Knowledge in Motion
SCI Education Series

Neuroregenerative Properties of Dental Pulp Stem Cells and their Future Potential to Improve Neurological Outcomes in SCI

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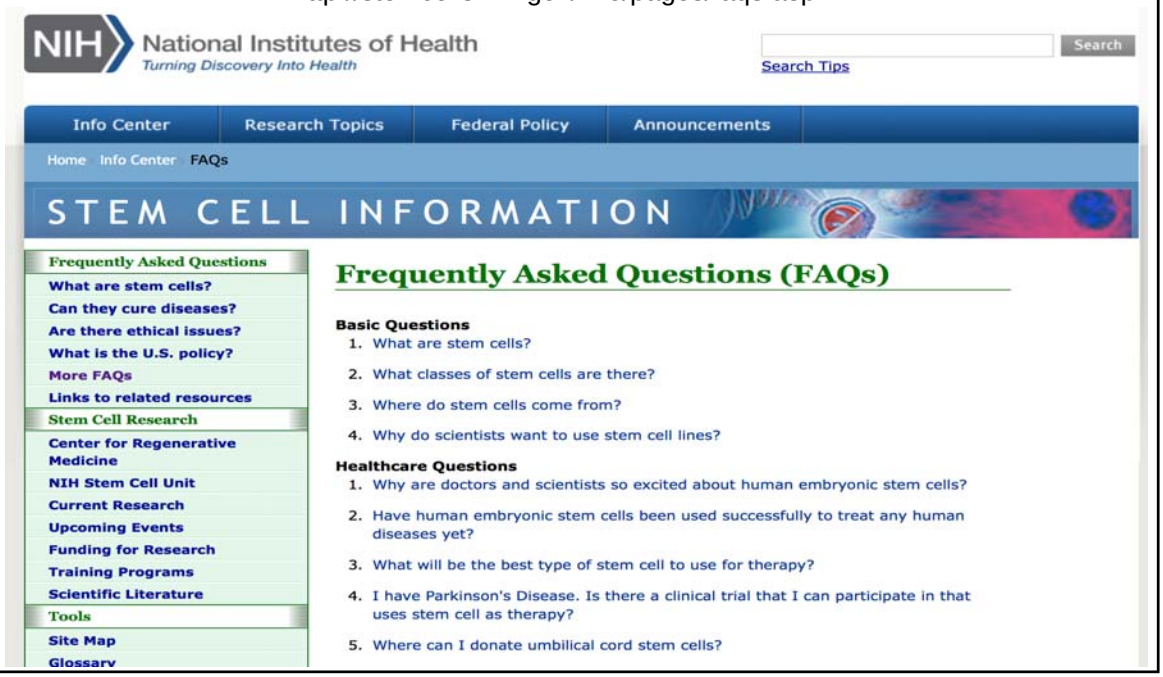
- The Ellen R. and Melvin J. Gordon Center for the Cure and Treatment of Paralysis
- Department of Education, NIDRR [H133N110010]

http://stemcells.nih.gov/info/pages/faqs.aspx

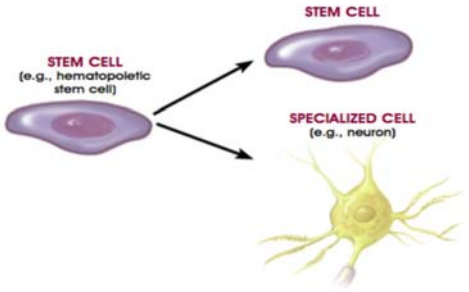


Objective 1

- Learn about the source of dental pulp stem cells and how they are obtained

What are Stem Cells?

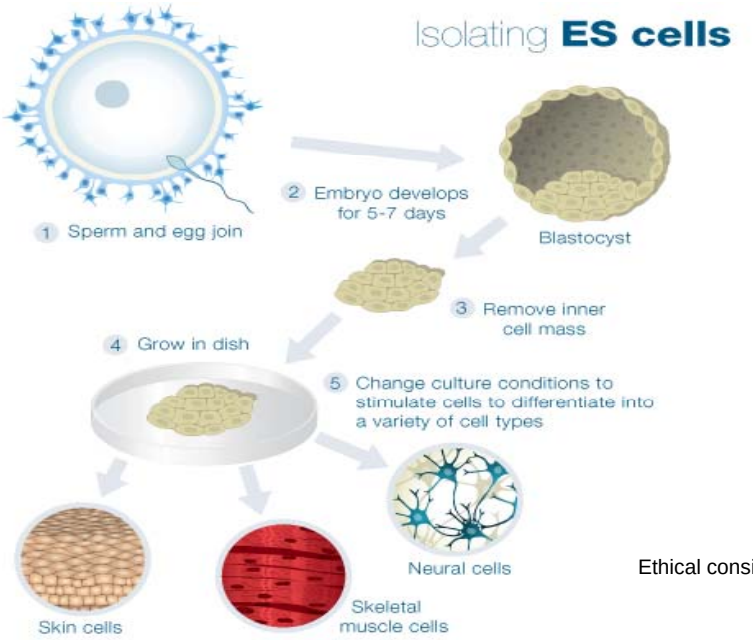
- ***Rare*** cells with unique properties:
  - **Self-renewal**: divide many times without developing into another cell type
  - **Potency**: develop into some or many different cell types in the body (neuron, muscle, fat)



- ***Repair system of the body***
  - Divide to replenish other cells lost because of disease or illness

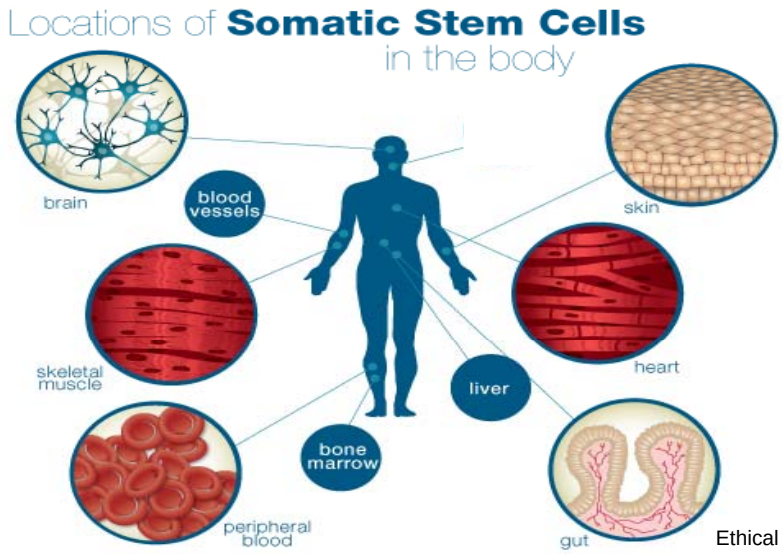
http://stemcells.nih.gov/info/pages/faqs.aspx

Embryonic stem cells (found in early embryos)

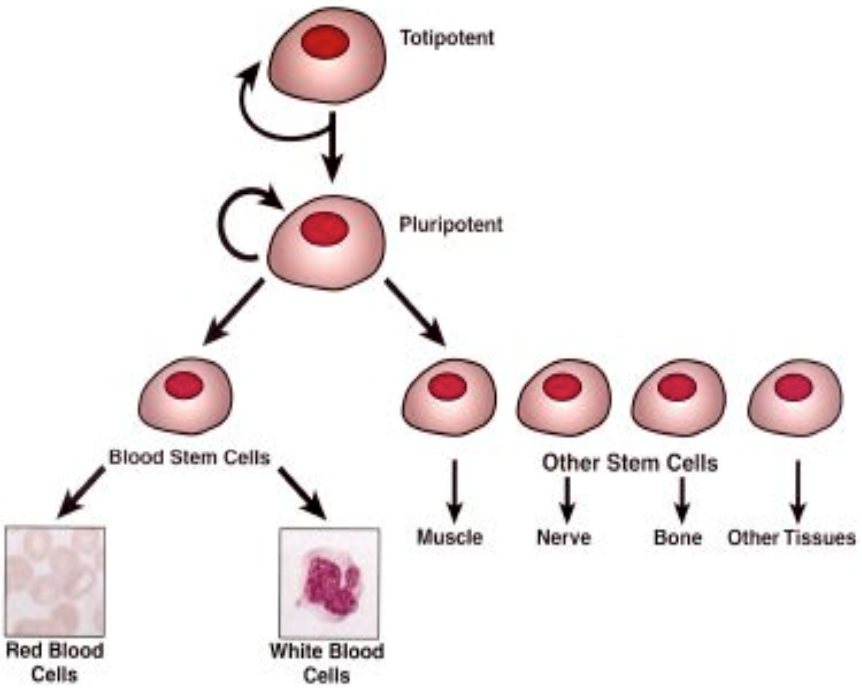


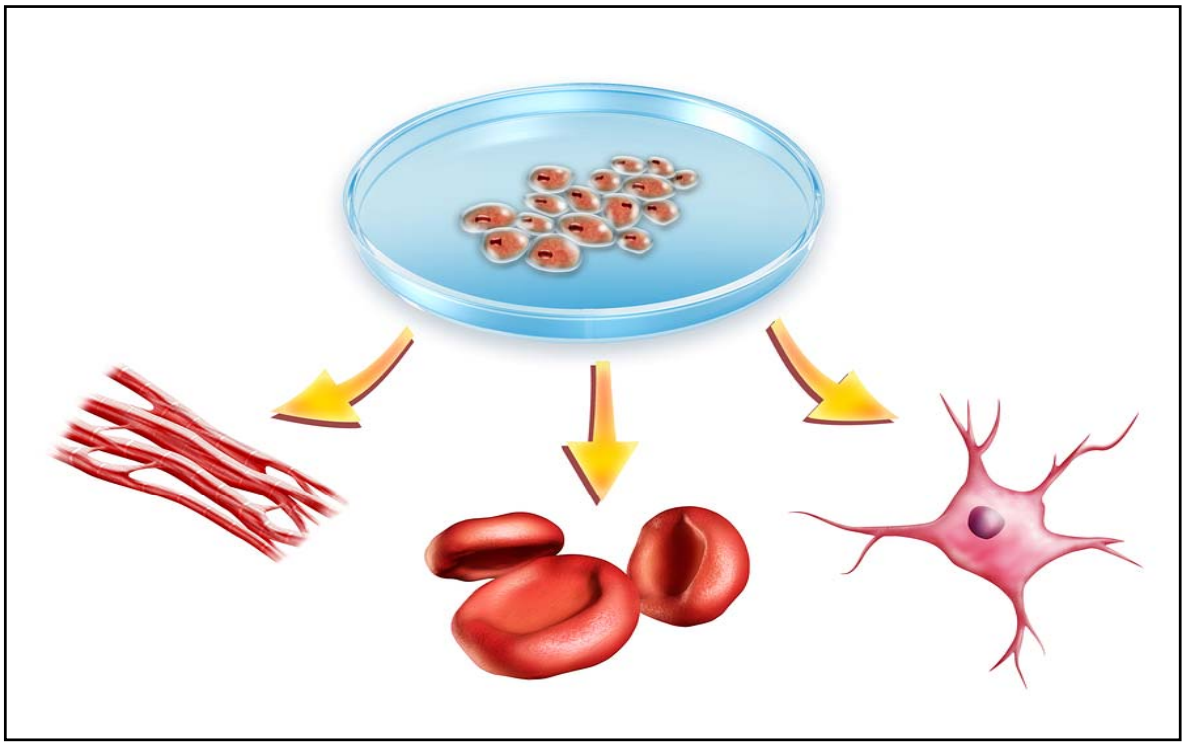
Ethical considerations

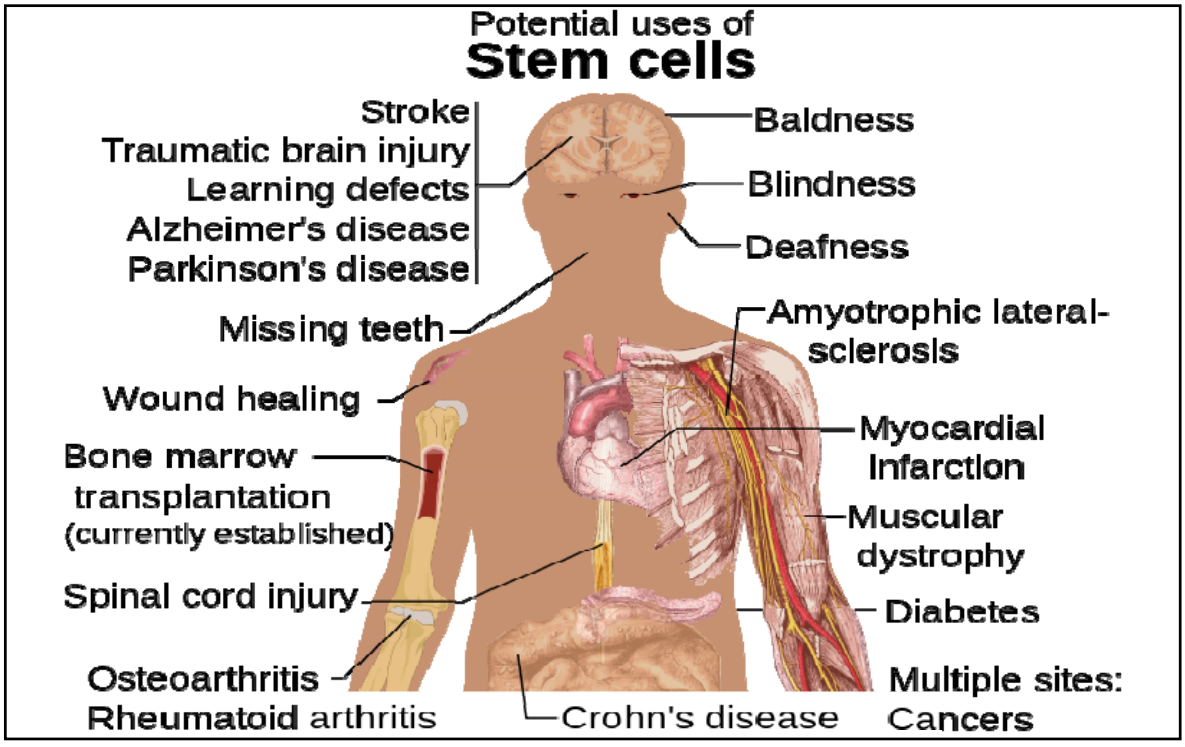
Adult stem cells (found in various tissues)



Ethical considerations

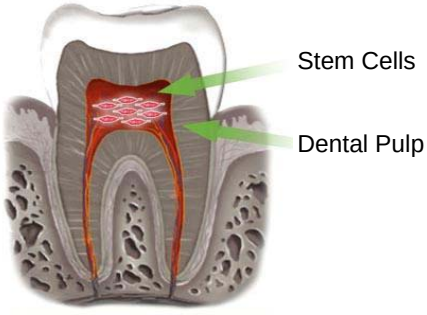





Potential uses of
Stem cells
Stroke
Traumatic brain injury
Learning defects
Alzheimer's disease
Parkinson's disease
Missing teeth
Wound healing
Bone marrow transplantation (currently established)
Spinal cord injury
Osteoarthritis
Rheumatoid arthritis
Baldness
Blindness
Deafness
Amyotrophic lateral-sclerosis
Myocardial Infarction
Muscular dystrophy
Diabetes
Crohn's disease
Multiple sites: Cancers

Dental Pulp Stem Cells

- First described in 2000 by Dr. Song Tao Shi*
- harvested from teeth
- Abundance: 20-30 million cells per tooth
- Less invasive to obtain than bone marrow stem cells



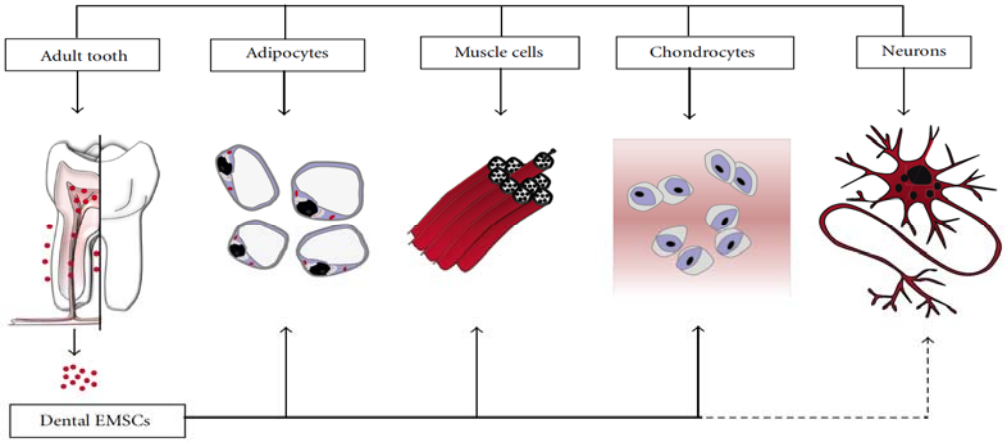
Shi, S. and Gronthos, S. (2003), Perivascular Niche of Postnatal Mesenchymal Stem Cells in Human Bone Marrow and Dental Pulp. *J Bone Miner Res*, 18: 696–704. doi: 10.1359/jbmr.2003.18.4.696

The use of dental pulp stem cells avoids the ethical issues associated with embryonic stem cells because wisdom teeth are destined to be thrown away and avoids the pain of bone marrow harvest

Neural Crest Stem Cells

Neural Crest Stem Cells from Dental Tissues: A New Hope for Dental and Neural Regeneration
Stem Cells Int. 2012;2012:103503. Epub 2012 Oct 4.

Neural crest stem cells give rise to the majority of the tissues in the skull and face



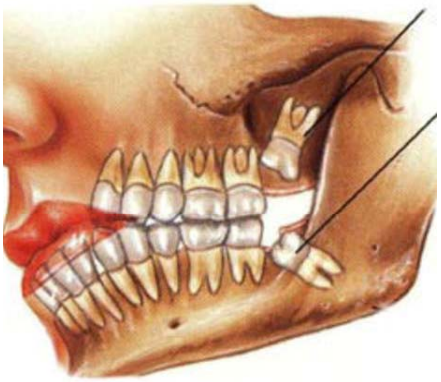
Neural Crest Stem Cells from Dental Tissues: A New Hope for Dental and Neural Regeneration. *Stem Cells Int.* 2012;2012:103503.

Objective 2

- Learn how dental pulp stem cells can develop into elements of the nervous system and how they have been used in rodents with spinal cord injury

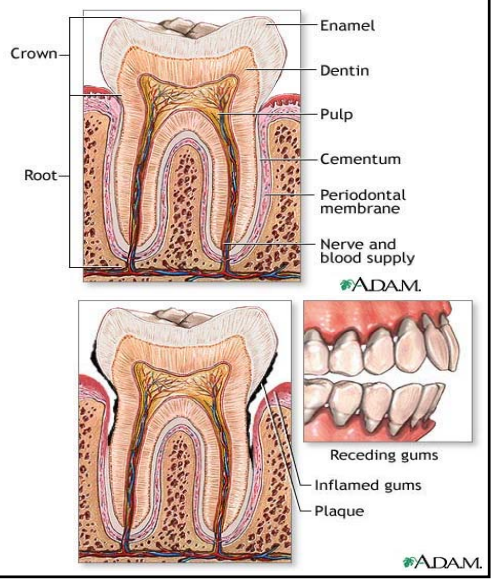
Dental Stem Cells

- Adult Teeth
  - Best sources:
    - Impacted third molars (wisdom teeth)
    - Erupted third molars without infection or decay
    - Teeth extracted for orthodontic reasons
    - ***Any health tooth is a source***
- Developing Teeth
  - Stem Cells from Human Exfoliated Teeth (SHED)
  - very high proliferative capacity
- Cell types
  - ***Dental Pulp Stem Cells (DPSC)***
  - Dental Pulp Pluripotent Stem Cells (DPPSC)
  - Periodontal Ligament Stem Cells (PDLSC)



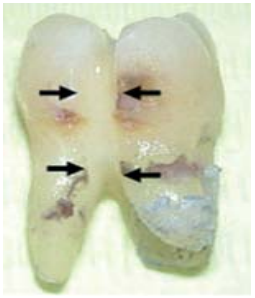
Dental and Periodontal Stem Cells in Regenerative Dentistry

- Dental tissue bioengineering to replace missing teeth and to regenerate the lost supporting tissues around the teeth (periodontium) including the alveolar bone due to periodontal disease

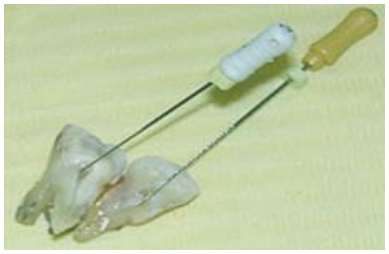


Dental Stem Cells and Neural Regeneration

- Generation of large pools of neural cells for cell therapy
- Share many properties of neural crest stem cells (NCSCs)
- Critical issues:
  - have higher proliferation rates and can be expanded in few weeks
  - No long-term immune rejection after transplantation



Isolation of Dental Pulp



Dental Pulp Stem Cells and Dental Pulp can be cryopreserved

- dental pulp and the dental pulp stem cells retain their stem cell characteristics after freezing, thawing, and growing in culture

Why doesn't the injured cord repair itself?

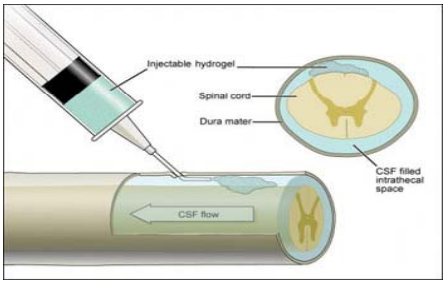
- inhibition of axonal growth
  - myelin-associated proteins
  - extracellular matrix molecules around injury site
- absence of growth factors after injury

He and Koprivica, 2004; Buchli and Schwab,2005, Fawcett, 2006; Fitch and Silver, 2008, Tuszynski and Lu, 2008

Goals for promoting functional recovery

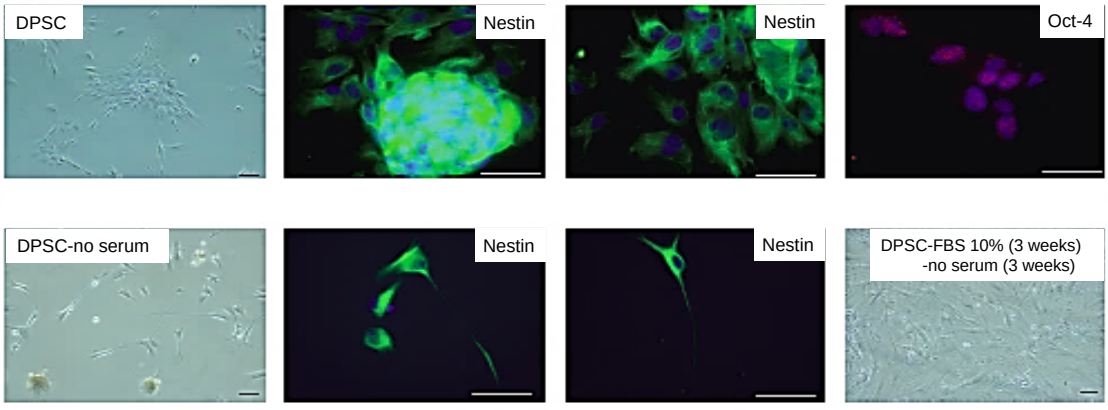
- Regain the ability to send and receive signals across the injured cord

Therapeutic uses for In vivo neuro-regeneration in animal models



Neural Differentiation Capacity and Pluripotency of Dental Pulp Stem Cells

- Strong expression of neural and glial cell markers even in basal conditions with no manipulation
- Cultured dental MSCs show neuron-like electrical activity
- Exogenous cells can integrate and survive in the host neural tissue and adopt specific phenotypes according to the location in nervous system
- Promote *de novo* neurogenesis



Neural Crest Stem Cells from Dental Tissues: A New Hope for Dental and Neural Regeneration. *Stem Cells Int.* 2012;2012:103503.

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Long-Distance Growth and Connectivity of Neural Stem Cells After Severe Spinal Cord Injury

Paul Lu[1,2,*], **Yaozhi Wang**[1], **Lori Graham**[1], **Karla McHale**[1], **Mingyong Gao**[1], **Di Wu**[1], **John Brock**[1], **Armin Blesch**[1], **Ephron S. Rosenzweig**[1], **Leif A. Havton**[3], **Binhai Zheng**[1], **James M. Conner**[1], **Martin Marsala**[4], and **Mark H. Tuszynski**[1,2,*]

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Adult rats with T3 SCI injected with neural stem cells 2 weeks after injury. Stem cells were co-grafted in a fibrin matrix with a growth factor cocktail.

Stem cell sources: embryonic rat spinal cord, human embryonic cells, or human fetal cells

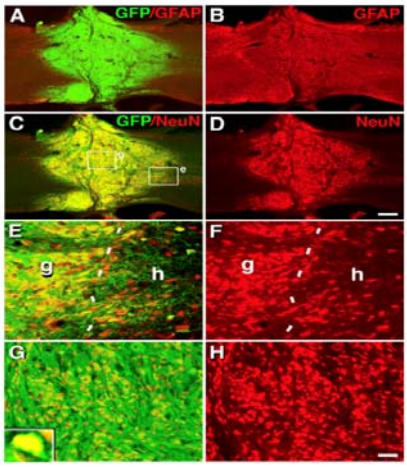
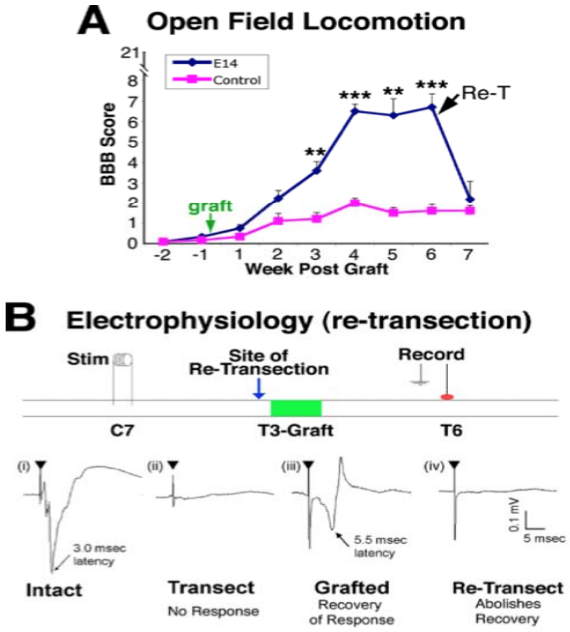


Figure 1. Survival, Filling and Differentiation of Neural Stem Cell Grafts in T3 Complete Transection Site

(A–B) Overview of **GFP** and **GFAP** fluorescent immunolabeling in a horizontal section demonstrates excellent graft survival, integration and filling of T3 complete transection site, seven weeks post-grafting. **(C–D) GFP** and **NeuN** labeling confirm extensive neuronal differentiation/maturation of grafted rat neural stem cells. **(E–F)** Higher magnification from **e** showing excellent integration and transition from host (**h**) neurons to grafted (**g**) neurons (dashed lines) **(E: GFP, NeuN; F, NeuN** alone). **(G–H)** Higher magnification from center of graft showing high density of NeuN-labeled neurons (inset) **(G: GFP, NeuN; H, NeuN** alone). Scale bar: A–D, 320 μm; E–H, 48 μm. Also see Figure S1 and S2.

Cell. 2012 September 14; 150(6): 1264–1273



Cell. 2012 September 14; 150(6): 1264–1273

Findings

- Early stage neurons from different sources and species are able to survive, integrate, and extend axons over very long distances and form functional relays in the adults injured cord

Cell. 2012 September 14; 150(6): 1264–1273

Research article

Human dental pulp-derived stem cells promote locomotor recovery after complete transection of the rat spinal cord by multiple neuro-regenerative mechanisms

Kiyoshi Sakai,[1] Akihito Yamamoto,[1] Kohki Matsubara,[1] Shoko Nakamura,[1] Mami Naruse,[1] Mari Yamagata,[1] Kazuma Sakamoto,[2] Ryoji Tauchi,[3] Norimitsu Wakao,[3] Shiro Imagama,[3] Hideharu Hibi,[1] Kenji Kadomatsu,[2] Naoki Ishiguro,[3] and Minoru Ueda[1]

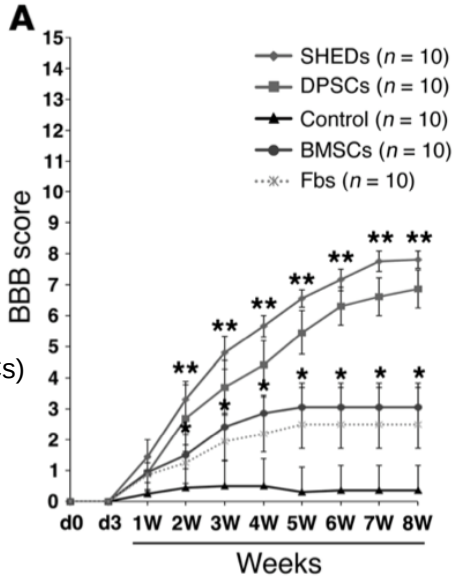
[1]Department of Oral and Maxillofacial Surgery, [2]Department of Biochemistry, and [3]Department of Orthopedic Surgery, Nagoya University Graduate School of Medicine, Nagoya, Japan.

Spinal cord injury (SCI) often leads to persistent functional deficits due to loss of neurons and glia and to limited axonal regeneration after injury. Here we report that transplantation of human dental pulp stem cells into the completely transected adult rat spinal cord resulted in marked recovery of hind limb locomotor functions. Transplantation of human bone marrow stromal cells or skin-derived fibroblasts led to substantially less recovery of locomotor function. The human dental pulp stem cells exhibited three major neuroregenerative activities. First, they inhibited the SCI-induced apoptosis of neurons, astrocytes, and oligodendrocytes, which improved the preservation of neuronal filaments and myelin sheaths. Second, they promoted the regeneration of transected axons by directly inhibiting multiple axon growth inhibitors, including chondroitin sulfate proteoglycan and myelin-associated glycoprotein, via paracrine mechanisms. Last, they replaced lost cells by differentiating into mature oligodendrocytes under the extreme conditions of SCI. Our data demonstrate that tooth-derived stem cells may provide therapeutic benefits for treating SCI through both cell-autonomous and paracrine neuroregenerative activities.

Sakai, *et al. J Clin Invest.* 2012;122(1):80–90.

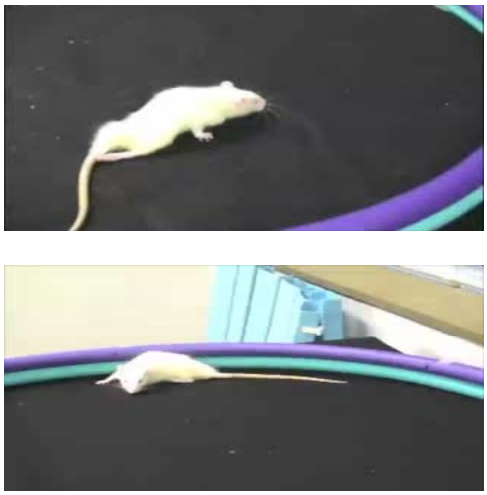
Adult female rats with T10 SCI injected with neural stem cells at the time of injury.

Stem cell sources:
-human deciduous teeth SHEDS
-human dental pulp stem cells
-human bone marrow stem cell line (BMSCs)



Sakai, *et al. J Clin Invest.* 2012;122(1):80–90.

In vivo neuro-regeneration



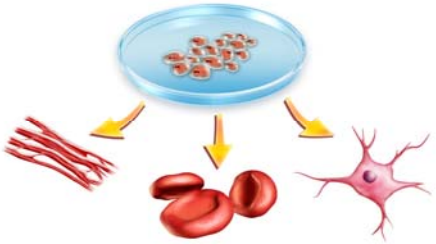
Sakai, *et al. J Clin Invest.* 2012;122(1):80–90.

Objective 3

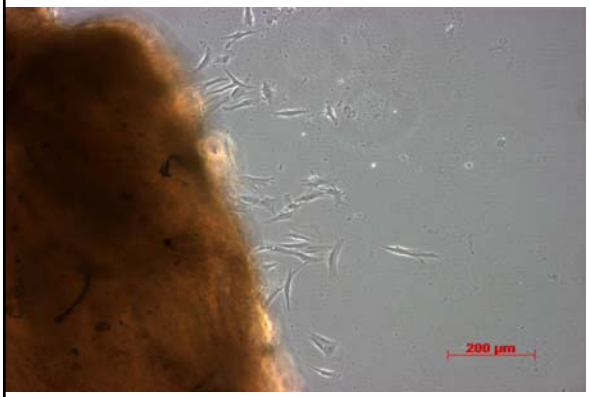
- To learn how dental pulp stem cells might be used to promote neuro-recovery for people with spinal cord injury

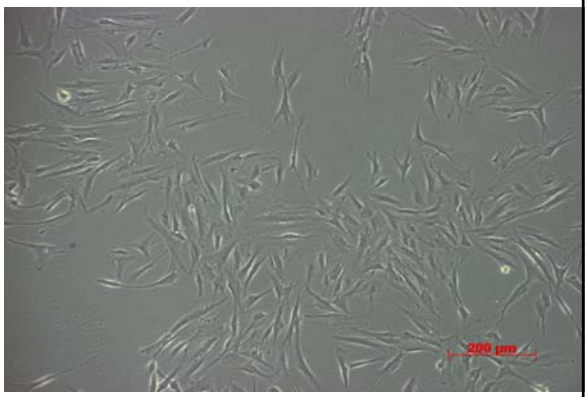
Ongoing Work

- Demonstrating stem cell characteristics:
  - Self renewal
  - Multipotency
  - Ability to promote *in vivo* regeneration

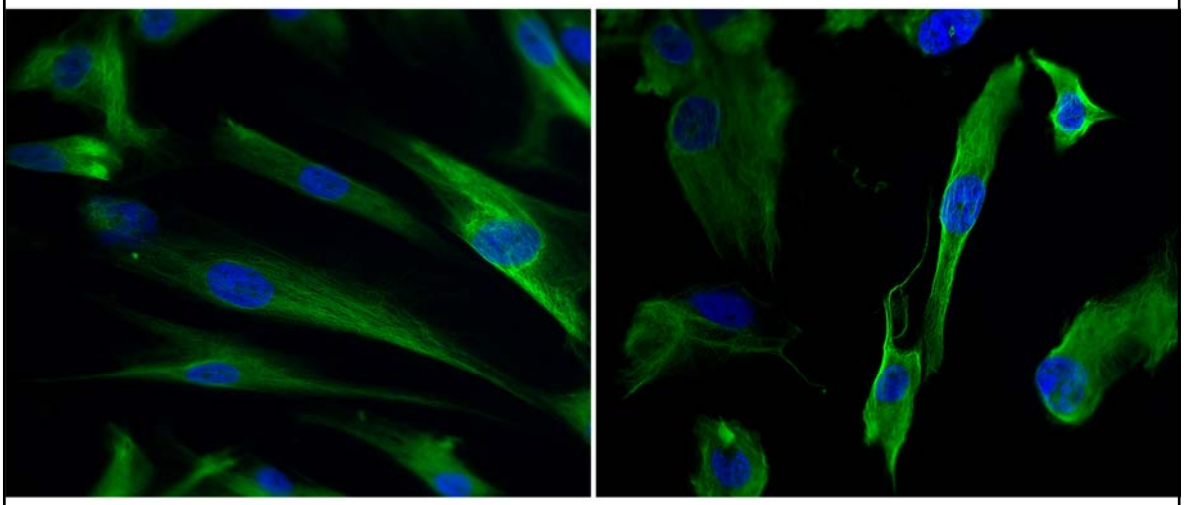


Ongoing Work

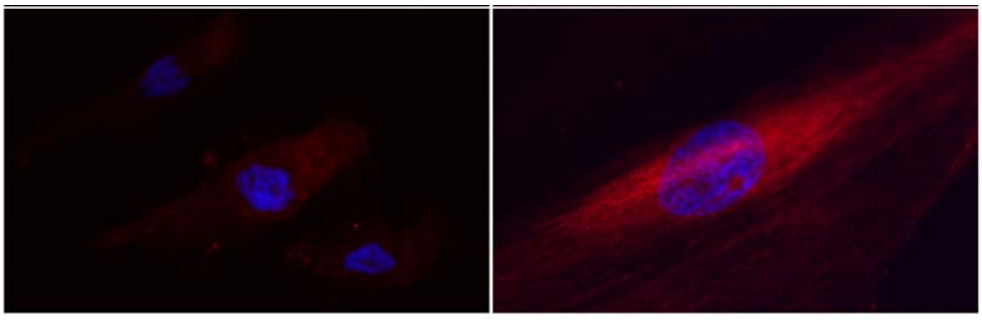




Ongoing Work



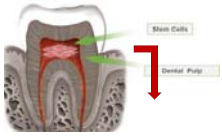
Ongoing Work

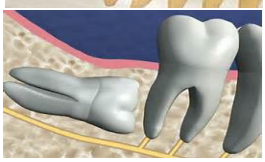


Neural cell differentiation

Processing and digestion of dental pulp, and initial culture of DPSC

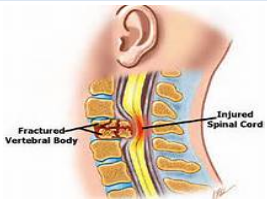
Extraction → Initial Culture → Passage → In vitro neural differentiation





Characterization
- Immunocytochemistry
- Karyotyping
- Flow cytometry
- Gene expression

Engraftment



Department of Physical Medicine & Rehabilitation
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SPAULDING-HARVARD SCI MODEL SYSTEM
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