Nerve Graft Immunosuppression

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Immunoengineering vs Tissue Engineering

**Tissue Engineering**

- **Scaffold**
- **Cells**
- **Growth factors**

Approximate a living tissue for the purpose of regeneration

**Immunoengineering**

- **Living donor nerve tissue**
- **Engineered immune modulators**

A living tissue that strongly supports regeneration

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

**Gold Standard**
Autologous sensory nerve harvested and transferred from the same patient
- Live nerve

**Pitfalls of using autografts**
- Morbidity
- Mismatch
- Non-restorative regeneration

**Alternatives to Autografts**
- Conduits/Wraps (11 in the market)
  - Acellular Allograft (Axogen)

**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

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

Nerve graft sutured into patient with nerve injury

Tregs mixed with gel and applied around graft

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
Process 2: Development of Biomaterial Hydrogel Vehicle for Treg Delivery

Collaboration with Professor John Oakey (Chemical Engineering)

**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
Key Results from Nerve Regeneration Experiments

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

- Best conduit achieves regeneration 27% of autograft.

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

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

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