Tissue Engineered Nerve Grafts Facilitate Muscle Reinnervation Following Long-Gap Facial Nerve Injury in Swine

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BACKGROUND

- With current strategies for surgical repair of peripheral nerve injury (PNI), recovery of motor and sensory function is generally inadequate owing to a lack of treatments aimed at preserving the regenerative environment of distal nerve segments and the reinnervation capacity of target muscle(s).
  - Insufficient bridging strategies across segmental nerve defects results in diminished axonal regenerative capacity, creating a *low ceiling for the extent of possible functional recovery*.
  - Peripheral nerve *regeneration is a race against time* as long distances for axonal re-growth (i.e. from a point of transection to end targets) often requires many months, over which time endogenous Schwann cells (SCs) and myofibers gradually loose their capacity to support axonal regeneration and reinnervation, respectively.

➢ ***To address this need, we have developed tissue engineered nerve grafts (TENGs) consisting of living neurons and aligned axonal tracts which we routinely generate in custom-built mechanobioreactors at densities of >100,000 axons and lengths of ≥5cm through the controlled process of axon "stretch-growth".***

➢ ***TENG axons mimic the developmental action of "pioneer" axons, where targeted axonal outgrowth can be achieved along pre-existing axonal tracts in vivo.***

➢ ***TENGs are bio-fabricated "living scaffolds" that provide an environment rich in structural and trophic cues necessary to drive host axon regeneration, sustain a pro-regenerative environment, and quickly facilitate reinnervation of muscle.***

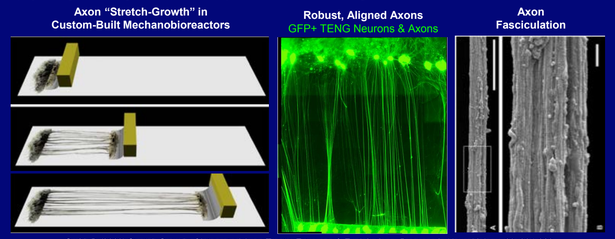
OBJECTIVES: Demonstrate that TENGs

- **Provide optimal "BRIDGING" and "BABYSITTING" strategies to facilitate long-distance axon regeneration, muscle reinnervation, and functional recovery in an extremely challenging 4-5cm segmental nerve defect models in swine.**
- **Having advanced our TENG technology with the development of mixed motor and sensory constructs, we hypothesize these mixed modality TENGs would facilitate functional recovery by increasing host axon regeneration or by babysitting the denervated muscle end target.**

METHODS: *TENG Bio-Fabrication*

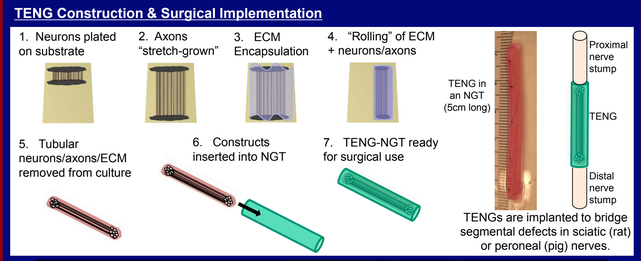
Axon "Stretch-Growth" in Custom Mechanobioreactors

- Controlled separation of two integrated dorsal root ganglion (DRG) and/or spinal motor neuron (SMN) populations: stretching increases axon length & thickness, and stretch-grown axons from fascicular bundles.
- Replicates endogenous developmental process: accelerated axon growth without chemical cues, physical guides or growth cones. **Unprecedented axon length: 5 - 10 cm in 14 - 21 days.**

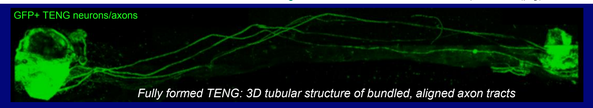


Smith D (2009) Stretch Growth of Integrated Axon Tracts: Extremes & Exploitations. Progress in Neurobiology. 89: 231-239

TENG Construction & Surgical Implementation



TENGs are implanted to bridge segmental defects in sciatic (rat) or peroneal (pig) nerves.



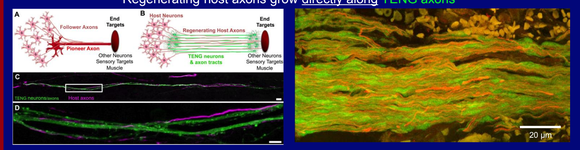
Fully formed TENG: 3D tubular structure of bundled, aligned axon tracts

- Allogeneic TENGs were created at lengths from 1-5cm and used to bridge segmental nerve defects in rats or pigs, with terminal time points ranging from 2 weeks to 12 months. Histological outcomes included host axon re-growth, SC presence and alignment, and DRG and SMN health. Nerve conduction and muscle action potentials were assessed at chronic time points.

RESULTS: Sensory *TENGs "BRIDGE" Segmental Defects in Rats (1-2cm) and Swine (1-5cm) and Facilitate Functional Recovery*

Demonstration of "Living Scaffold" for Directed Axonal Regeneration in Rats and Pigs

Regenerating host axons grow directly along TENG axons



Acute axon regeneration was equivalent between TENGs and Autografts, 3-4x faster than NGTs

Rates of Axon Regeneration (Regenerative Front – Leading Regenerators)

Species	Nerve	TENGs	Autograft	NGTs/Nerve Wraps
Rat	Sciatic (mixed)	0.44 – 1.11 mm/day	0.52 – 0.77 mm/day	0.17 – 0.27 mm/day
Pig	Deep Peroneal (sensory branch)	1.20 – 1.45 mm/day	*0.87 – 1.20 mm/day	0.29 – 0.53 mm/day
	Deep Peroneal (motor branch)	0.41 – 1.02 mm/day	**0.75 – 0.97 mm/day	0.26 – 0.44 mm/day

*Reverse isograft: autograft that perfectly matches modality of nerve lesion (not done clinically)
**Dual nerve autograft; repairing a motor nerve with a sensory autograft, as is the case clinically

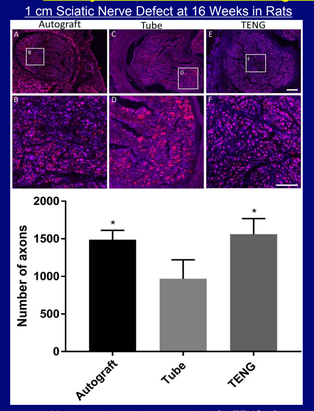
New Mechanism: "Axon-Facilitated Axon Regeneration"

Mimics developmental mechanisms for axonal growth
We are the first to exploit this mechanism for nervous system repair

TENGs were the only group to achieve >1 mm/day regeneration across the lesion for all nerves/species

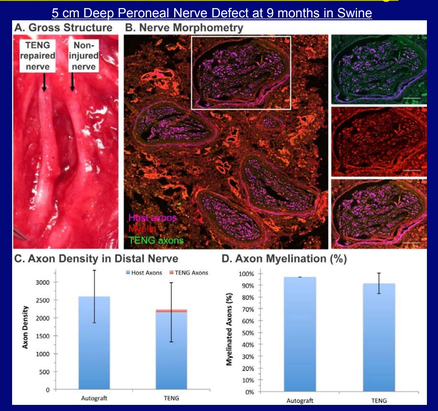
Sensory TENGs Accelerate Axon Regeneration and Schwann Cell Infiltration at Chronic Time Points in Rats and Pigs

1 cm Sciatic Nerve Defect at 16 Weeks in Rats



Nerve morphometry was matched for TENGs & Autografts vs NGTs at 16 weeks (p<0.01)

5 cm Deep Peroneal Nerve Defect at 9 months in Swine

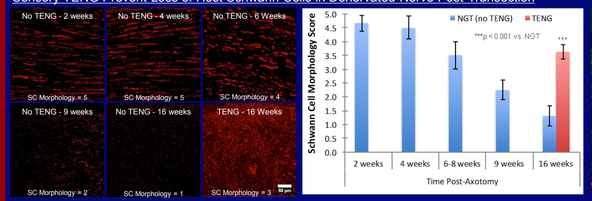


Regenerated nerve structure, axon density, and myelination were equivalent between animals repaired with TENGs vs Autografts

RESULTS: Sensory *TENGs "BABYSIT" Host Spinal Motor Neurons, Pro-Regenerative Schwann Cells, and Target Muscle in Rats*

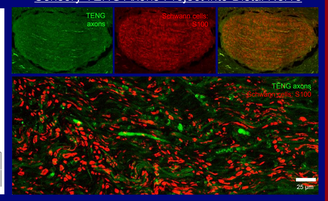
Sensory TENGs Maintain Pro-Regenerative Host Schwann Cells in Model of Chronic Axotomy in Rats

Sensory TENG Prevent Loss of Host Schwann Cells in Denervated Nerve Post-Transection



After nerve transection, the denervated distal nerve sheath gradually degrades over 16 weeks; however, implanted TENGs maintain pro-regenerative host SCs

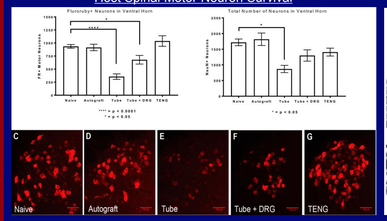
Sensory TENG Axons Project into Distal Nerve



A relatively small "dose" of TENG axons are sufficient to maintain entire fascicles of host SCs

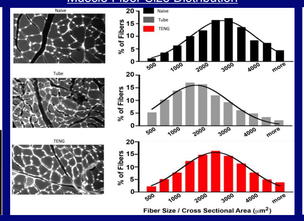
Sensory TENGs Preserve Host Spinal Motor Neuron Health and Muscle Function When Used to Bridge Segmental Defects in Rats

Host Spinal Motor Neuron Survival

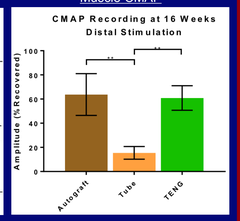


NGT repairs resulted in >60% loss of host SMNs; this effect was abolished with TENGs or Autografts

Muscle Fiber Size Distribution



Muscle CMAP



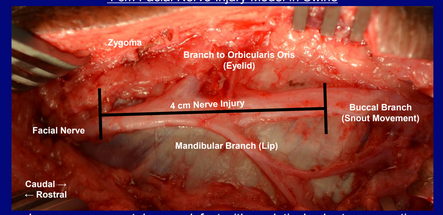
Muscle fiber size and electrophysiology show that TENGs drive reinnervation and significantly improve muscle function by 16 weeks after nerve transection and repair

These comprehensive results – across multiple species and nerve repair models – demonstrate that TENGs are a "living scaffold" that drive axonal regeneration to facilitate robust functional restoration.

RESULTS: *Mixed Sensory/Motor TENGs Facilitate Functional Recovery Following Facial Nerve Segmental Defects in Swine (4cm)*

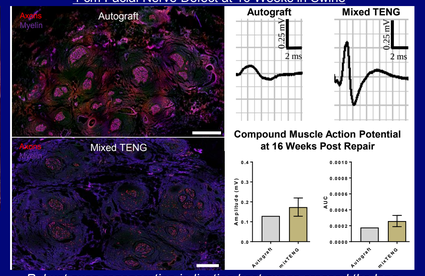
Mixed Sensory/Motor TENGs Enhance Axonal Regeneration at Chronic Time Points in Pigs

4 cm Facial Nerve Injury Model in Swine



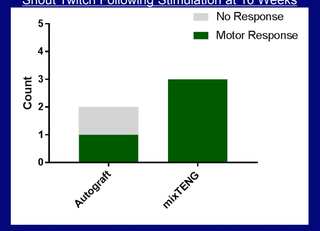
Long gap segmental nerve defect with a relatively short regenerative distance to target muscle compared to hind limb model; 8 cm vs 27 cm total regenerative distance

4 cm Facial Nerve Defect at 16 Weeks in Swine



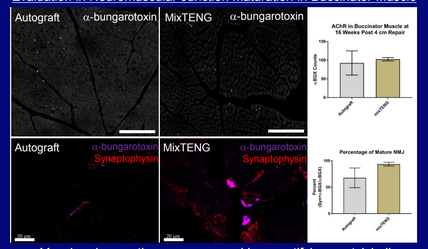
Robust axon regeneration indicating host axons crossed the long gap nerve injury and reached the distal nerve segment

Snout Twitch Following Stimulation at 16 Weeks



Following nerve stimulation, an evoked snout twitch with a measurable CMAP was observed with Mixed TENG repair

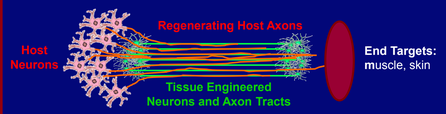
Evaluation in Neuromuscular Junction Maturation in Buccinator Muscle



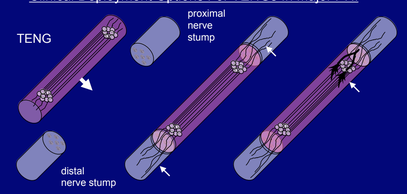
Muscle reinnervation was assessed by quantifying acetylcholine receptors co-labeled with bungarotoxin and synaptophysin

CONCLUSIONS & FUTURE DIRECTIONS

- TENGs exploit our recently discovered mechanism of axon-facilitated axon regeneration to serve as ***living bridges*** across segmental nerve defects while ***babysitting*** distal structures to facilitate muscle reinnervation.



Clinical Deployment Options For TENGs In Major PNI

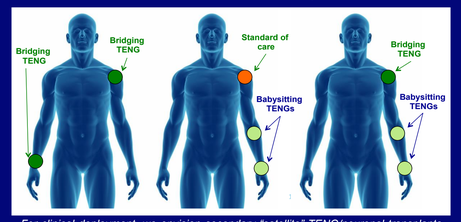


BRIDGING
Reconstruct proximal & large distal PNI using TENGs at site of injury

BABYSITTING
Reconstruct proximal PNI w/ SOC (autograft), implant babysitting TENGs distally to preserve distal structures

BRIDGING & BABYSITTING
Reconstruct proximal PNI w/ bridging TENG, add babysitting TENGs to preserve distal structures

- Early findings of the current study suggests the capacity for mixed TENGs to preserve host SC regenerative capacity, SMN health, and myofibers mass in challenging rat and pig models of PNI:
  - Axons projecting from TENGs grew along host SCs in otherwise denervated nerve segments – a mechanism not possible with autograft repairs – that served to maintain pro-regenerative SC alignment over at least several months.
  - TENGS facilitated improvements in SMN health – equivalent to autografts – versus those repaired with acellular nerve guidance tubes (NGTs) or NGTs filled with disorganized (non-stretch-grown) neurons.
  - TENGs resulted in improvements in target muscle mass and fiber size distributions – equivalent to autografts – versus NGTs alone.
- TENGs present a "bridging" strategy that matches the performance of the clinical "gold standard" – the sural nerve autograft – while presenting axon-based "babysitting" mechanisms not possible with autografts.
- Additional enrollment is necessary to increase statistical power.



For clinical deployment, we envision secondary "satellite" TENG/neuronal transplants at metered locations distal to a primary repair, with the sole purpose to maintain pro-regenerative SCs ahead of regenerating axons.

- Overall, TENGs uniquely act as "living scaffolds" to promote a favorable environment for nerve regeneration by maintaining pro-regenerative capacity of host SCs, health of SMNs, and muscle fiber mass, collectively **raising the ceiling for potential levels of functional recovery beyond that attainable by conventional surgical repair strategies**.
- Axonova Medical and the University of Pennsylvania have initiated efforts for **TENG bio-fabrication under cGMP conditions** to facilitate IND-enabling safety, tolerability, and efficacy studies in NHPs and eventual Phase I/II Clinical Trials.

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