Nerve Graft Immunosuppression

University of Wyoming Board of Trustees

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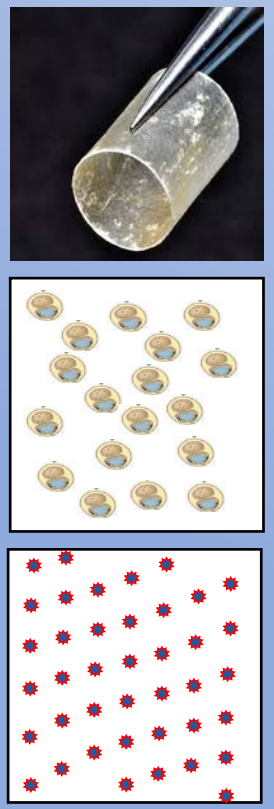
Immunoengineering vs Tissue Engineering

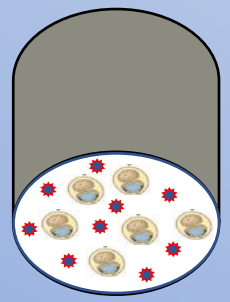
Tissue Engineering

Scaffold

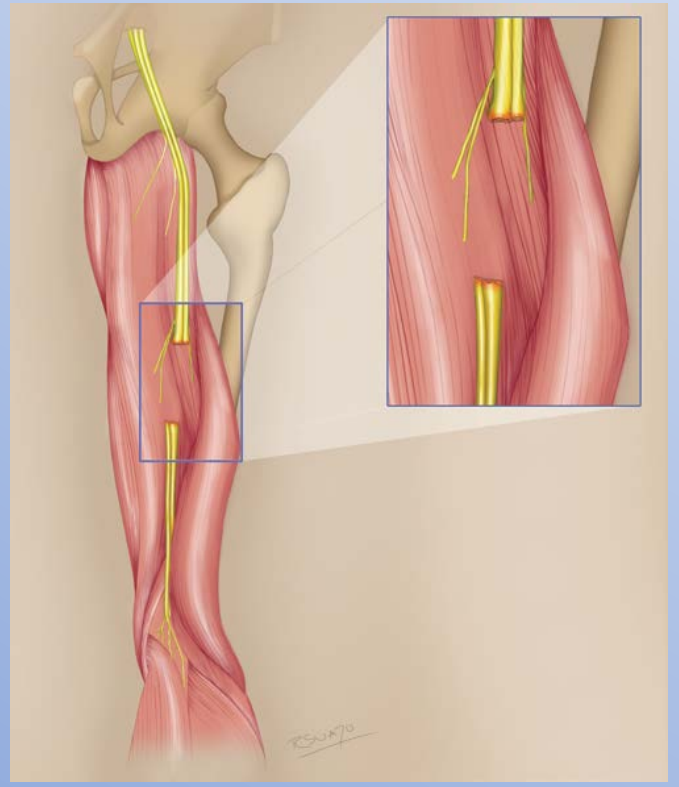
Cells

Growth factors





Approximate a living tissue for the purpose of regeneration



Immunoengineering

A living tissue that strongly supports regeneration

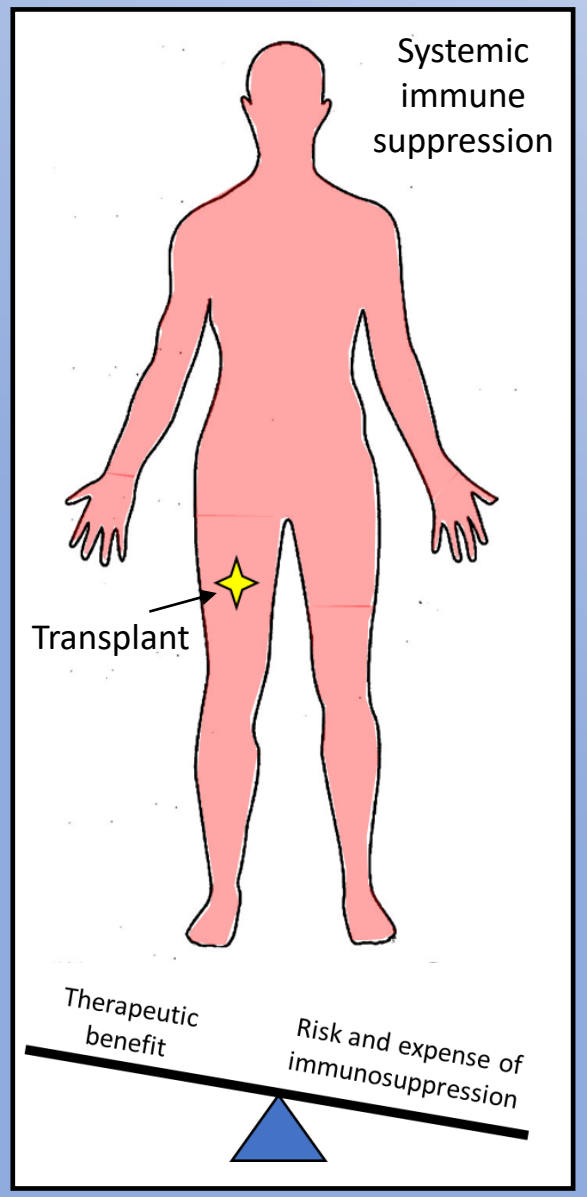
Living donor nerve tissue

+

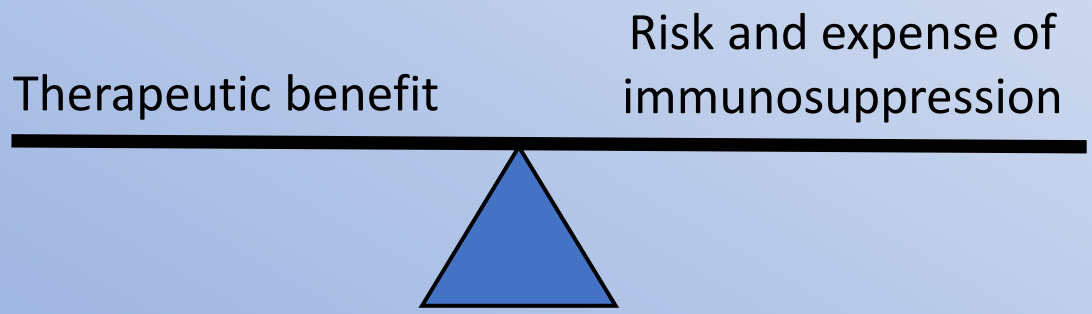
Engineered immune modulators

Living tissue provides some immediate functional replacement

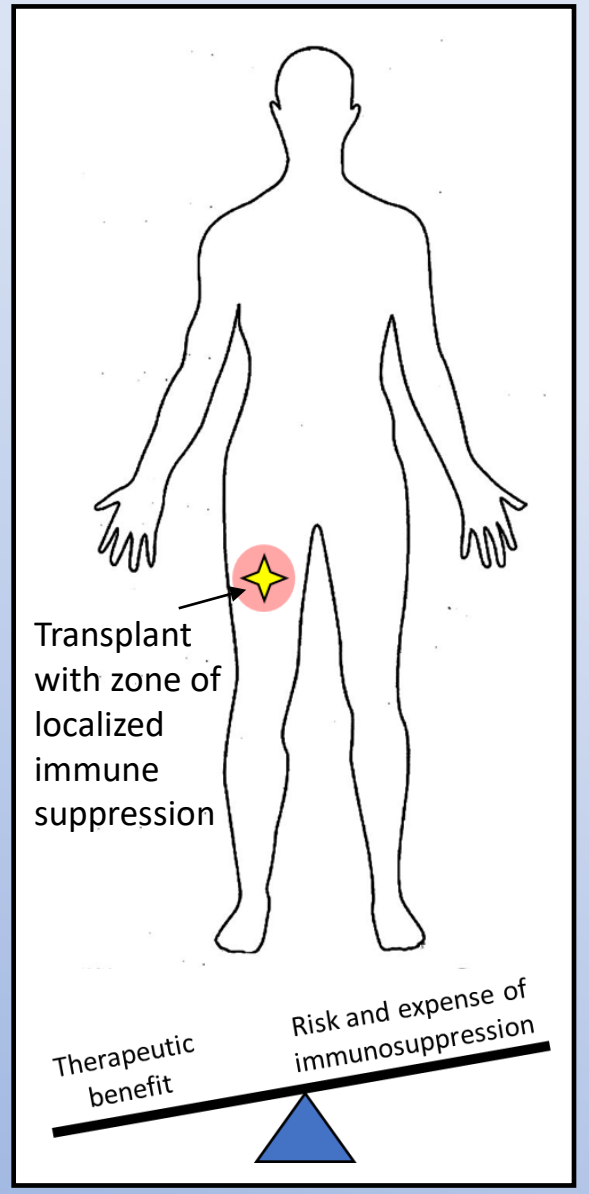
Problem and Concept



Balance between benefit of transplant versus risks and cost of immunosuppression

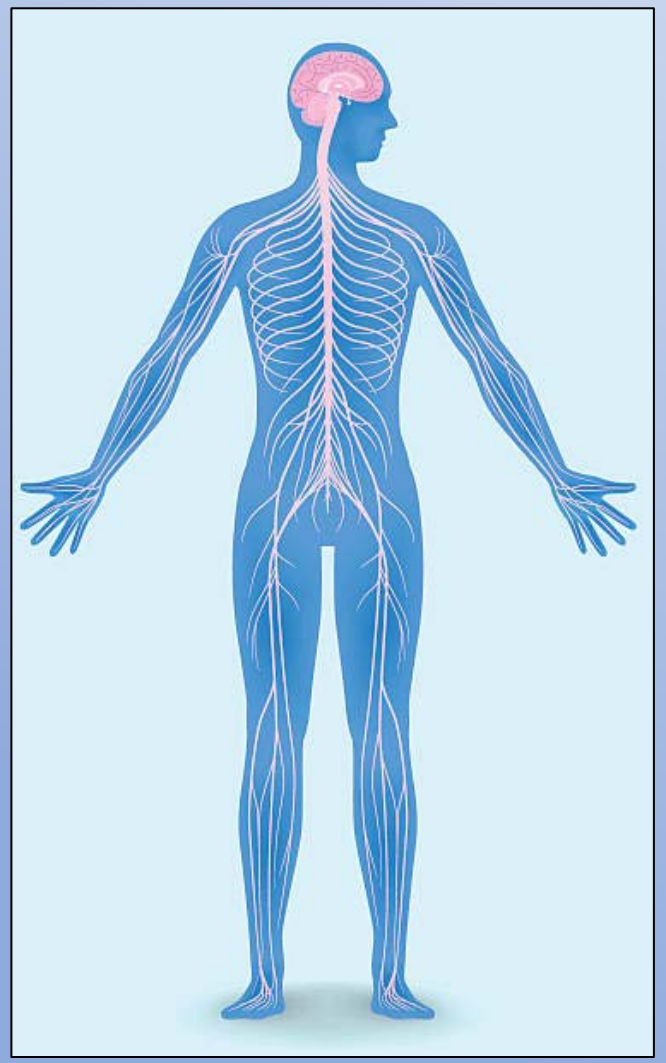


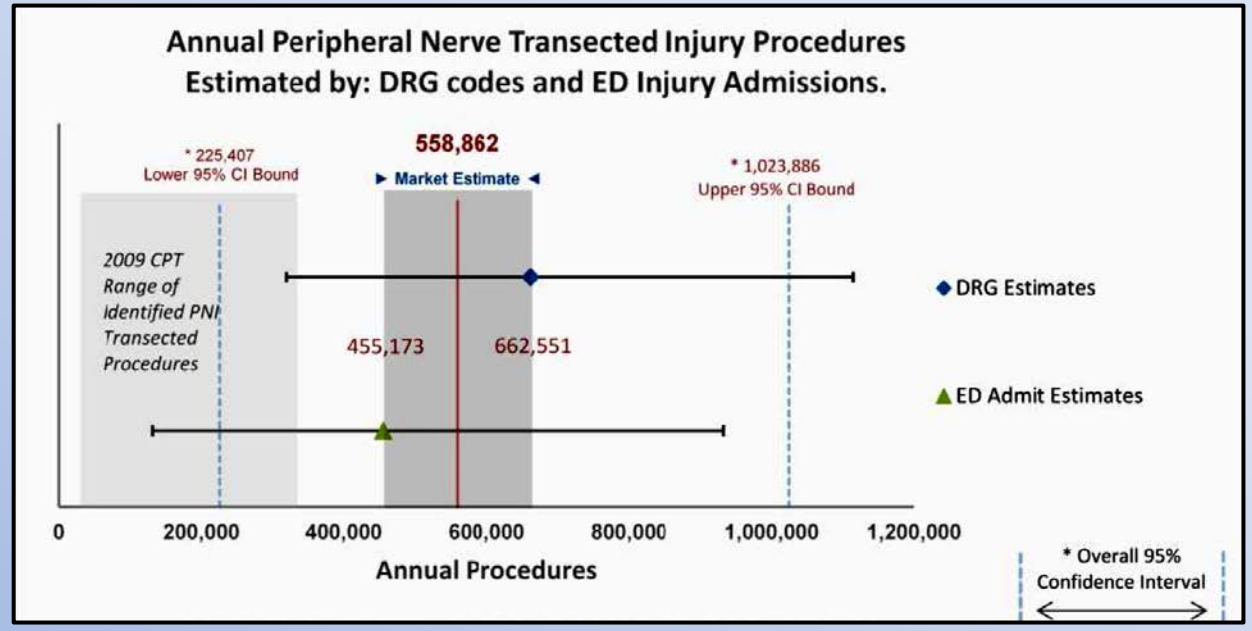
Objective is to reduce the costs and risks of immune suppression by localizing it only to the donor tissue graft by immunoengineering



Initial Application: Peripheral Nerve Injury

- ~50 miles of peripheral nerves in a human body
- Nerve frequently injured from trauma and disease
- ~550K annual surgical procedures in the United States





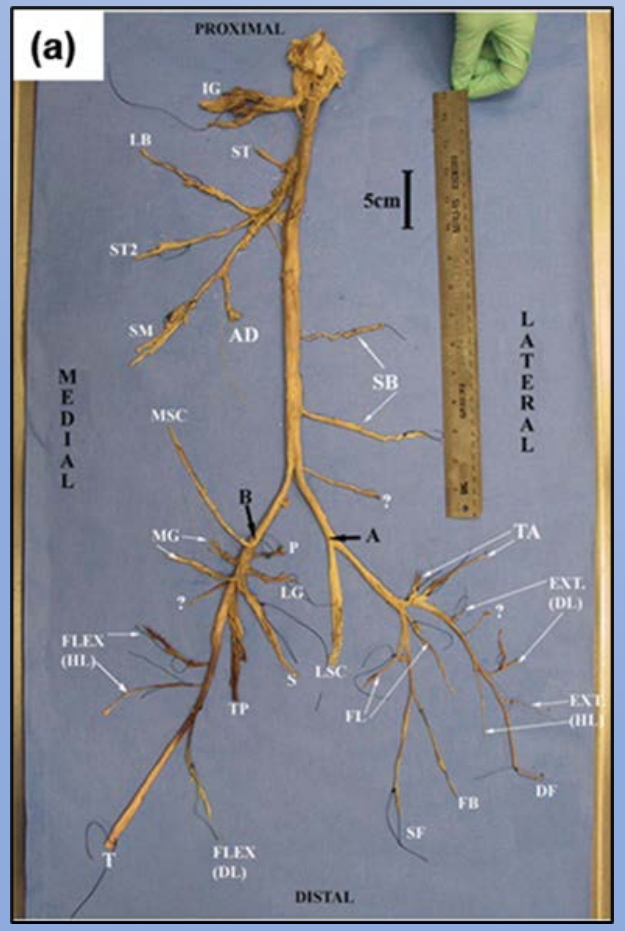
2010 is last known market analysis

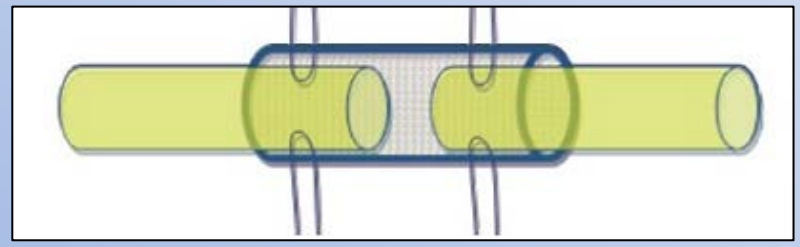
- ~550K annual surgical procedures in the United States
  - Market of 1.8 billion $US
- Outcomes with current therapies are poor, permanent disability is common

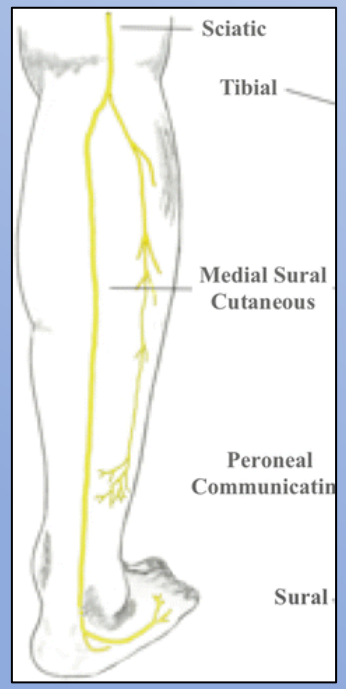
Market research data obtained from report by Magellan Medical Technology Consultants and published reports on frequency of PN injuries in 2010

First Application: Segmental Peripheral Nerve Repair

Human sciatic nerve







Gold Standard

Autologous sensory nerve harvested and transferred from the same patient

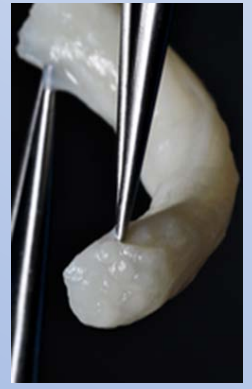
- Live nerve

Pitfalls of using autografts

- Morbidity
- Mismatch
- Non-restorative regeneration

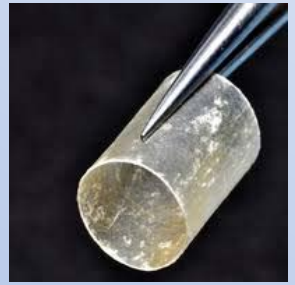
Injuries that create segmental peripheral nerve defects require a bridging device for any regeneration to occur

Alternatives to Autografts



Acellular Allograft (Axogen)

Conduits/Wraps (11 in the market)

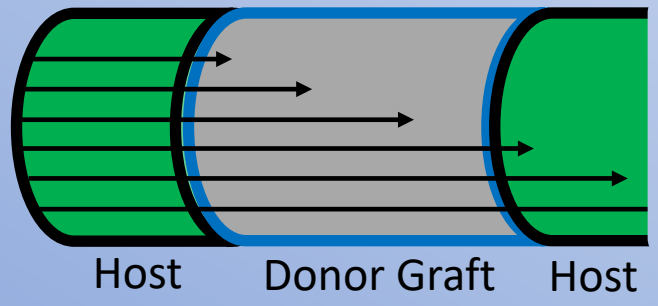


Pitfalls of engineered devices

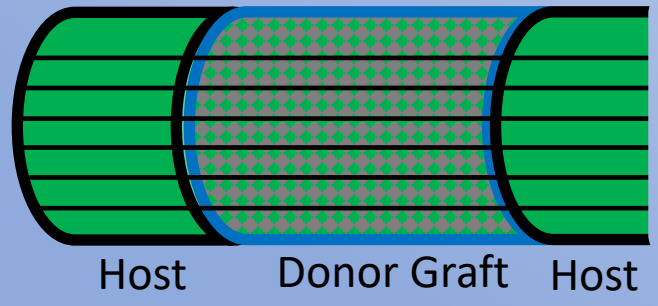
- Fractionally effective compared to autograft – not a live nerve graft
- Can only be used for short defects
  - Still inferior to autograft

Studied the immune response to peripheral nerve allografts to determine if/how immunoengineering could be accomplished

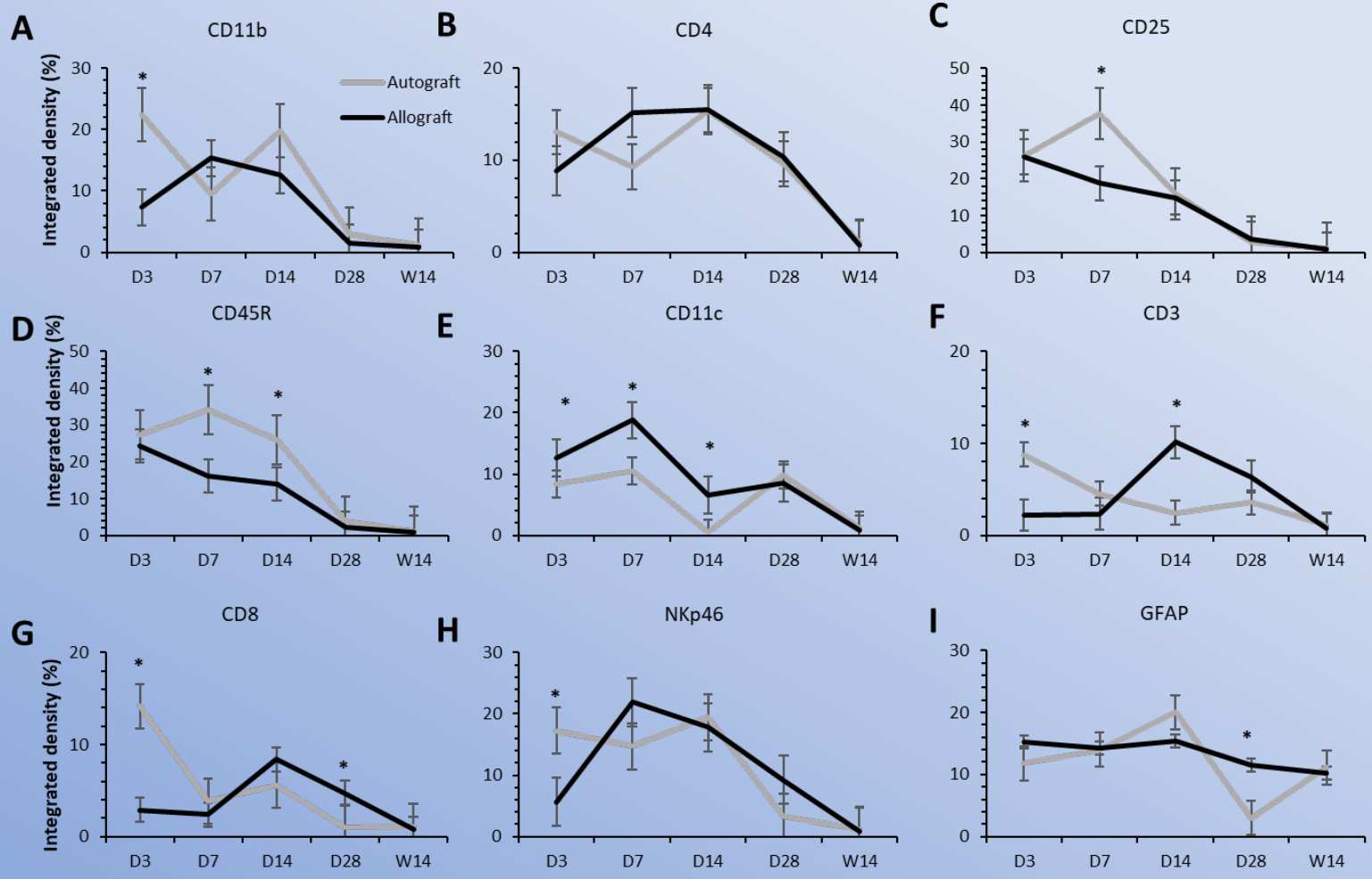
Regenerating host axons through the graft



Tissue rejection after axon regeneration

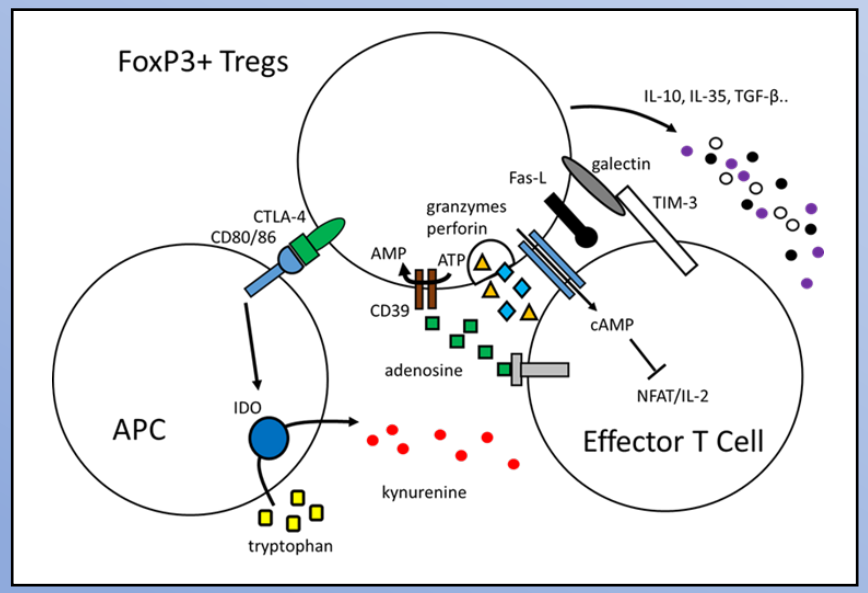


- Nerve allografts are a **temporary scaffold** for rejection of host axons
- Host immune response within nerve allografts is mild compared to other tissues



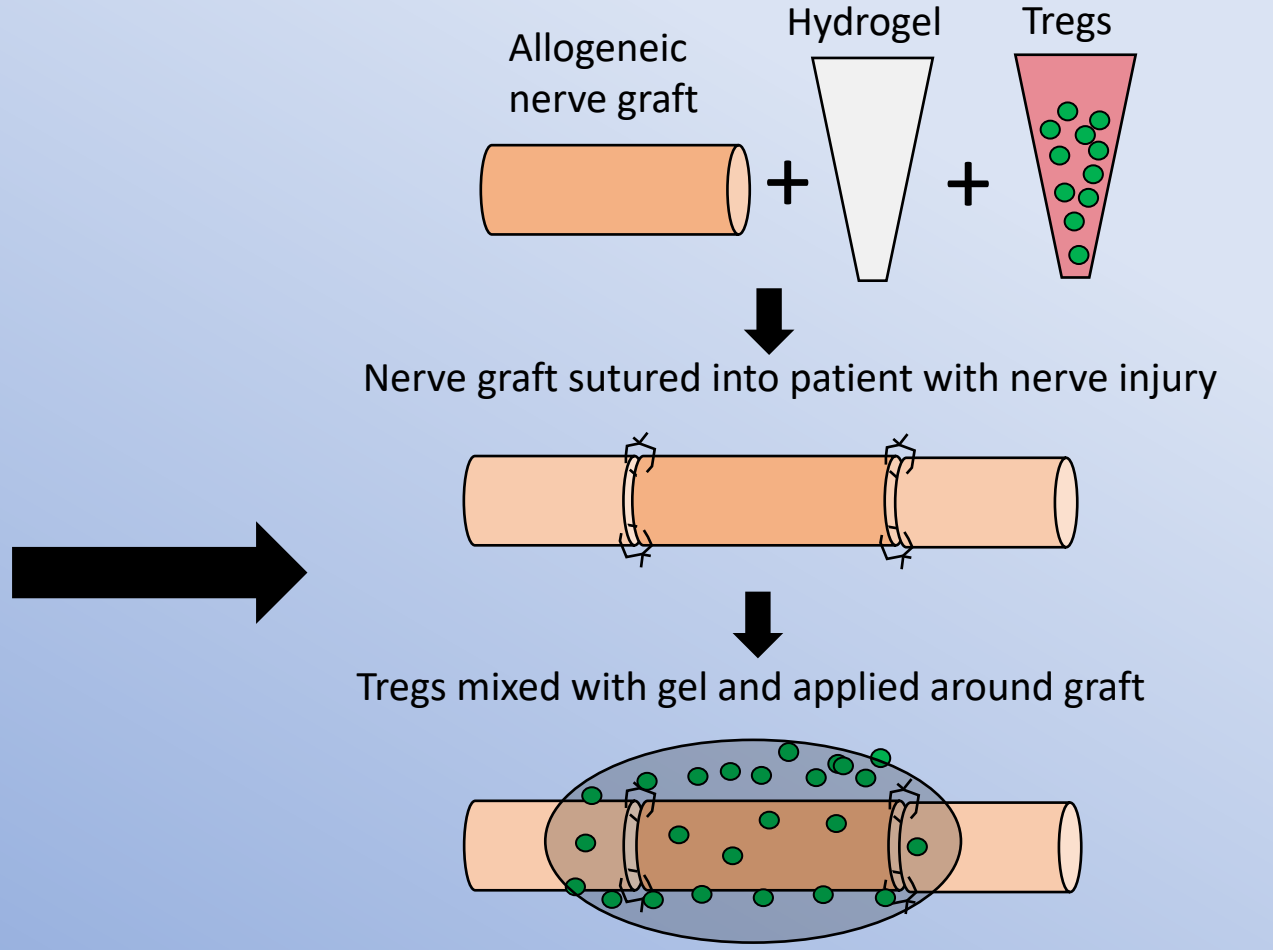
Roballo and Bushman, Transplant Immunology 2019

Immunoengineering by Localized Cell Delivery



Tregs suppress the immune cells that cause rejection of peripheral nerve allografts

Collaborated with industrial partner (Terumo) on method to expand human Tregs using a commercial bioreactor

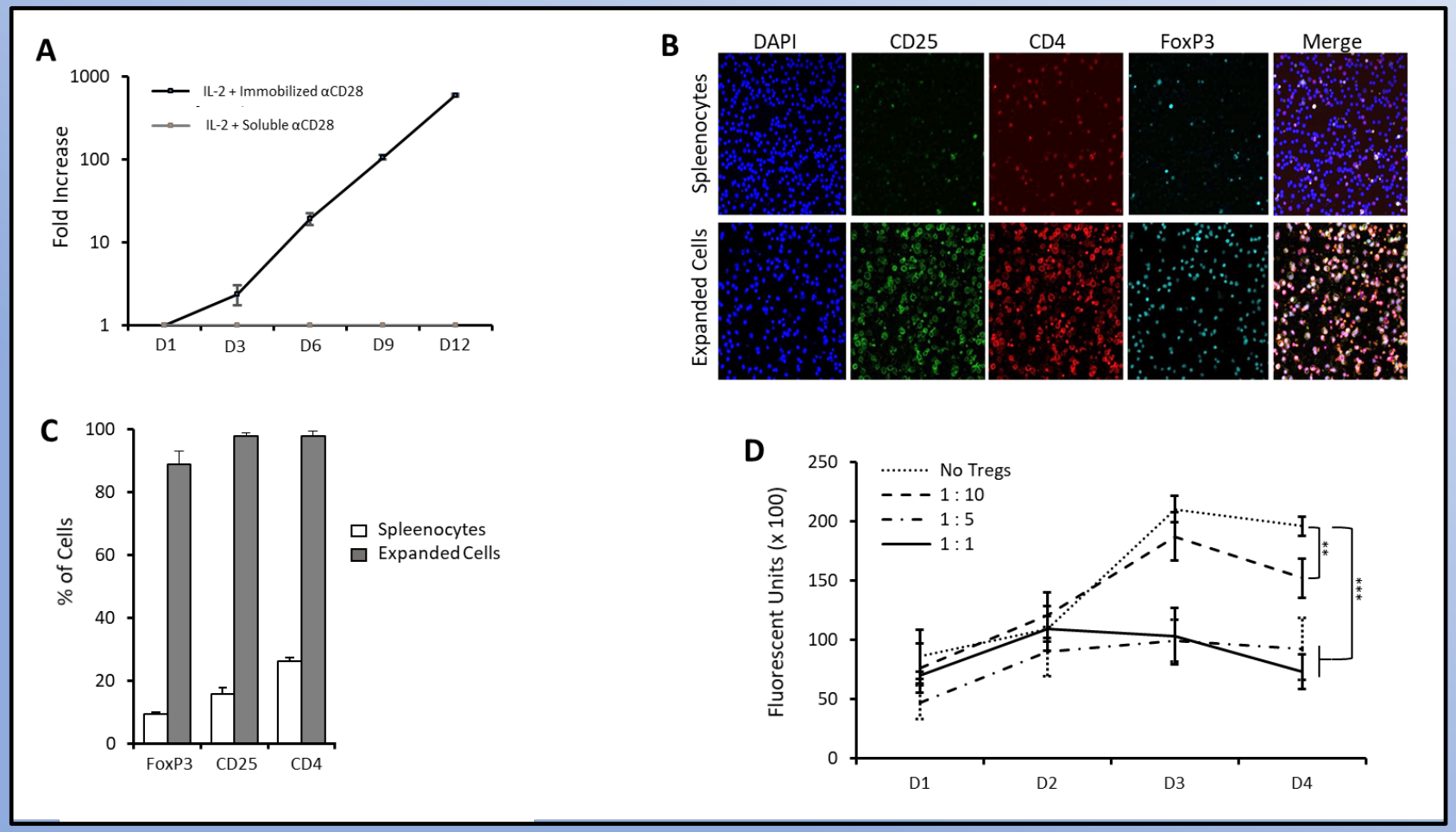


IP Portfolio

- US Patents: 10,064,938, 10,588,970, 10,683,408, 10,980,880
- US Patent Applications: 16/119,934, 16/049,343, 16/988,878

Jones, Bushman and Coeshott, Cell Transplant . Jan-Dec 2020;29:

Process 1: Treg Isolation, Expansion and Characterization

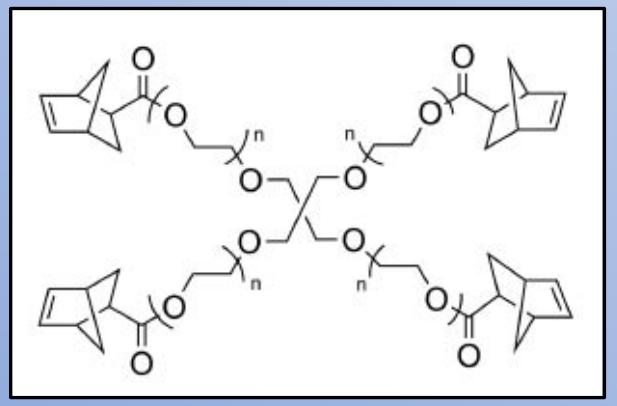


- Isolated from spleen (CD4+, CD25+)
- Expanded in vitro (IL-2, anti-CD28)
- Immunophenotype
  - >98% CD4+
  - >98% CD25+
  - >90% FoxP3+
- Functional assay
  - Inhibit expansion of allogeneic spleenocytes

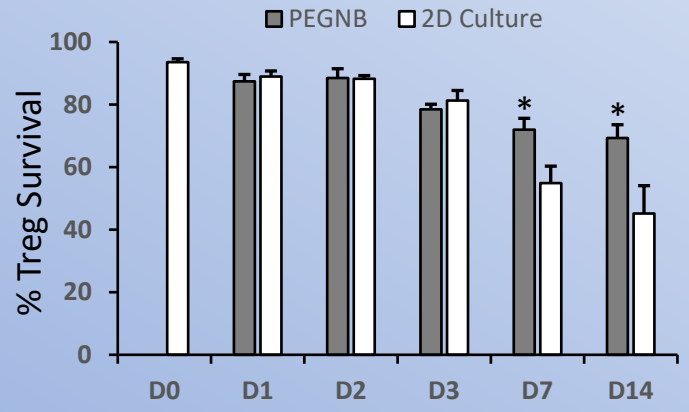
Roballo and Bushman, Biomaterials. 2019

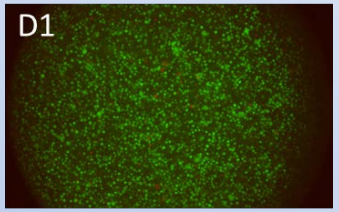
Process 2: Development of Biomaterial Hydrogel Vehicle for Treg Delivery

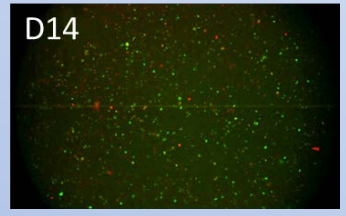
Collaboration with Professor John Oakey (Chemical Engineering)

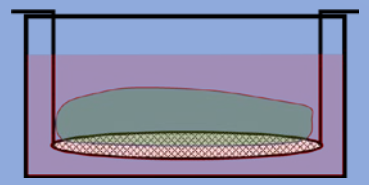


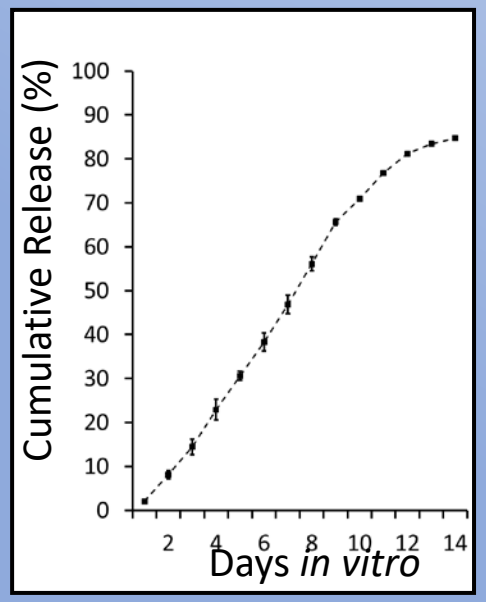
Poly(ethylene glycol) norbornene

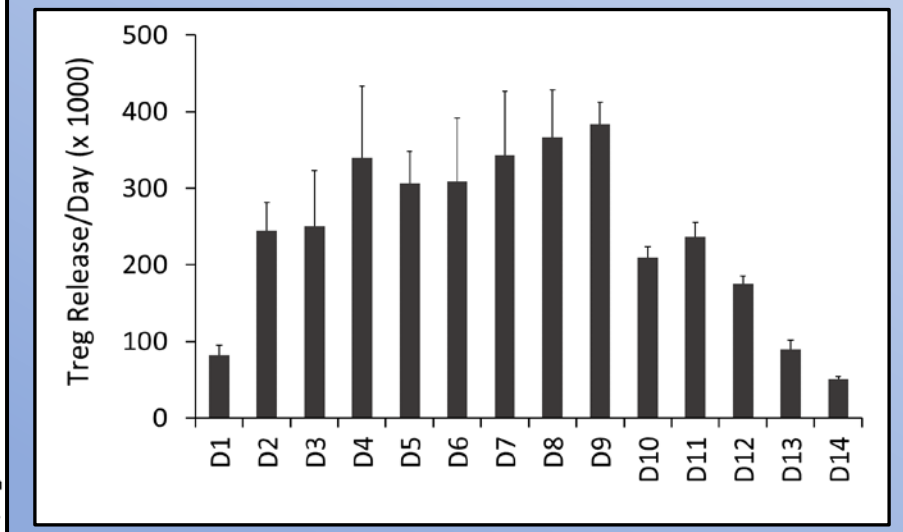










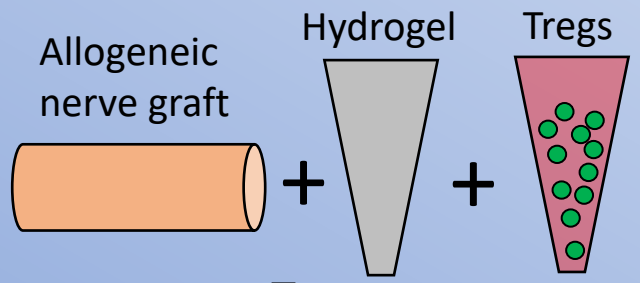


PEGNB Hydrogel Delivery Vehicle

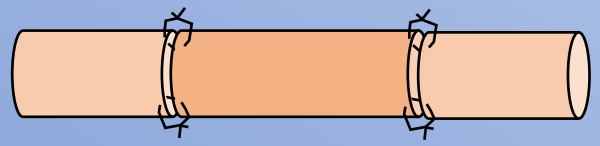
- Maintains high viability of Tregs for extended periods of time
- PEGNB formulation optimized to degrade over 14 days, the time frame in which host immune cells infiltrate the graft
- Over 14 day period, > 84% of Tregs initially embedded in the PEGNB are released as viable immune suppressing cells

Roballo and Bushman, Biomaterials. 2019

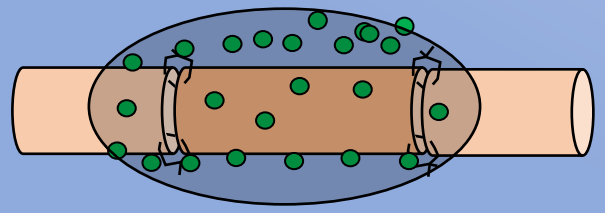
Process 3: Application in Surgical Rat Model of Nerve Injury



Nerve graft sutured into patient with nerve injury



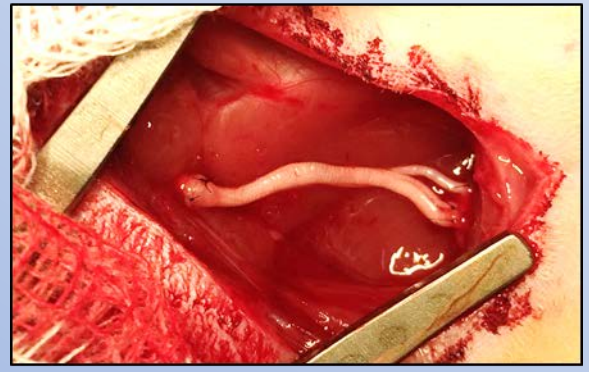
Tregs mixed with gel and applied around graft



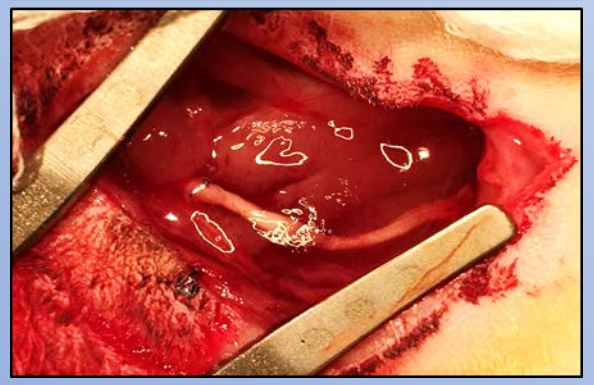
Proof of concept in 2 cm rat defect

- Single application of Tregs at time of graft implantation
- No additional immune suppression provided to animals

Nerve graft after harvest from donor

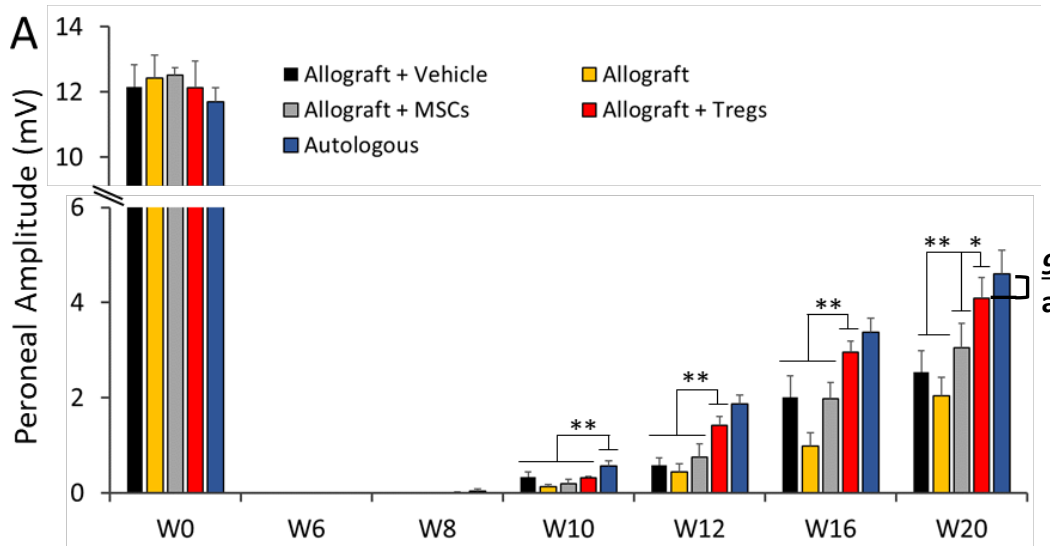


Nerve graft sutured into recipient animal



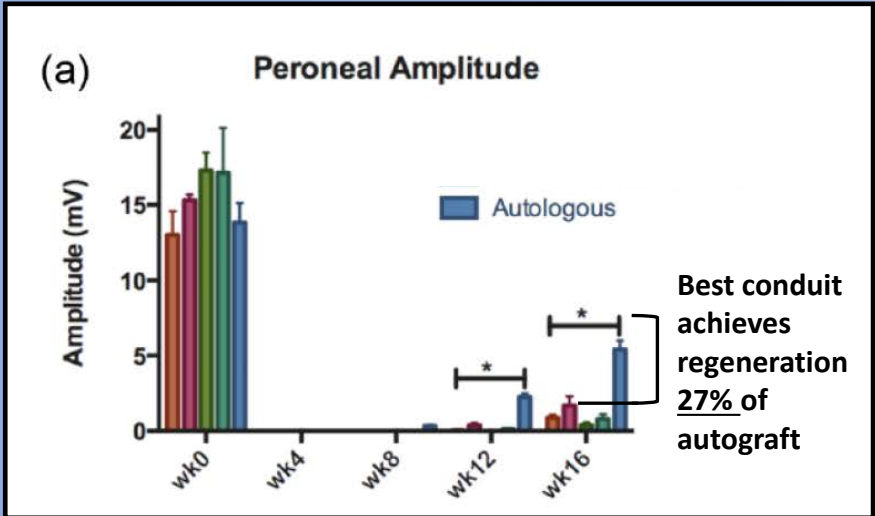
Translucent hydrogel + cells applied around nerve graft within recipient animal

Key Results from Nerve Regeneration Experiments



Regeneration with Tregs is equivalent to fresh mixed autograft

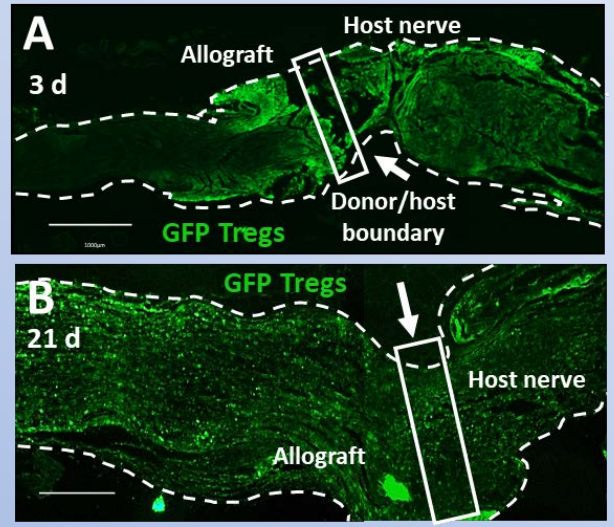
Comparison: Study with 1 cm defect using conduits in same animal model

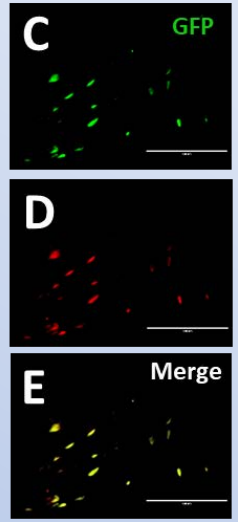


J Tissue Eng. 2016 Feb 5;7:2041731416629471.

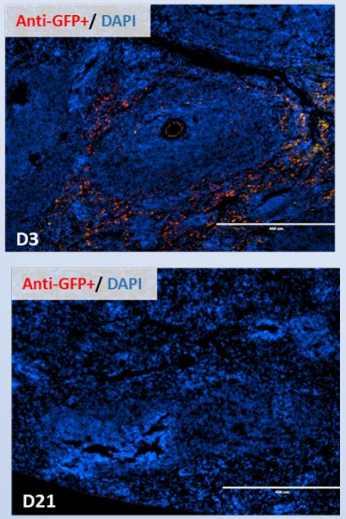
Locally-delivered Tregs infiltrate the graft and do not engraft in non-target tissues

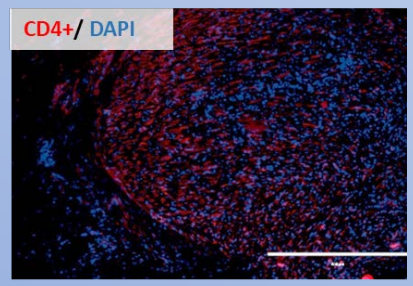
Nerves

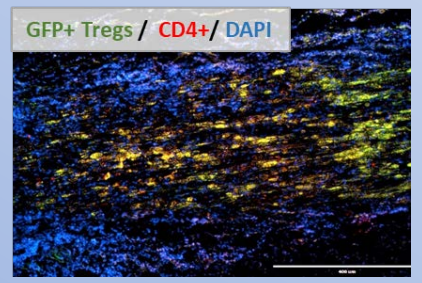


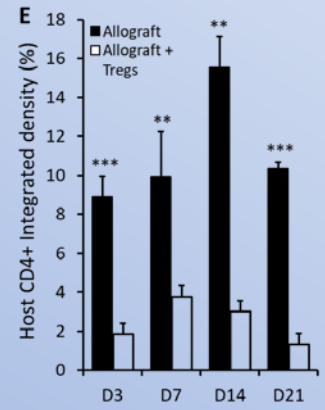


Spleen







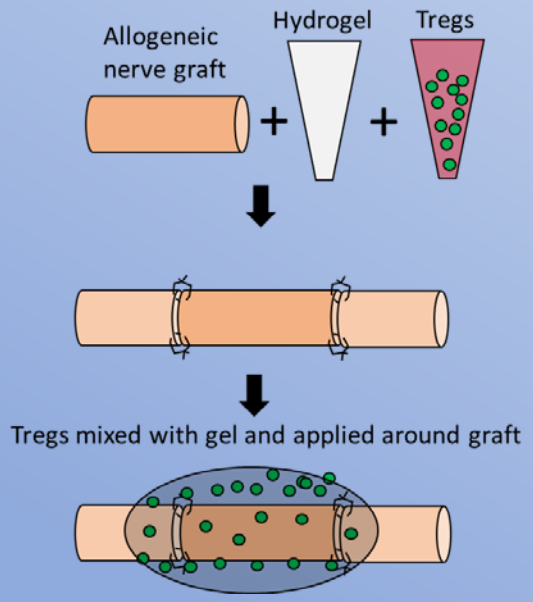


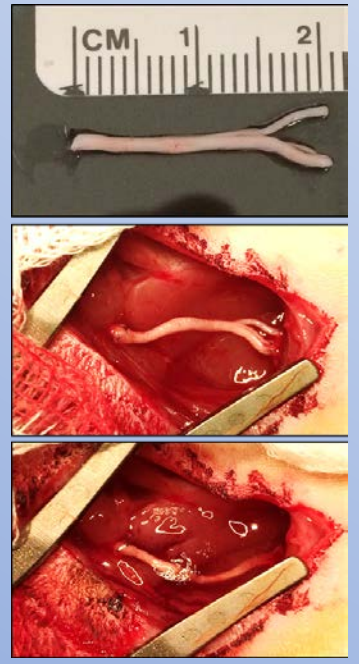
Tregs inhibit and kill host CD4+ effector T cells in graft

Roballo and Bushman, Biomaterials. 2019

Design Improvements – *Ex situ* Pre-assembly

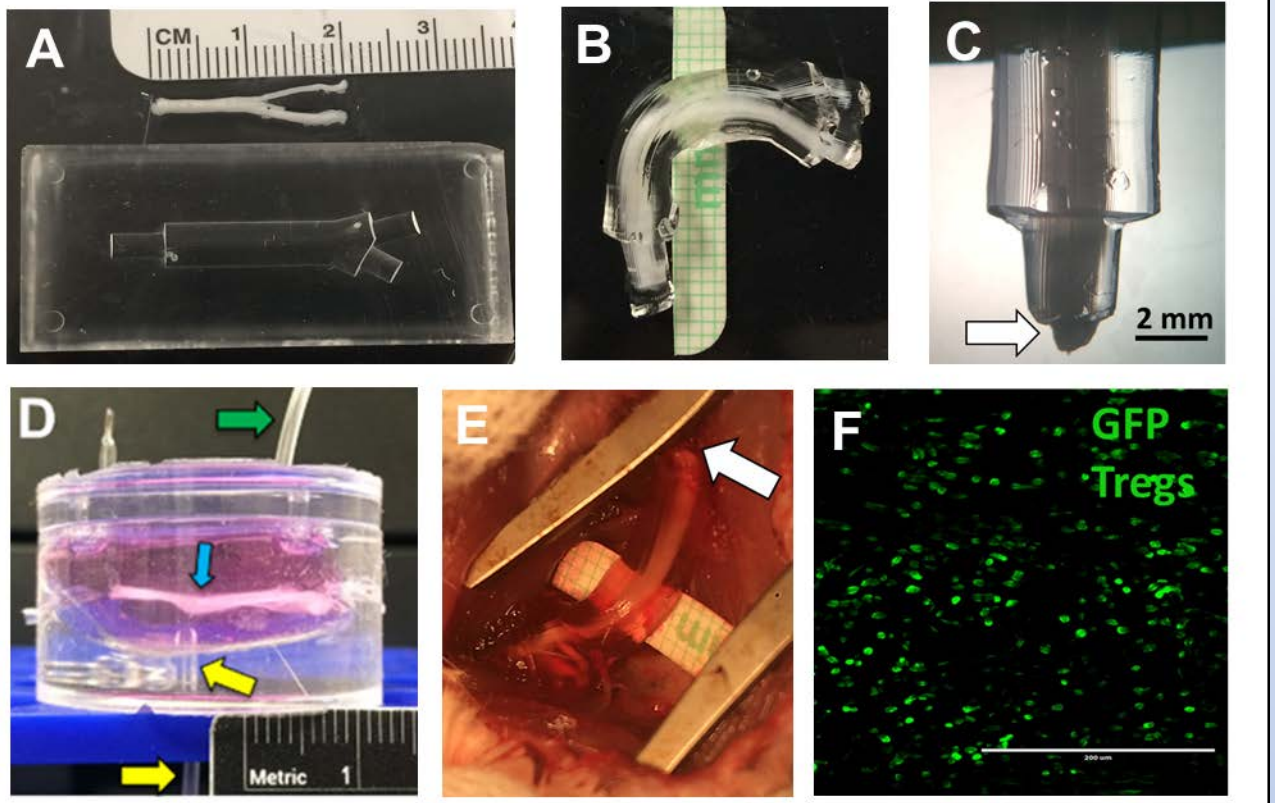
In situ assembly





- Assembly of the device *in situ* has the potential for variability
  - Dilution of gel by lymph/blood
  - Surgeon
- *Ex situ* pre-assembly would preclude variables and enable one-step implantation

Ex situ **pre-assembly** around living grafts via 3D printed molds



3D printed molds allowed for reproducible fabrication, hit targets for Treg number and viability with less variability.

Unpublished Data

Summary Localized Immunosuppression

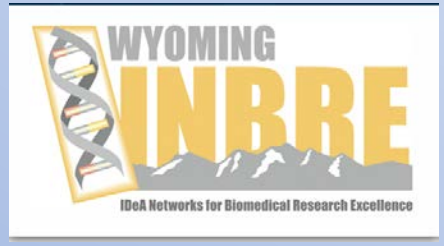
- Peripheral nerve allografts are an ideal starting point for immunoengineering
  - More effective than current options for nerve injury
  - Can serve an immediate functional role
- Immunoengineering by localized Treg delivery locally suppressed the immune response to the graft and enabled full functional recovery
  - No additional immune suppression was necessary
- Improvements in device design via 3D printing of molds enable pre-assembly and one step implantation
- First time that regeneration has been achieved through a segmental defect that included a branch point

Future of Localized Immunosuppression

- Localis Therapeutics LLC established as startup to develop the technology
- Commercialization partners are on board, aligning clinical partners
- Proximate objective is to obtain funding to show efficacy in pig preclinical model
- Combine with emerging technology of axon fusion

Funding, Partnerships & Collaborations

Wyoming Sensory Biology Center of Biomedical Research Excellence



Funding

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Kelly Roballo

JuliAnne Allgood

Osmanjan Wupu

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Jamie Shores, MD - Transplant Surgeon, Johns Hopkins University

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