use an autologous BH scaffold that mimics the properties of a naturally healing muscle hematoma. Further research may lead to a promising autologous treatment approach for functional muscle defect regeneration in clinical settings.