Biomimetic Hematoma–Mediated mRNA Delivery Restores Structure and Function in Volumetric Muscle Loss

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What was the question?
Volumetric Muscle Loss (VML) leads to permanent disability following severe extremity trauma or surgical resection, with current treatments often resulting in impaired mobility or amputation. Despite extensive efforts using a variety of scaffolds, biologic molecules, and cell–based therapies, achieving consistent and functional muscle regeneration remains a significant challenge. Hematoma formation is a crucial early step in tissue repair, significantly influencing both muscle and bone regeneration. This understanding led to the development of the Biomimetic Hematoma (BH) scaffold, designed to replicate the native fracture hematoma and serve as a growth factor delivery system. Preclinical studies have demonstrated that BH delivering rhBMP–2 effectively regenerates large bone defects without complications. Recognizing the shared role of hematoma in muscle and bone repair, we investigated the potential of BH to deliver mRNA encoding RSPO–2, a myogenic growth factor, to enhance functional regeneration of VML in a rat tibialis anterior model.

How did you answer the question?
A 30% full–thickness, 8 mm tibialis anterior defect was created in male Fischer–344 rats. Animals were assigned to four groups (n=6/group): 1) Natural Healing (NH, 3 mm defect), 2) Empty Defect (ED), 3) Biomimetic Hematoma alone (BH), and 4) 25 μg RSPO–2 mRNA + BH (mRNA+BH). The uninjured limb served as a control. Autologous blood combined with calcium and thrombin was used to generate the BH scaffold, with or without RSPO–2 mRNA. Muscles were harvested at 5, 10, and 21 days, and 12 weeks post–injury to assess revascularization (microCT), tissue morphology (histology), and functional recovery (tetanic force).

What are the results?
At 5 days, the ED group showed no vessel formation, while NH demonstrated modest early recovery; in contrast, the BH and BH+mRNA groups exhibited early capillary formation. By 10 days, vascularization increased in all groups, with NH, BH, and BH+mRNA showing further gains in vessel volume and number. By 21 days, NH, BH, and BH+mRNA demonstrated substantial revascularization versus ED, with BH supporting consistent tissue formation and maturation, and BH+mRNA showing the greatest vessel volume and number. At 12 weeks, BH and BH+mRNA had significantly higher vessel number and thickness than ED, with BH+mRNA slightly exceeding BH and control. Histologically, all treatment groups showed disrupted muscle morphology at 5 days with presence of the BH scaffold, while the 3 mm defect showed limited repair versus control. By 10 days, BH scaffold resorption and early myofiber alignment were evident in BH–treated groups, more pronounced in BH+mRNA; ED remained poorly organized, and the 3 mm defect showed partial regeneration. At 21 days, BH+mRNA exhibited the most advanced regeneration with aligned myofibers approaching native morphology, BH showed substantial but less mature repair, the 3 mm defect was moderate and heterogeneous, and ED remained fibrotic. At 12 weeks, the ED group exhibited predominantly fibrotic and adipose tissue with minimal organized muscle compared to control. The BH group demonstrated muscle regeneration with aligned but heterogeneous myofibers, while the BH + mRNA group showed more organized and mature muscle architecture, more closely resembling control muscle morphology. Functional recovery as measured by tetanic force improved over time in all groups, with both BH and BH + mRNA demonstrating significantly greater functional recovery compared to the empty defect (~50%). The BH and mRNA+BH groups achieved the highest recovery approaching near–normal control limb levels by 12 weeks.

Biomimetic Hematoma–Mediated mRNA Delivery Restores Structure and Function in VolumetricMuscle Loss *continued*

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What are your conclusions?

These findings demonstrate that replicating the native hematoma environment is a critical driver of muscle regeneration following VML. The BH scaffold alone supported consistent muscle formation, vascularization, and functional recovery, highlighting the importance of early provisional matrix cues by 12 weeks. Importantly, the addition of RSPO–2 mRNA further enhanced vascularization and improved the organization and maturity of regenerated muscle, resulting in tissue morphology more closely resembling native muscle. This enhanced vascular response likely contributed to the improved morphological and functional outcomes observed at later time points. In contrast, the empty defect group exhibited failed regeneration, with tissue dominated by fibrotic and adipose infiltration and poor functional recovery. BH–based delivery of mRNA represents a promising, biologically inspired strategy to achieve functional muscle regeneration in VML, with clear clinical relevance for improving limb salvage and restoring function in patients with severe muscle loss.