Bioengineered Human Skeletal Muscle as a Platform to Model Injury, Regeneration and Drug Response

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Published as: Fleming, J.W., Capel, A.J., Rimington, R.P. *et al.* Bioengineered human skeletal muscle capable of functional regeneration. *BMC Biol* **18**, 145 (2020). https://doi.org/10.1186/s12915-020-00884-3

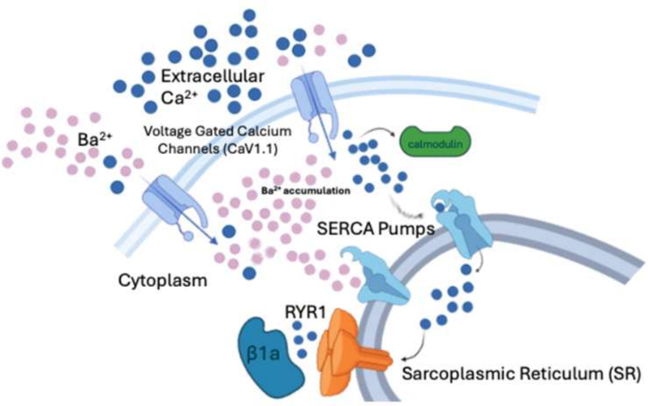
Overview Engineered human skeletal muscle tissues provide a powerful *in vitro* model for studying injury, regeneration, and pharmacological modulation in a physiologically relevant setting. Using 2D monolayer, 3D muscle tissues, and 3D neuromuscular co-cultures, Myomaker Bio has tested various compounds spanning injurious, anabolic, metabolic, inflammatory, and neuromuscular functions. Each integrated model can support translation between traditional 2D cell culture and *in vivo* muscle physiology.

Methods 3D human skeletal muscle tissues were engineered in 50 µL collagen–Matrigel hydrogels (4×10^6 cells/mL), which self-aligned within 3D-printed moulds. At day 14, iPSC-derived motor neuron spheroids were added in a type I collagen overlay to generate innervated neuromuscular tissues. Contractile function was assessed by evoked electrical tissue contraction, while immunostaining, confocal imaging, and molecular assays quantified myotube structure and pathway activation. Mouse and human myoblast cell line cultures were used in parallel for mechanistic or metabolic analyses.

Barium Chloride ($BaCl_2$): Isomorphic Mimicry Mechanism

1. Dissociation

$BaCl_2$ quickly dissociates into **Ba^{2+} and Cl^-**, and water stabilises these ions, **allowing Ba^{2+} to diffuse** through the cell and **bind** key proteins.



2. Ca^{2+} Ion Mimicry

Ba^{2+} ions imitate Ca^{2+} ions as they share a similar charge and ionic radius. This isomorphic mimicry allows Ba^{2+} to:

- Pass through voltage-gated calcium channels
- Enter via sodium channels
- Compete for intracellular Ca^{2+} binding sites

However, Ba^{2+} cannot perform the biological functions of calcium, leading to disrupted signalling.

3. Mechanism of Action

Once inside the cell, Ba^{2+} interferes with **calcium-dependent processes**, including:

- Excitation–contraction coupling (ECC)
- Calcium-dependent proteins (e.g., calmodulin)

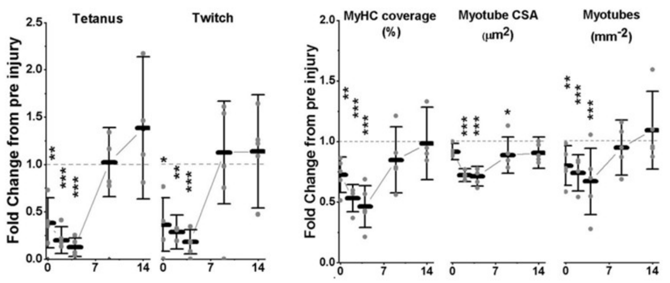
4. Utility Within Injury Modelling

Applied to skeletal muscle cultures $BaCl_2$ produces:

- **Diffuse myofibre necrosis**
- **Reproducible injury patterns** for studying regeneration

5. Experimental Findings

- **2D skeletal muscle cultures*:*** Myotube ablation is observed, however myogenic cell numbers are relatively stable pre- and post-injury. Poor regeneration is observed.
- **3D skeletal muscle cultures:** Injury characterised by reductions in evoked functional contraction, myosin heavy chain and F-actin coverage; however, **regeneration is observed.**



Engineered human skeletal muscle regeneration (0, 2, 4, 9, 14 days post-injury). **Left:** Normalised contraction. **Right:** Normalised morphology. Graphs display control normalised means ± SD. Dashed line represents level at control, normalised to 1.

6. Consequences:

- Reduced Ca^{2+} availability
- Impaired contraction, weakness following Ba^{2+} accumulation (can't be removed by SERCA)
- Cytotoxicity, membrane damage, oxidative stress and **myonecrosis**

Conclusion Bioengineered muscle (and neuromuscular) systems reproduce human injury, repair, and pharmacological responsiveness. Distinct classes of compounds, injury agents, anabolic nutrients, anti-inflammatory mediators, and metabolic stressors elicit predictable, quantifiable outcomes in tissue function. The Myomaker Bio tissue platform enables mechanistic screening of therapeutics targeting muscle health, regeneration, and disease.