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Muscle Diseases and Spinal Cord Injuries

ALS (amyotrophic lateral sclerosis): degeneration of motor neurons in spinal cord (progressive).


Spinal cord injury: spinal cord severed at certain point, results in loss of function below that point.


* solutions: 1) bypass spinal cord control using brain-machine interface, 2) targeted reinnervation/FES, 3) repair link using either gene therapy or stem cell therapy.
Role of Denervation in Muscle

What is denervation and why is muscle special?

* in development, motorneurons (MNs) establish connections with target tissues.
* if tissues not innervated, lose their function/structure.

Muscle size and force output (strength) requires regular stimulation.

* space flight, long-term bed rest, disease: muscle does not encounter forces and resistance (atrophy).
* long-term exercise, rehabilitation: muscle encounters significant forces and resistance, stimulates repair, IGF-1 (hypertrophy).

Denervation ~ catabolism (process of atrophy).

Tetanic stimulation: high-frequency, extended firing of action potentials onto a target.

* implicated in LTP, affects contraction ability of muscle (fatigue).
* used here to test efficacy of implanted MNs (under fatigue – chemical changes due to sustained activity).
Gastrocnemius Muscle Location and Structure

Anatomical location of lateral Gastrocnemius muscle:

* hindlimb muscle: involved in pushing off during movement (force production).

Muscle fiber types: defined by different isoforms of myosin.

* FF muscle fibers (produce lots of force quickly, but also fatigue quickly): limited force production in pathological state.

Anatomy of skeletal muscle body:

a) Muscle body (e.g. gastrocnemius, $n$ muscle fibers).

b) single muscle fiber ($n$ myofibrils).

c) sliding filament structure of myofibril (produces contractions, bungee cord-like).
Muscle Fibers, Motor Units, and Force Production

Motor Unit (MN): single motor neuron and all muscle fibers that it innervates. Differences in composition of types between individuals in single muscle.

- Motor units contribute linearly to force production output, performance of controlled tasks.
- Same type of MNs innervate same types of muscle fibres (slow MNs = slow muscle fibers). In some cases, MNs can convert muscle fibers to its own type (selectivity).
- Activation of several different fiber types simultaneously required for controlled, ballistic movements.
- Some species (toadfish) have highly-specialized muscles (all “ultrafast” fibers) for specific tasks (mating calls).
Hypothesis and Assumptions

Hypothesis: treating atrophied muscle with neuronal stem cells will result in a rehabilitation of the treated muscle, which will further lead to a recovery of function.

ES-cell-derived motorneurons differentiate *in vitro*, injected into nerve.

* HBG3 transgenic mouse ES cell lines (uses HB9 promoter to drive eGFP expression – visualizes differentiated motorneurons).

* ES-cell derived MNs cultured and differentiated using retinoic acid and *Shh* agonist (see Miles, J. Neuroscience, 24, 784, 2004). Normally present in development.

* 5 days after differentiation, cells dissociated using Papain system and maintained in DFK10 medium.

Injection site: tibial nerve just proximal to medial gastrocnemius nerve. Nerves transected to channel axonal outgrowth.
Nerve Block and Injection Method

Method:

* nerve block on tibial nerve (denervation).

* pipette placed in section of tibial nerve distal to block.

* Injection of ES-cell derived motorneurons.

* axons project down nerve towards gastrocnemius.

* reinnervation of muscle by therapeutic means.

* only a short way to project compared with MNs in lower spinal cord.
Results (Figure 2)

GFP-expressing neurons project axons into muscle (stains A and B = tibial and medial gastroc nerves).

* stain C shows myelin protein surrounding newly sprouted axons (myelination via Schwann cells).

* Stain D shows nerve branching near NMJ. Distributes axon to multiple innervation sites.

* Stain E’ = bungarotoxin-labeled Ach receptors.

* Stain E” = eGFP-positive axons.

* Stain E’’’ = E’ and E” colocalized, confirms innervation.
Results (Figure 3)

Stem cell treatment responds to both regular electrical stimulation and tetanic stimulation:

* frame A shows trace of control and treatment for force production (mega-newtons).

* frame B shows trace for force production given tetanic stimulation.

  * about 40% of control achieved, similar waveform.

* frame C shows NMJ mediation of force production

  * C’ = baseline, C’’ = treatment with D-tubocucaraine, C’’’ = after 3 hrs.
Results (Figures 4 and 5)

Are ES-cell-derived motor units fatigue resistant?

Stimulation:
* 13 pulses at 40Hz per second for 2 minutes.

* Figure 4 = force recordings (time-series) and fatigue index (bar graph).

* Implants about 1/3 as resistant to fatigue, effects of fatigue asymptotic after 1 min (see lower fatigue at 2 min.) in both cases.

* Figure 5 shows motor unit analysis. Incremental force steps and EMG recordings show that distribution of motor units (in terms of size) in control ~ treatment.

* efficacy of treatment axonal innervation of multiple muscle fibers ~ control.
Results (Figure 6)

Three Conditions:
1) control (no lesion)

2) surgical control (spinal block only)

3) ES implant (stem cell treatment). Underwent increase in slow muscle fiber number and attenuated muscle fiber atrophy.

Slow (S58) fibers, fast fibers (myosin), and merged (slow and fast).

Whole midbelly of muscle (top rows for each condition), close-up box (bottom rows).

Stain F shows proliferation of slow fibers (S58) for ES implant, indicative of reinnervation.
Results (Figure 7)

Quantitative analysis:
* slow twitch fiber (S58) count (A)

* slow twitch fiber cross-sectional area (~ power output, innervation) – (B)

* MG muscle weight (mg, detect rescue from atrophy) – (C)

* MG muscle cross-sectional area (~ power output) – D)

* implant treatment is well > control for A, = control for B.

* whole muscle weight and cross-section for implant are significantly < control.

Curious result for A…artifact of targeted treatment?
Discussion and Conclusions

ES-cell-derived MNs can be functionally similar to endogenous MNs (e.g. electrophysiology, transmitter expression, ion channel dynamics).

* ES-cell-derived MNs quite heterogeneous.

Other studies using ES cells in spinal cord: rat model, kill subset of endogenous MNs, implant cells.

* axons can innervate target muscle, but motor units could not be formed (due to pre-existing motor units).

Increase in the number of slow muscle fibres after implantation: immunohistochemistry (myosin heavy chain antibodies), implants at tibial nerve change fiber biochemistry.

* slow fibres localized to a specific region of muscle. Different types of ES-cell derived MNs (type S) might innervate a certain fiber type and expand their territory.

ES-cell derived MNs form motor units comparable in size with endogenous MNs, but no larger (despite fewer MNs innervating muscle in implant group).

* prolonged denervation can hinder the reinnervational capacity of endogenous MNs.

* but ES-cell-derived MNs not as responsive to axonal sprouting cues (e.g. CNTF from Schwann cells)
Clinical Benchmarks

1) Proliferate extensively and generate sufficient quantities of tissue? MAYBE (what is a sufficient # of MNs? For $10^6$ ES-cell-derived MNs, 42% muscle x-sectional area, 44% of tetanic force, and 44% # of motor units in single muscle).

2) Differentiate into the desired cell type? YES.

3) Survive in the recipient after transplant? CONDITIONAL (didn't test spinal cord implantation, issues with axon guidance and maintenance?).

4) Integrate into the surrounding tissue after transplant? CONDITIONAL (see last point – experimental technique not viable in everyday life).

5) Function appropriately for the duration of the recipient's life? MAYBE (muscle ages – gains adipocytes - and is constantly being damaged/repai red, can differentiated neurons deal with those challenges?).

6) Avoid harming the recipient in any way? YES.