

Supplementary Materials for

Acute multidrug delivery via a wearable bioreactor facilitates long-term limb regeneration and functional recovery in adult *Xenopus laevis*

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The PDF file includes:

Figs. S1 to S3
Legends for movies S1 and S2

Other Supplementary Material for this manuscript includes the following:

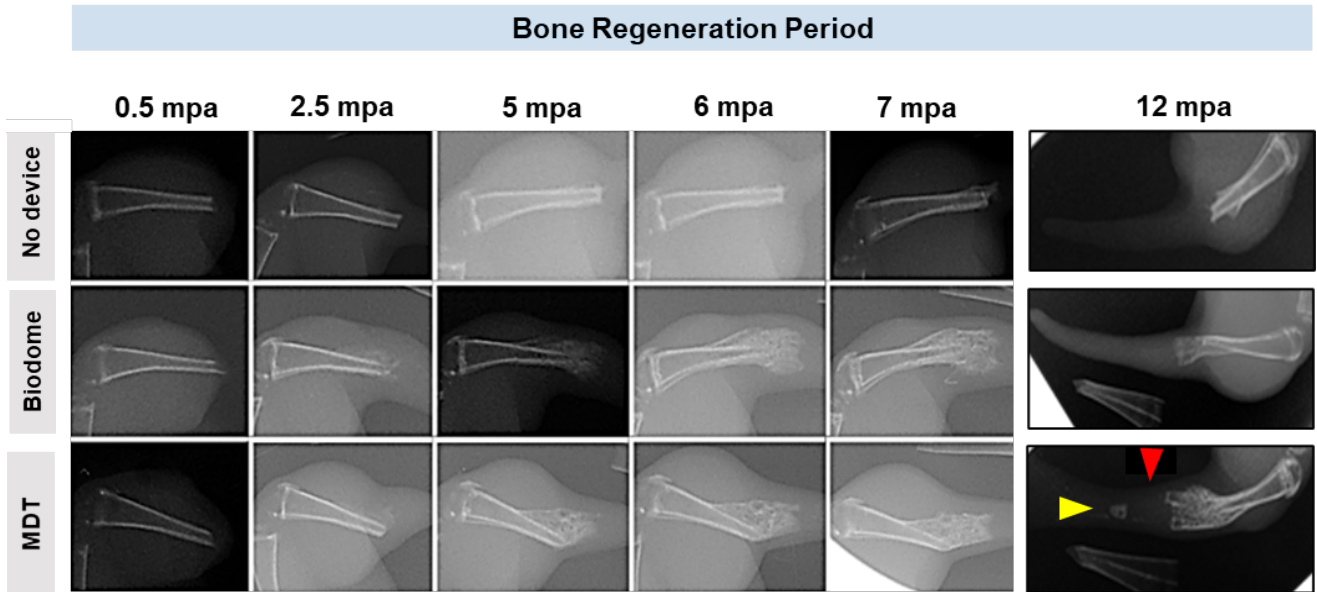
Movies S1 and S2

Supplement Figure 1. Interruption in Hindlimb Bone Regeneration. **(A)** X-ray imaging of the Bone Regeneration Period ranging from 0.5 mpa to 12 mpa for ND, BD, and MDT groups. The red arrow indicates the interruption in bone growth. The yellow arrow indicates discontinuous bone growth. **(B)** MicroCT 3D tomograms revealed unique fragmentation phenotypes associated with the MDT condition relative to the unremarkable structures associated with ND and BD conditions. As indicated by the yellow arrows, bone growth was interrupted as indicated by a reliable and anatomically consistent gap across the transverse plane of the regenerate. Bone fragments and continues tissues resumed formation caudal to the gap. It should be noted that the BD condition displayed a limited fragmented structure without the notable growth gap that was only apparent for MDT-exposed animals. The red dashed line indicates the original site of amputation; all tissues past the red line are indicative of new growth.

Supplement Figure 2. Gene Modules Related to Biological Functions Altered by MDT. Gene network analysis revealed parent modules related to **(A)** ECM organization and collagen biosynthesis module (M1), **(B)** cell-organization and cell communication (M2), **(C)** muscle contraction and myogenesis (M3), and **(D)** glucose metabolism and gluconeogenesis. Vertical dotted lines across graphs indicate the threshold for significance; only significant components are displayed. Each module contains several sub-factors that relate to theme which are rank-ordered here from top-to-bottom. **(E-G)** Volcano plots show the genes associated with the GO pathway “extracellular matrix”. Expression dynamics appear to incrementally arise over time and display the greatest dynamic levels at day 7 post-MDT treatment.

Supplement Figure 3. Cumulative Release for Drugs in MDT. In order to assess cumulative release for the multidrug treatment (MDT) in the device hydrogel, hydrogel loaded with each of the MDT drugs was suspended in 1x DPBS (ThermoFisher) and incubated for 25 minutes at at 37°C. Supernatant was collected every 5 minutes for 1 hour, then hourly for up to 24 hours and concentration of each drug was determined via a microplate reader. Each drug/hydrogel mixture exhibited similar release kinetics, releasing around 70% of the total drug concentration within 10 minutes and not releasing more than 80% of the total amount loaded (except for retinoic acid, which released all of the drug by 25 minutes).

Supplement Video 1 and 2. (Separate Files) Functional recovery of sensorimotor pathways in the regenerated limb 18 months after amputation was assessed using stimulation force threshold tests. Video 1 shows a ND animal non-responsive to incremental force stimulation. Video 2 shows an MDT animal, despite not exhibiting a patterned regenerate, the animal is responsive to the smallest force stimulation, revealing the re-establishment of the animal’s sensorimotor system within the regenerate.

A**B**