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Temporally Regulated GDNF Delivery Prevents Axon Trapping in Long Peripheral Nerve Injuries

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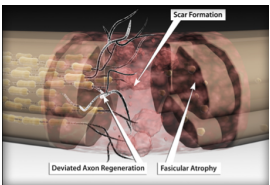
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²Division of Plastic and Reconstructive Surgery, Washington University School of Medicine

ASNR
Chicago, IL
October 15th, 2015

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Peripheral Nerve Injury (PNI)

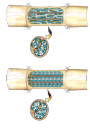
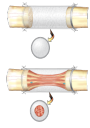
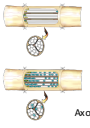
- Accounts for 1-3% of all traumatic injuries in the US each year with 200,000 peripheral nerve repair surgeries per year¹
- Results in loss of motor function and sensation



¹Taylor, C, et. al. AM J Phys Med Rehab. 2008.
²Archibald, S, et. al. J of Neurosci. 1995.

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Current Clinical Treatment Options

<p>Nerve Autograft</p>  <ul style="list-style-type: none"> • Host tissue • Neurotrophic/cellular support • Donor site morbidity • Multiple surgeries required 	<p>Hollow Tube</p>  <ul style="list-style-type: none"> • Non-immunogenic • Off-the-shelf • Lack of physical/neurotrophic support • Poor regenerative results 	<p>Acellular Nerve Allograft</p>  <ul style="list-style-type: none"> • Non-immunogenic • Native nerve architecture • Lack neurotrophic/cellular support <p style="font-size: x-small;">Axogen, 2014.</p>
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Can we improve ANA outcomes with cell transplantation and /or growth factor delivery ?

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Glial cell-line derived neurotrophic factor (GDNF)

- 15 kDa member of TGF β superfamily¹
- Affects neuron survival and extension¹
- Implicated in SC migration, proliferation, and differentiation²

In neurons:
GDNF binds to RET (CLD1-CLD4) and GFR α (G3-G4) on the cell surface. RET is a tyrosine kinase (TK) associated with a lipid raft. Signaling involves Ca²⁺ and PLC β .

In SCs:
GDNF binds to Heparan sulfate proteoglycan (Agrin) and GFR α 1. This complex is associated with NCAM and a lipid raft. Downstream signaling involves Fyn, Ras, Raf, and MEK.

Alirakinen, M. Nat Rev. 2002
Iwase, T. J Neurochem. 2003

¹Alirakinen, M. Nat Rev. 2002
²Hbke, A. J Neurosci. 2003



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Glial-cell line derived neurotrophic factor (GDNF) delivery methods

diffusion-based release suspension affinity-based delivery microsphere encapsulation

Daly, W. L, Yao, D, Zeugolis, A, Winderbank, and A, Pandit. J Roy Soc Interface. 2012. 9(67):202-2



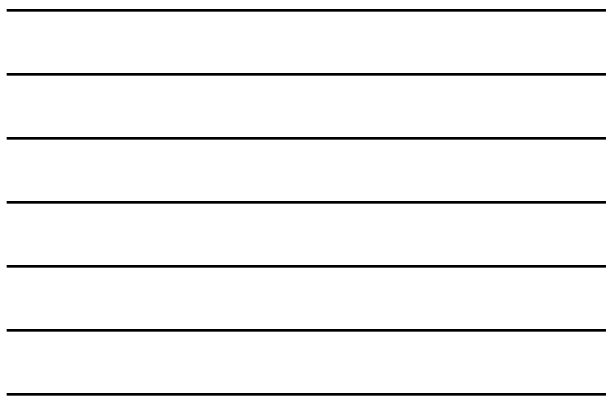
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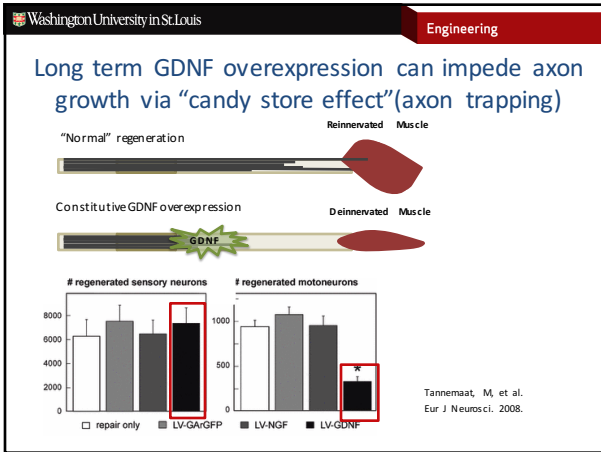
Short term affinity-based delivery shows improved, yet limited regeneration

Number of Nerve Fibers

Condition	Number of Nerve Fibers
Isograft	~12500
SC-GDNF-DS	~4500
KDF-DS	~3000
SC-GDNF-DSL	~3500

Wood, M, et. al. Acta Biomater. 2009.



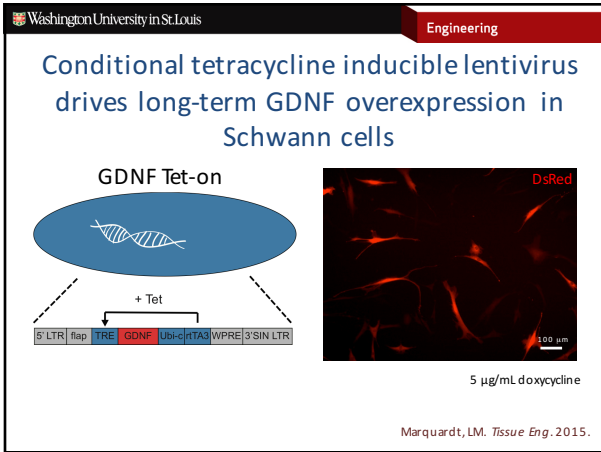


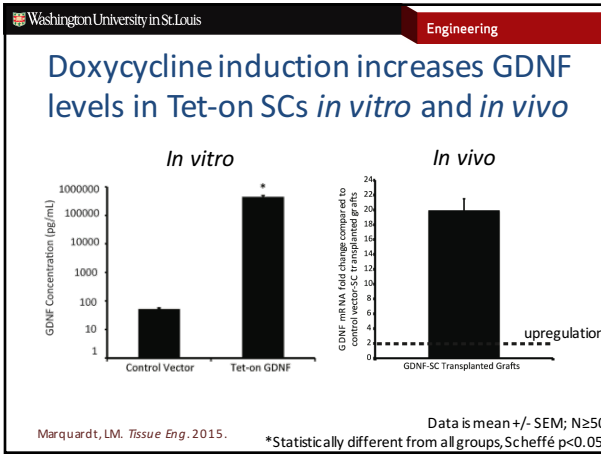
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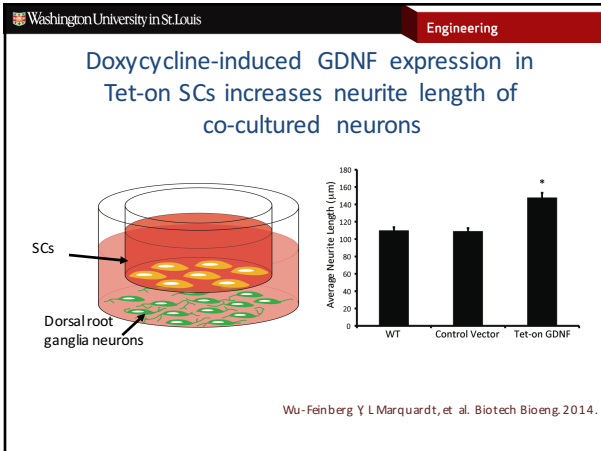
Objectives

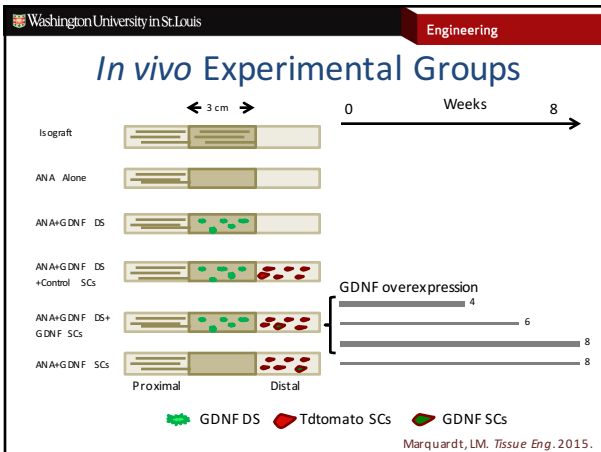
Overall : Enhance axonal regeneration in a long nerve defect model through a temporal and spatial GDNF delivery platform

- Dual GDNF delivery platform: modified ANA with affinity-based GDNF release and transplanted GDNF expressing Schwann cells (SCs)
- Determine timing of controlled GDNF delivery through affinity-based delivery system and LV GDNF-SCs in 3 cm rat sciatic nerve defect model





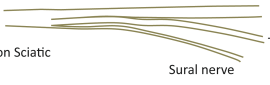




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Surgical Procedure


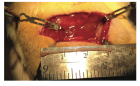
- Expose nerve and isolate sural nerve to be spared.

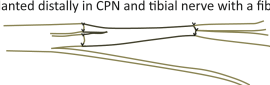



Common peroneal nerve (CPN)

Tibial nerve

Sural nerve
- Transect CPN and tibial nerve about 5 mm distal to the trifurcation.



- Graft 3 cm reverse isograft or ANA to CPN and tibial nerve. SCs are transplanted distally in CPN and tibial nerve with a fibrin gel.

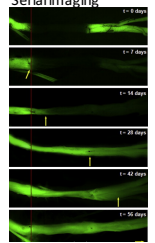



Marquardt, LM. *Tissue Eng.* 2015.

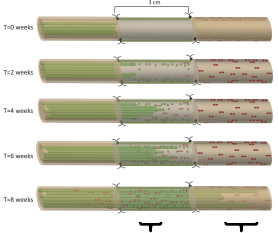
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Thy1-GFP⁺ axons allow for serial tracking of nerve regeneration

Serial imaging



Moore, A. *J Neurosci Met.* 2012.

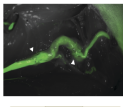


Histomorphometry

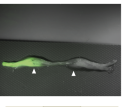
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Fluorescent imaging highlights differences between transplant groups

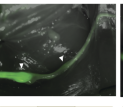
Isograft



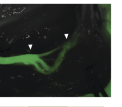
ANA



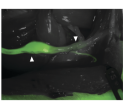
GDNF DS



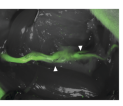
Control vector-SCs + GDNF DS



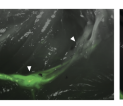
GDNF-SCs + Dox 4 DS



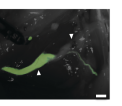
GDNF-SCs + Dox 6 DS



GDNF-SCs + Dox 8 DS

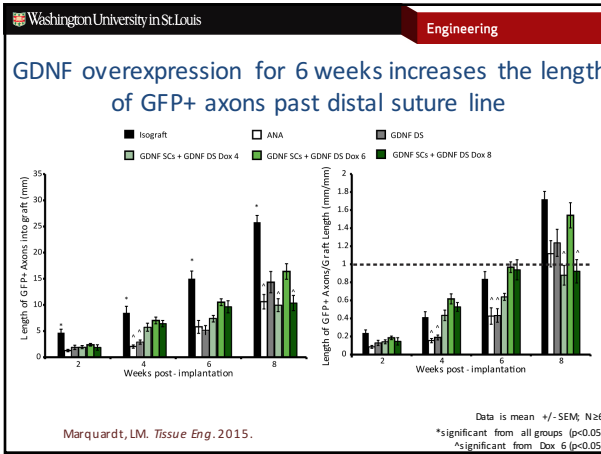


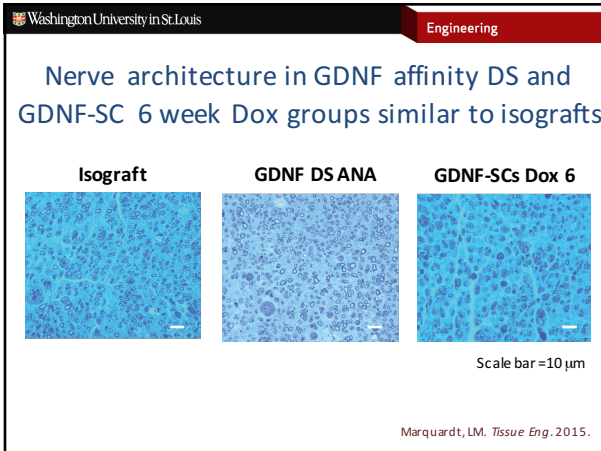
GDNF-SCs + Dox 8 no DS

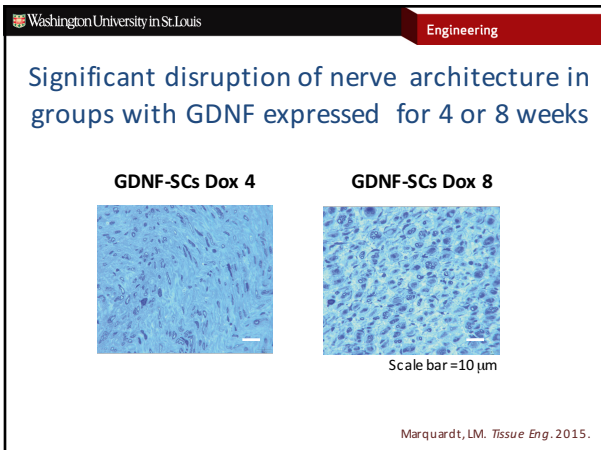


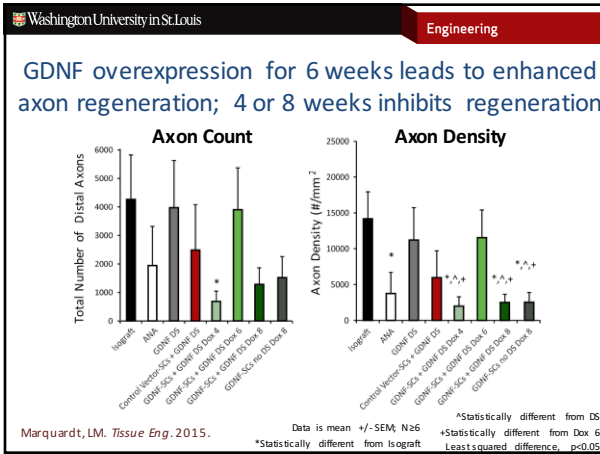
Scale bar = 3 mm
Arrows denote graft length

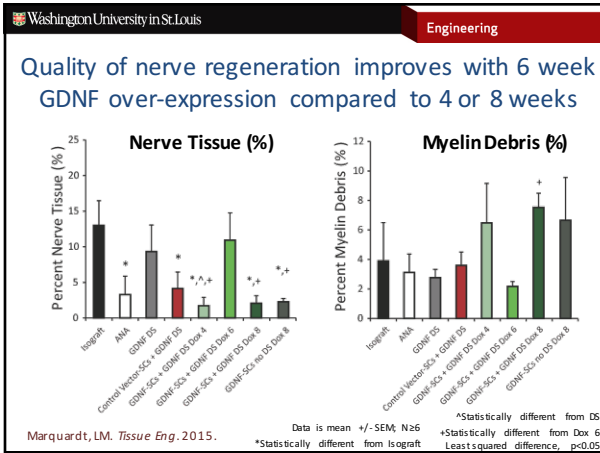
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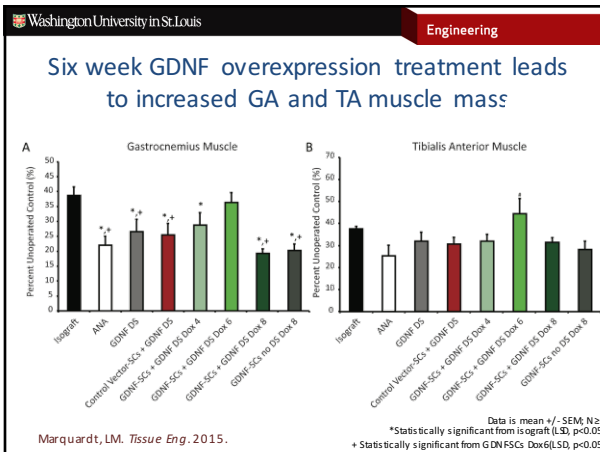












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Summary

- GDNF released from GDNF-SCs remains biologically active and promotes significant neurite extension of DRG neurons.
- *In vivo* metrics indicate an intermediate time point of 6 weeks induced GDNF expression and a GDNF modified ANA prevents the “candy store effect” and enhances axonal regeneration.
- Increased muscle mass of GA and TA observed in 6 week GDNF overexpression similar to that of isografts

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