Spinal cord reconstruction

Aleš Hejčl, MD, PhD.

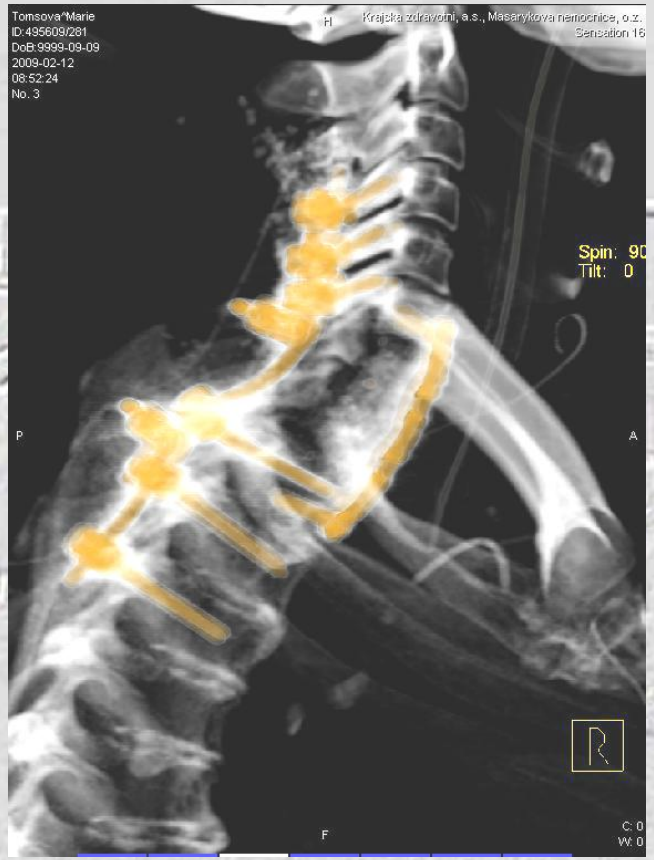
Neurosurgery Dept, Ústi nad Labem, Czech Republic

Institute of Experimental Medicine, Academy of Sciences, Prague, Czech Republic

Spinal cord injury

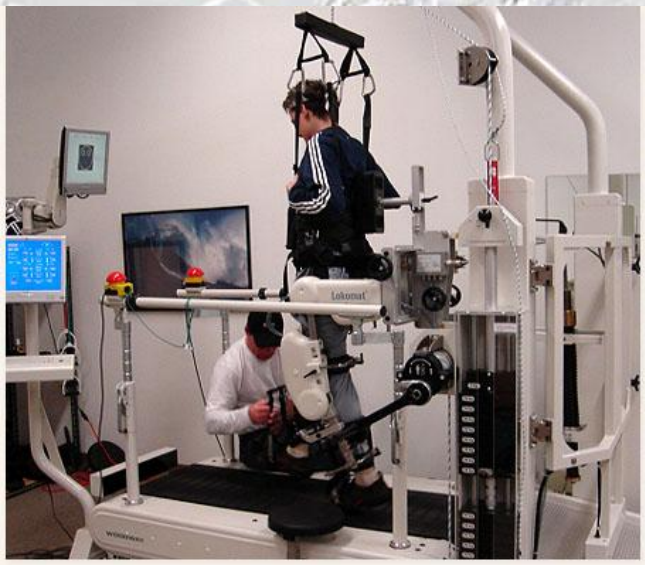
current clinical therapeutic modalities

1. surgery *(decompression, stabilisation)*

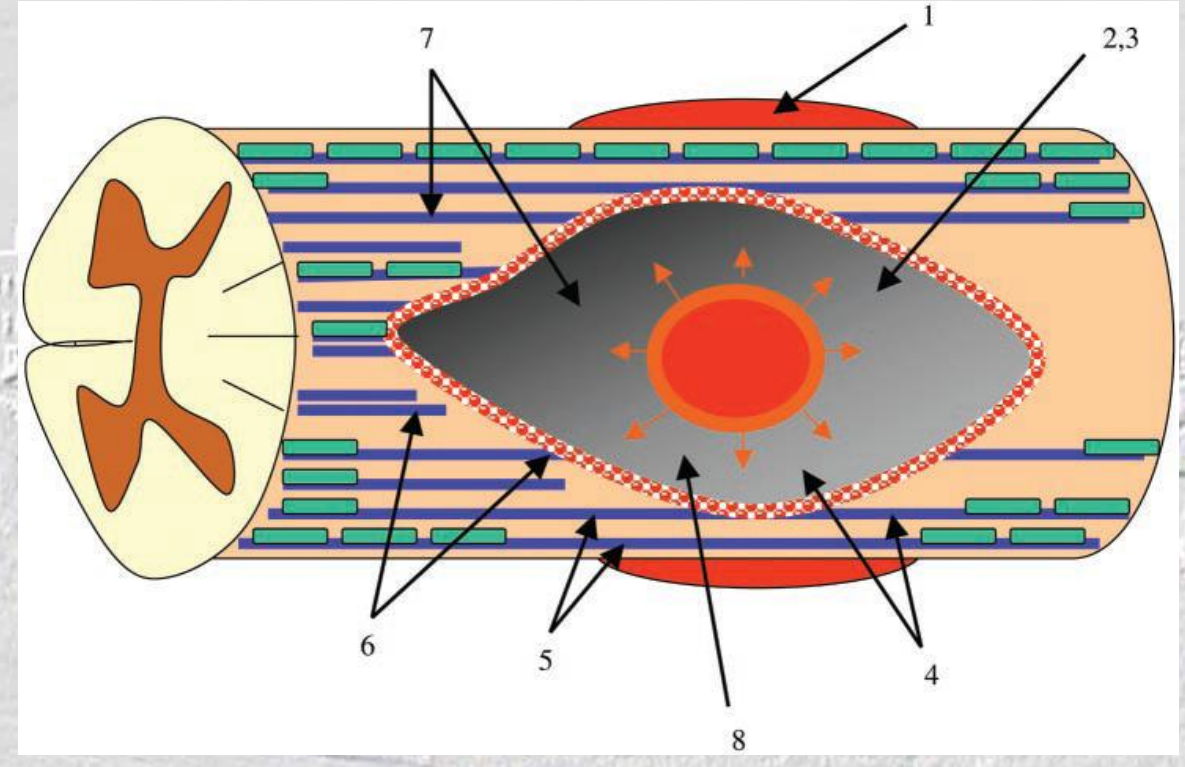


2. methylprednisolone *(NASCIS study)*

3. rehabilitation



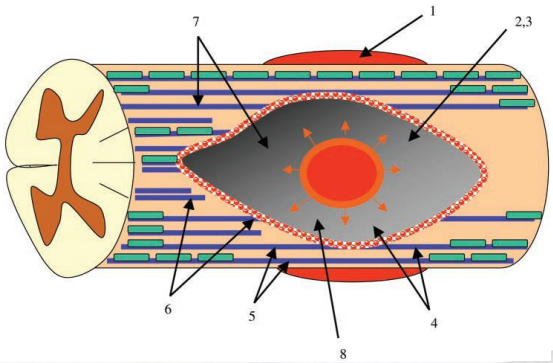
How do we reconstruct the spinal cord itself?



1) reduction of edema and free radical production, 2) rescue of neural tissue at risk of dying in secondary processes such as abnormally high extracellular glutamate concentrations, *3) control of inflammation, 4) rescue of neuronal/glial populations at* risk of continued apoptosis; *5) repair of demyelination and conduction deficits, 6)* promotion of neurite growth through improved extracellular environment, *7) cell* replacement therapies, *8) efforts to bridge the gap with transplantation approaches.* Not shown on the schematic diagram but discussed in the text are the *9) efforts to* retrain and relearn motor tasks, *10) restoration of lost function by electrical stimulation,* and *11) relief of chronic pain syndromes.*

How to reconstruct the SCI?

- Replace/substitute the damaged cells
- Direct the immune response
- Create permissive environment at the lesion site
- Overcome the obstacle of glial scarring
- Guide newly growing axons across the lesion



Experimental therapies

- **I. cellular therapies** (stem cells, Schwann cells, olfactory ensheathing glia – OEG)
- **II. protective autoimmunity** (activated macrophages)
- **III. neurotrophic factors**
- **IV. overcoming the obstacle of the glial scar**
- **V. guidance therapies** (natural and synthetic polymers)

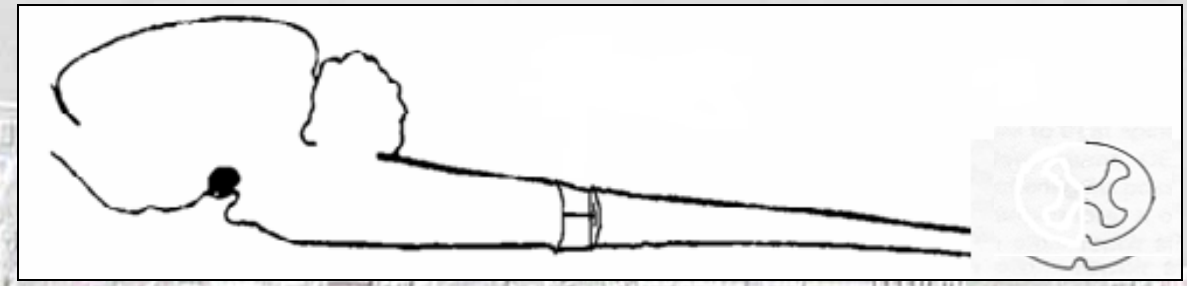
Design of experimental SCI

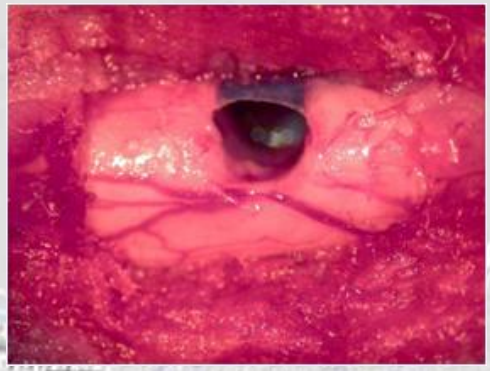
- experimental models of SCI
- evaluation – end points

Experimental models of SCI

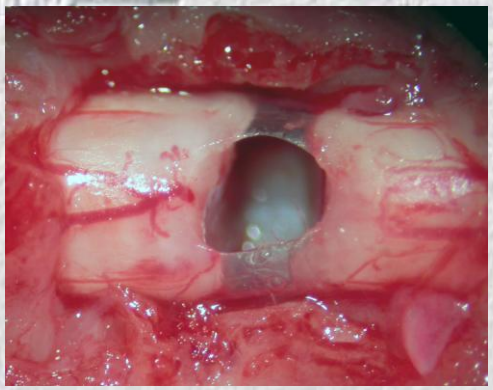
1. sharp

- hemisection





- transection

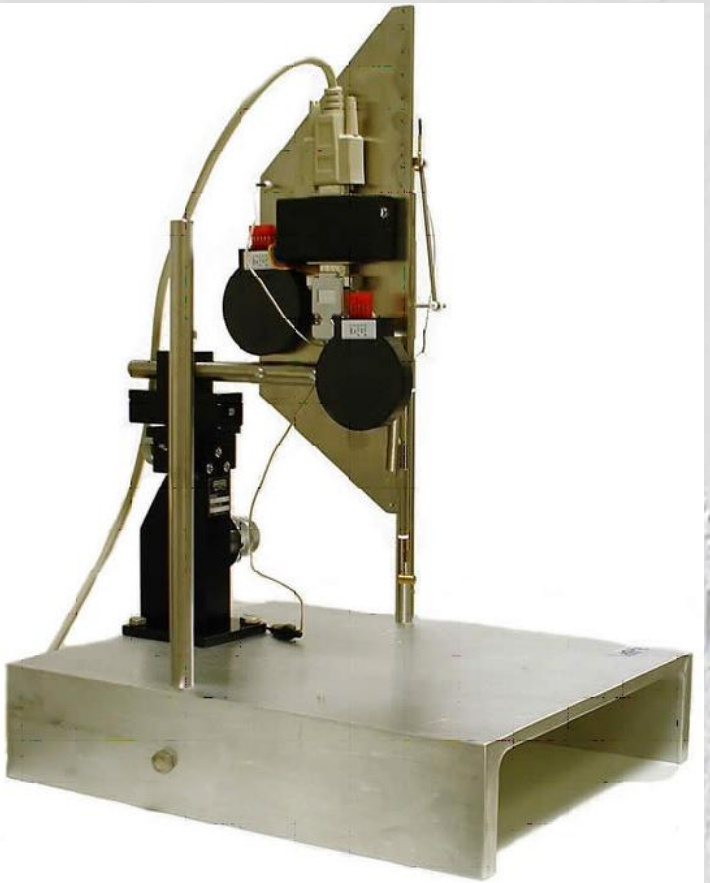


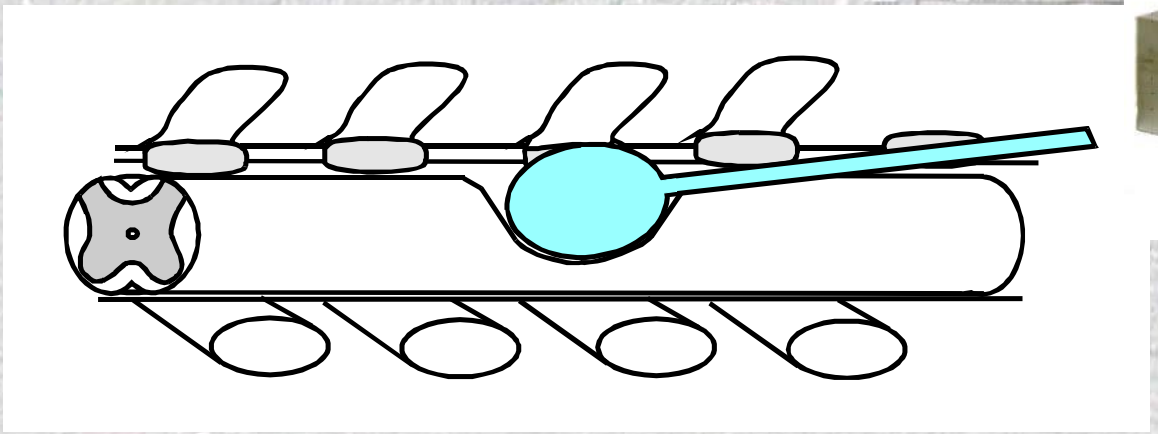
Experimental SCI

2. blunt

contusion
(NY impactor, OSU impactor, MASCIS)

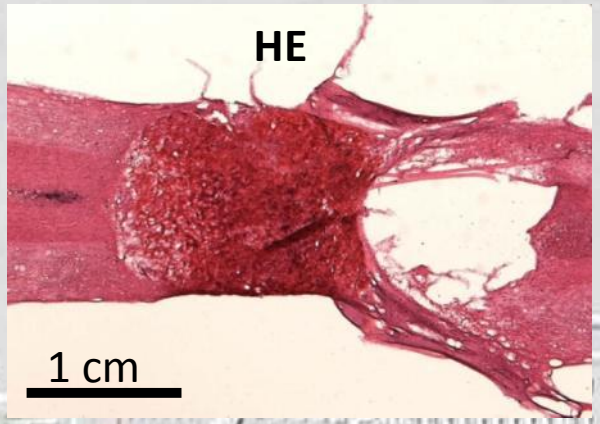
compression (BCL)



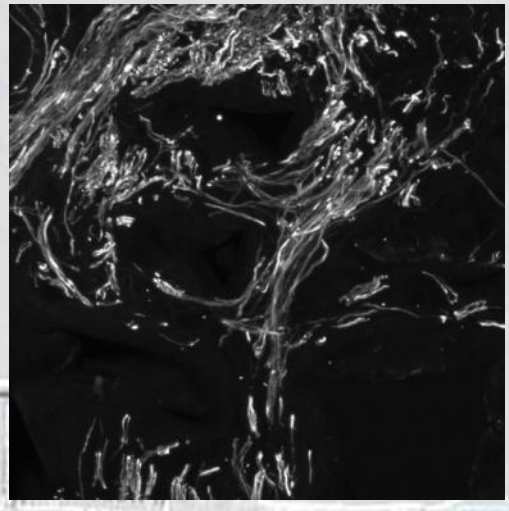


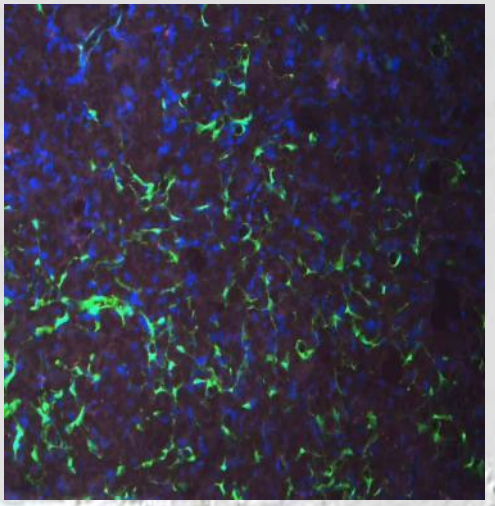
Morphology evaluation

classical histology

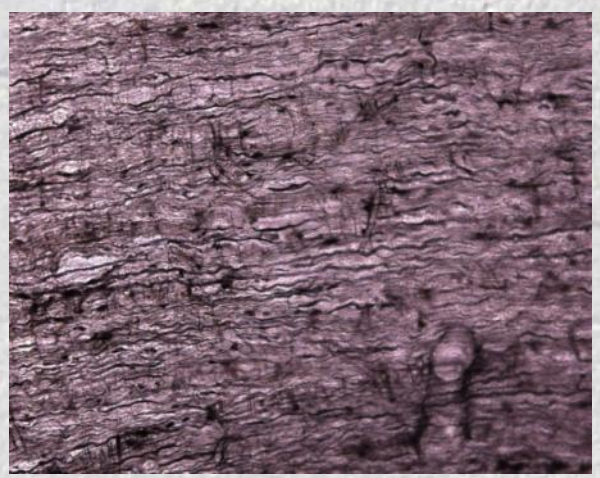


immunohistochemistry

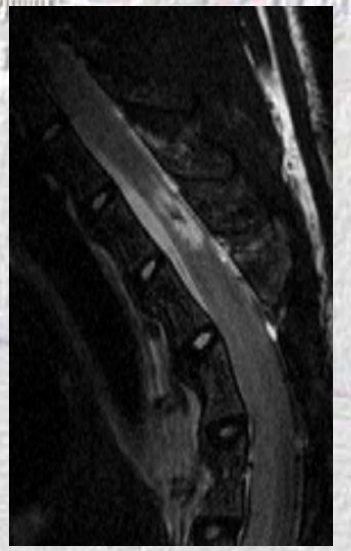


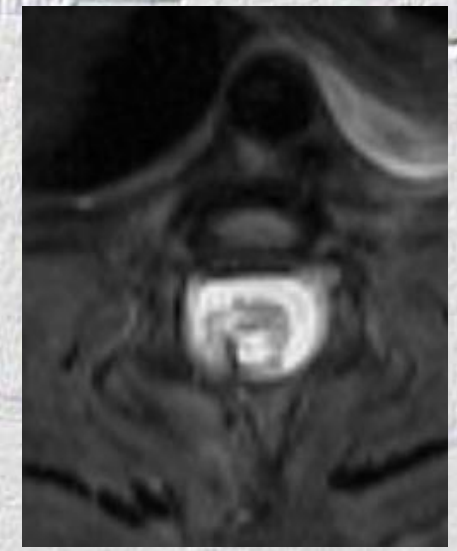


retrograde/anterograde staining



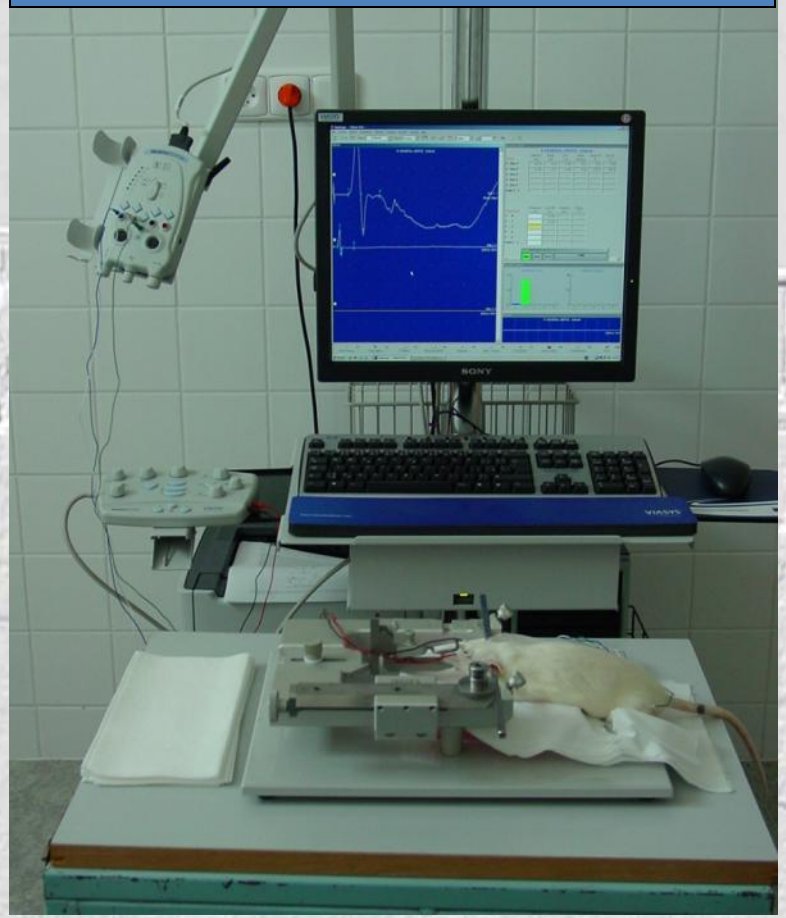
non-invasive evaluation - MRI

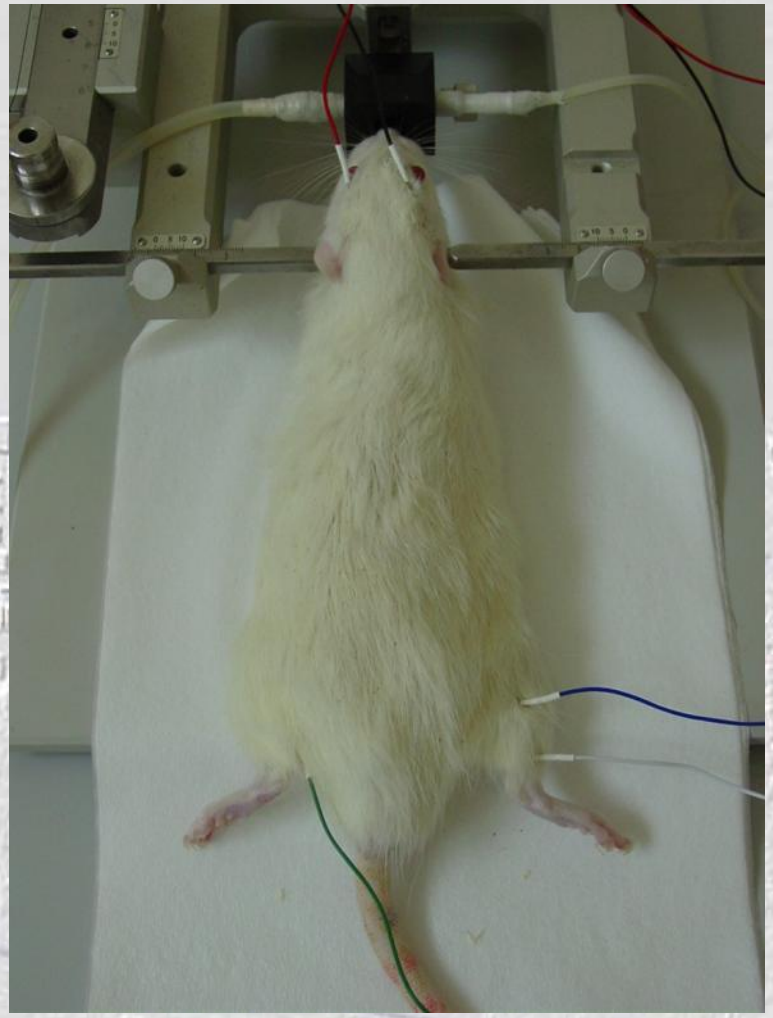




Electrophysiology

Motor Evoked Potentials





Courtesy of Takashi Amemori, Prague

Function: behavioral testing

sensory function

- von Frey filaments
- paw compression test
- hot plate-based tests, etc…

plantar test

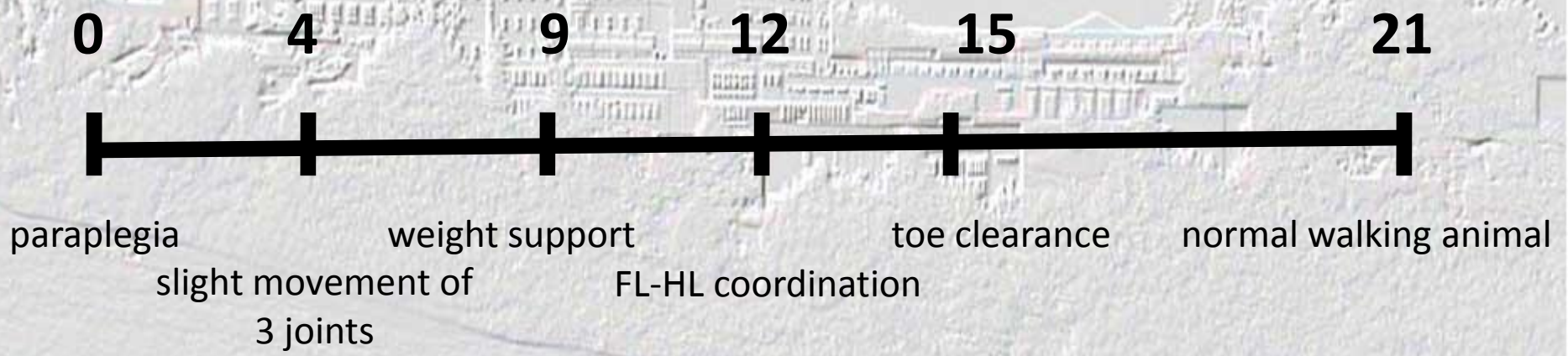
motor function

- open field locomotor test
- inclined plane
- swim tests, etc…

open field locomotor test

Functional evaluation

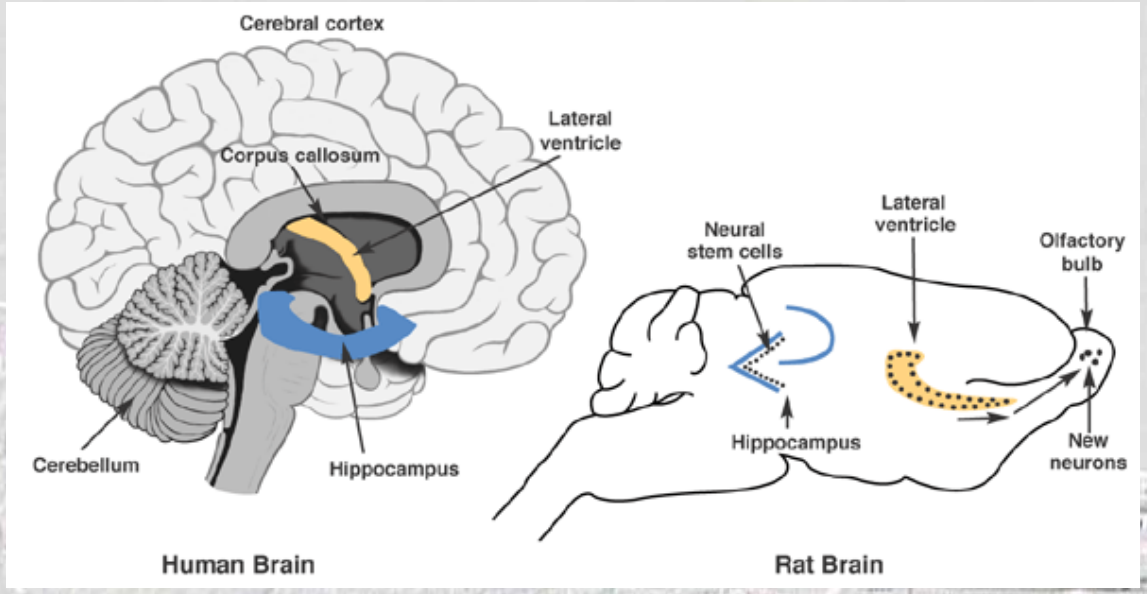
BBB test (Basso-Beattie-Bresnahan score)



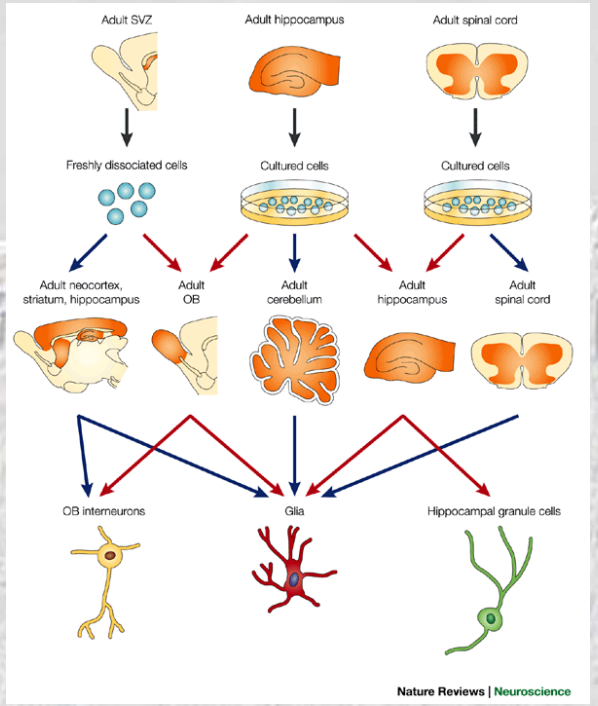
I. Cellular therapies

- **Stem cells:**
  - **adult neural stem cells**
  - **MSCs**
  - **embryonic stem cells**
  - **fat-derived stem cells**
- **Schwann cells**
- **OEG**

Adult neural stem cells (NSCs)



Gage, Science, 2000



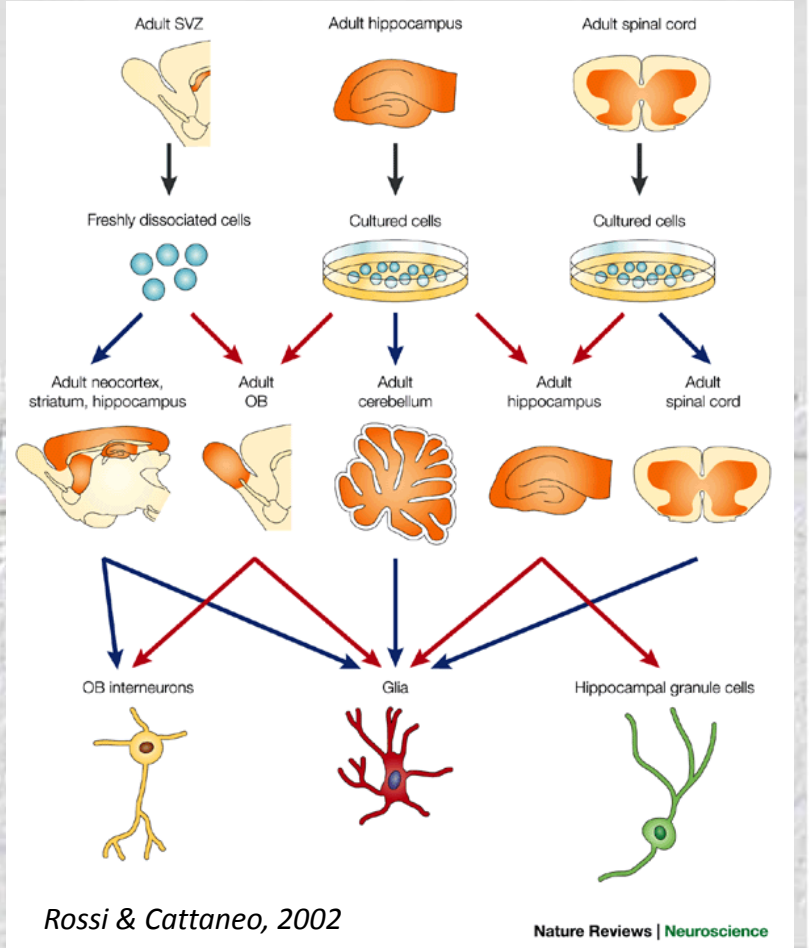
in vivo – neurons

in vitro – neurons, astrocytes, oligodendrocytes

The role of NSCs

activation of endogenous cells to provide “self-repair“

CNS injury (BI or SCI) leads to increase in NSCs (Frisén et al., 1995).

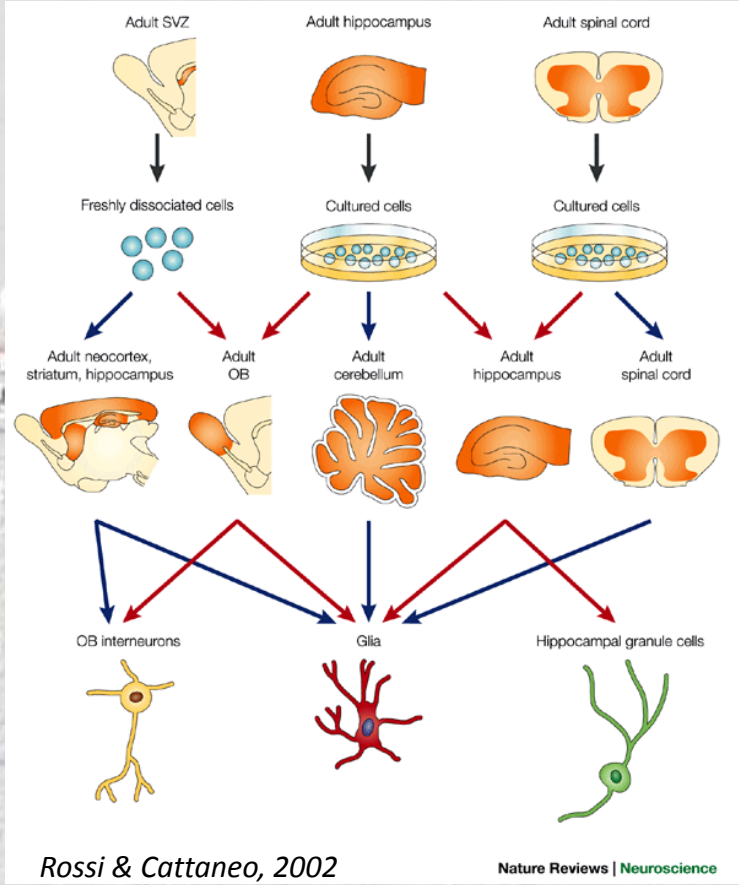


Rossi & Cattaneo, 2002

The role of NSCs

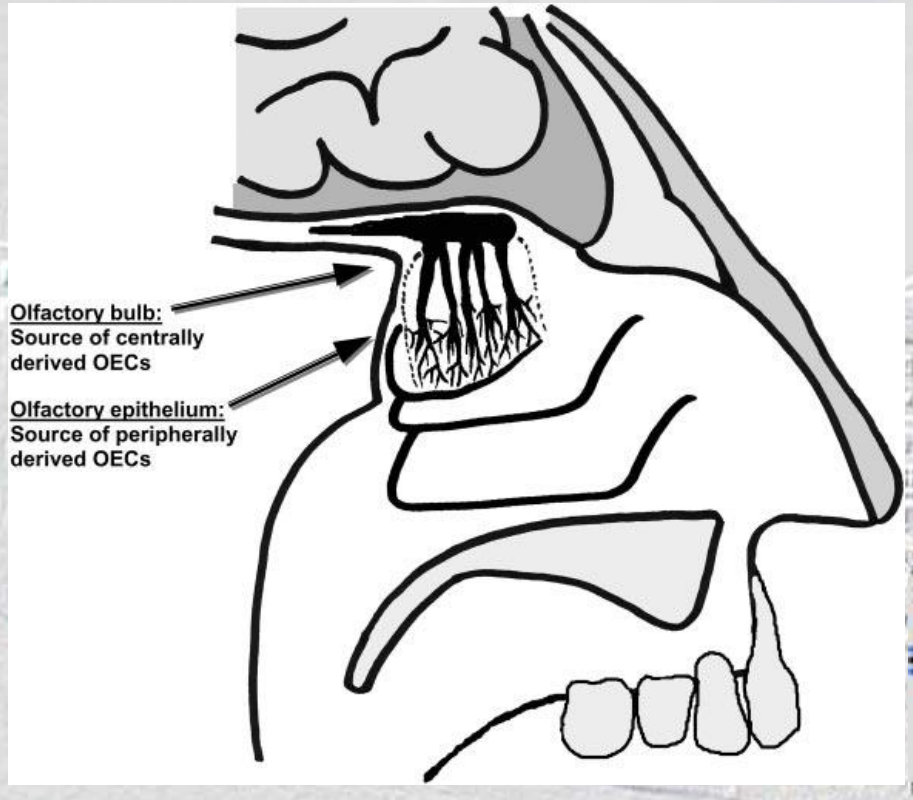
transplantation to substitute missing/lost cells

implanted NSCs 2 weeks after SCI leads in 50% of cells to genesis of oligos and myelinization of axons, which results in improved motor function of hindlimbs(Karimi-Abdolrezaee et al., 2006).



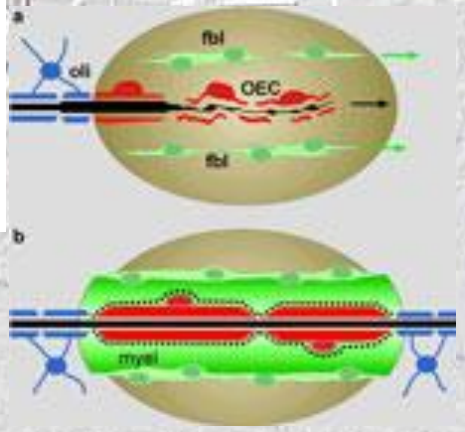
Rossi & Cattaneo, 2002

Olfactory ensheathing glia (OEGs)

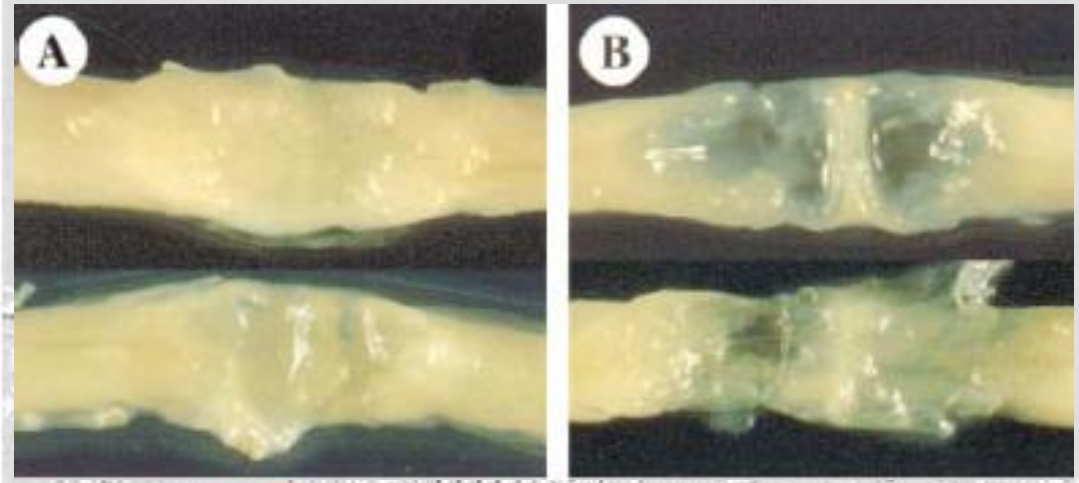


- guide regenerating axons
- provide permissive environment across the glial scar (Ramon-Cueto *et al.* 1994; Ramon-Cueto *et al.* 1998)
- show + effect on PNS/CNS regener.
- OEGs showed axonal regeneration across a long distance

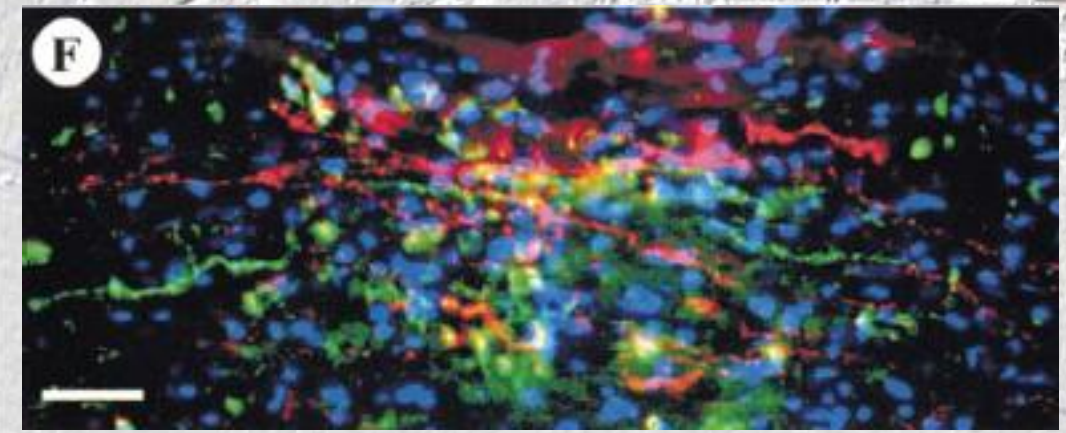
Ramón-Cueto, Madrid



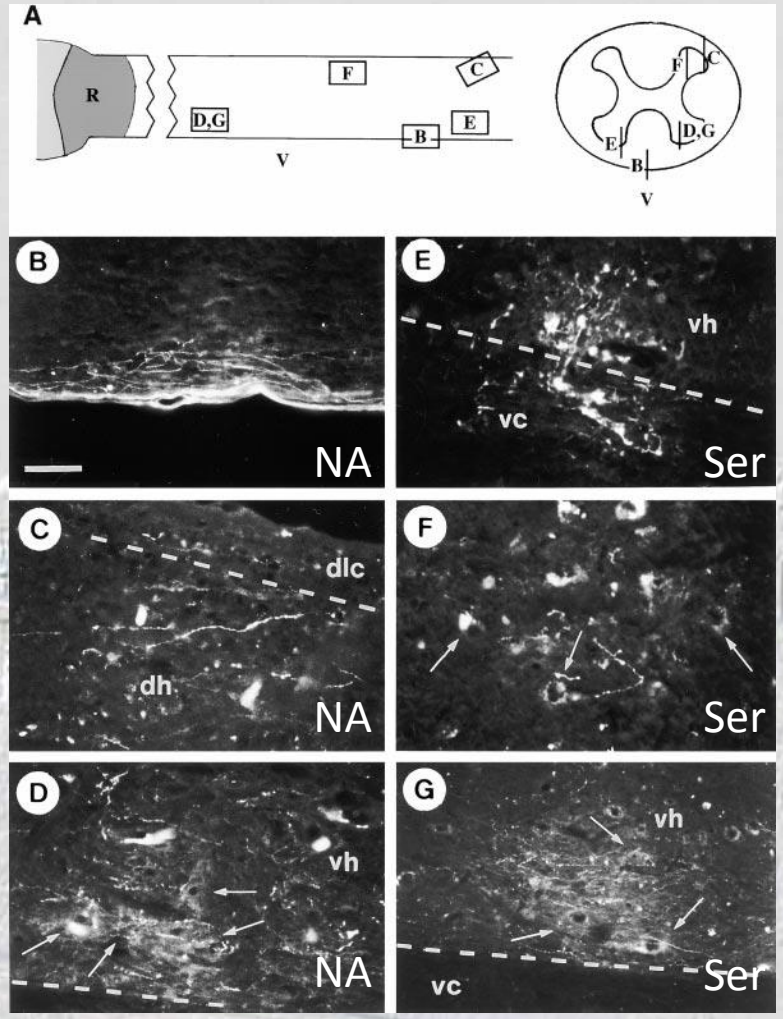
OEGs in transection SCI - morphology



A. OEG transplanted **B. untreated**



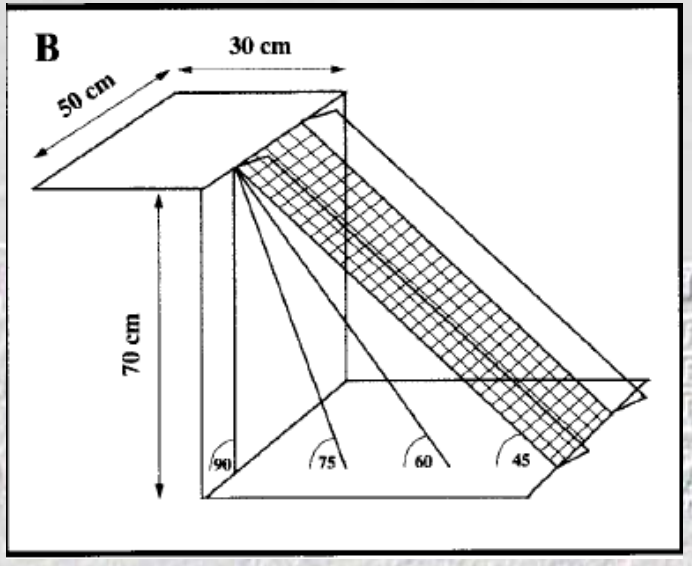
F. CST (green) and NA axons (red) crossing the epicenter of the lesion



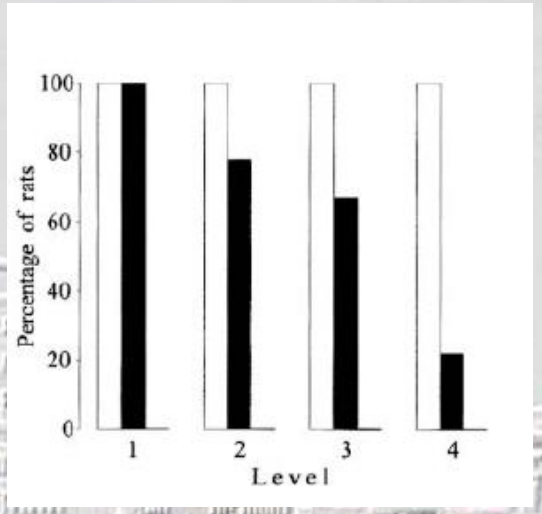
Ramón-Cueto, Neuron, 2000

OEGs in transection SCI - function

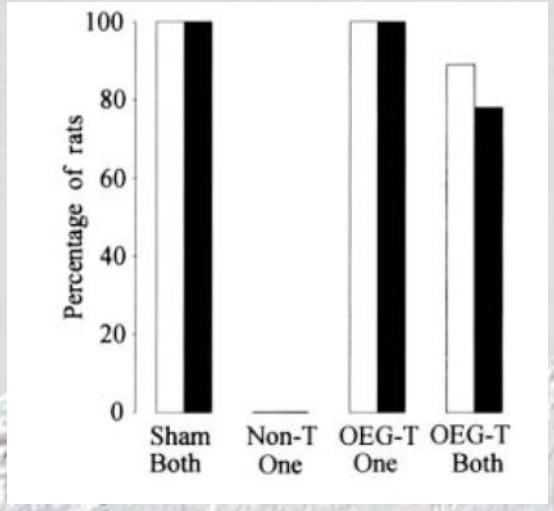
vertical grid

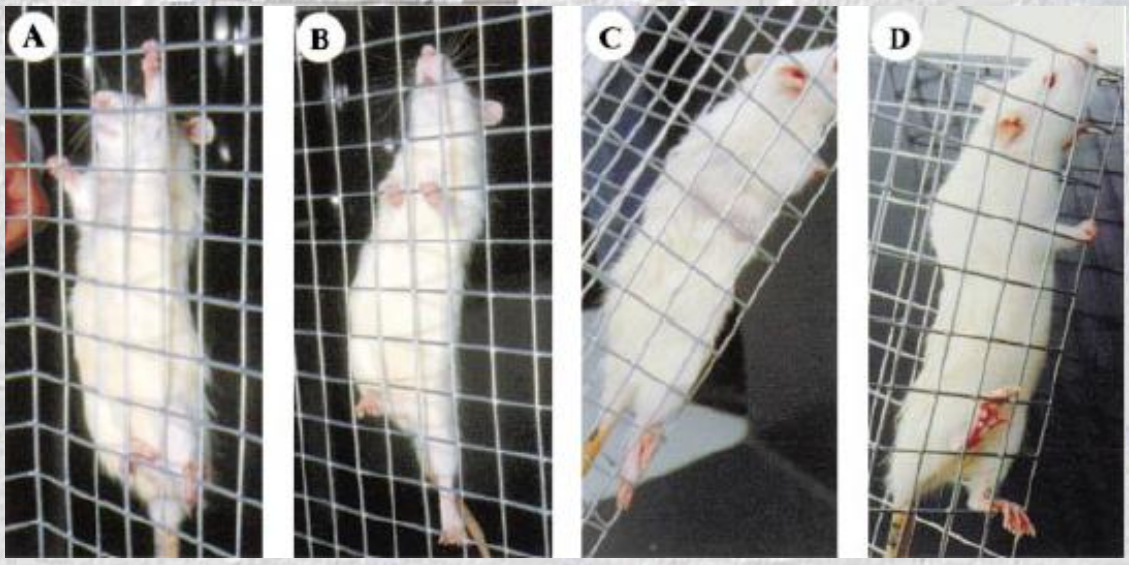


motor function



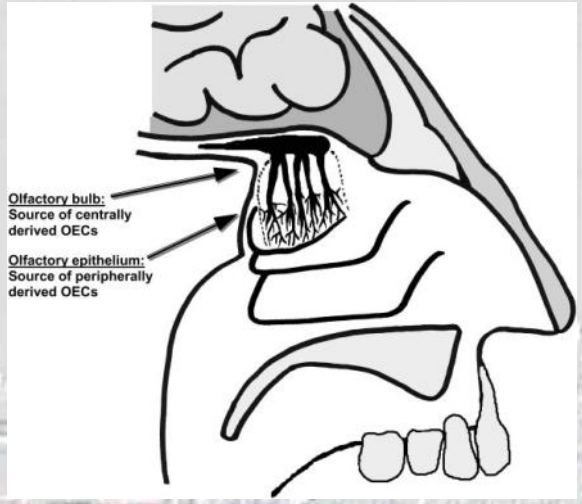
sensory testing





Drawbacks / critics about OEGs:

- Most research has been done with centrally-derived OEGs (Lu et al., 2002).

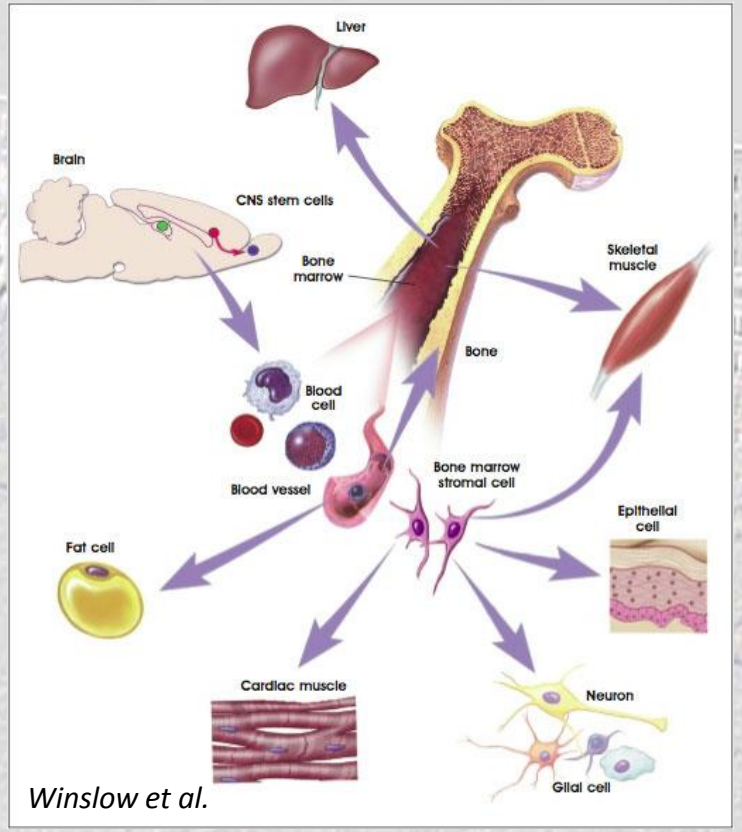


- The OEGs may have been contaminated with Schwann cells, as pure cultures of OEGs did not form myelin and did not show association with axons (Plant 2002)

Bone marrow stem cells

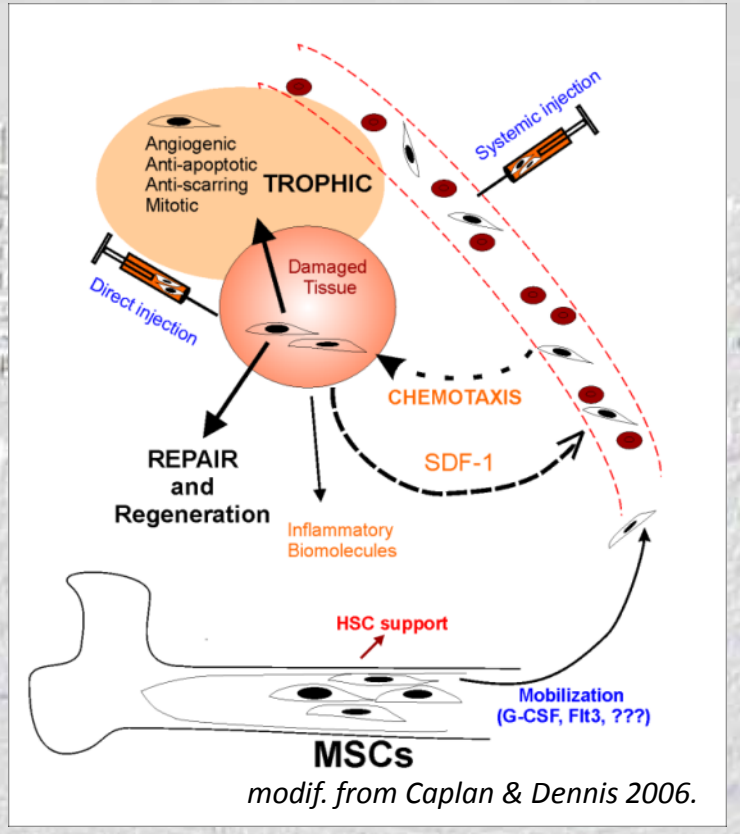
Prockop JD. *Science*, 1997.
J. Cohnheim. *Arch. Path. Anat. Physiol. Klin. Med,* 1867.

BMSC – source of stem cells for nonhematopoietic tissue



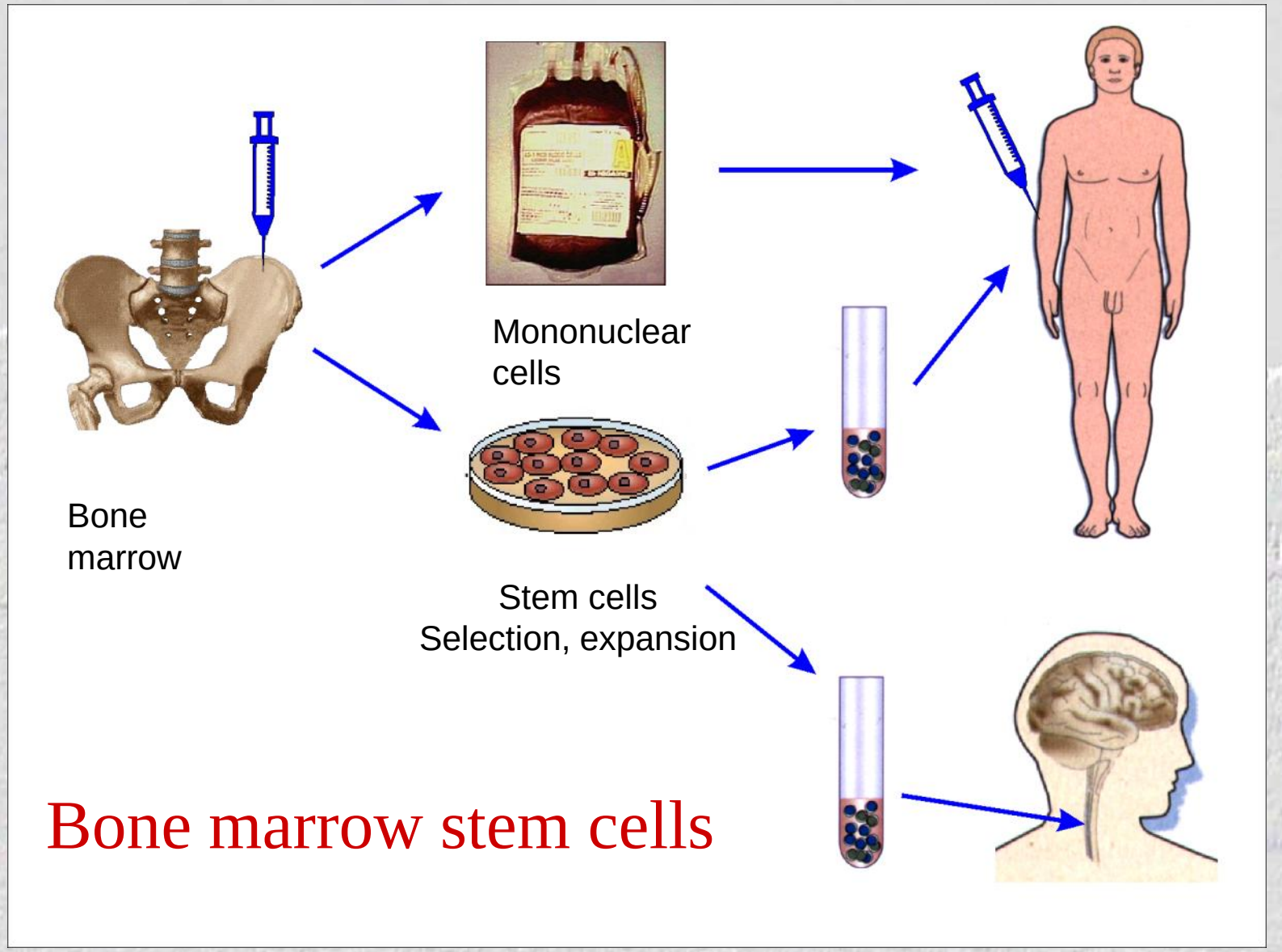
Winslow et al.

Delivery and function of MSCs in the host organism



modif. from Caplan & Dennis 2006.

BMSC in clinical application



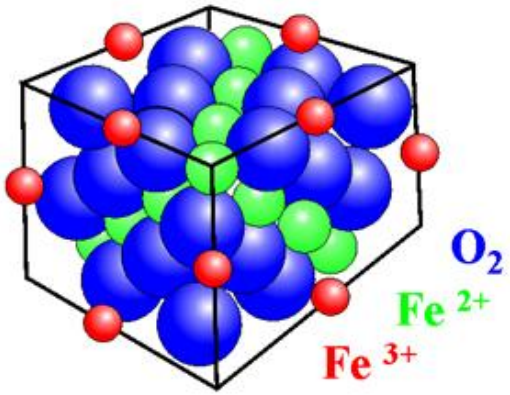
Bone marrow stem cells

Functions of bone marrow stem cells

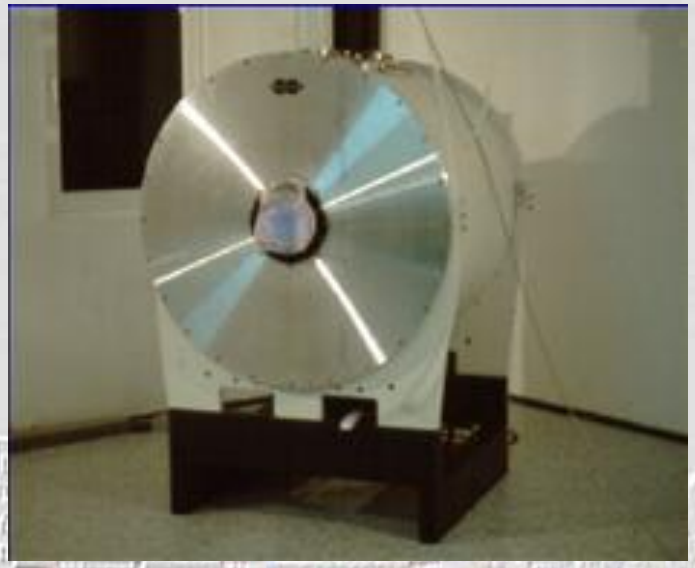
- Substitute cells (neurons, astrocytes, oligos) – REPAIR
- Save damaged cells - RESCUE
- Trophic support.
- Support regeneration of synapses and axons.
- Myelinisation.
- Mobilisation of endogenous stem cells.
- Induction of other genes/proteins.
- Reduction of scarring.
- Support in revascularisation.
- Modification of immune response.

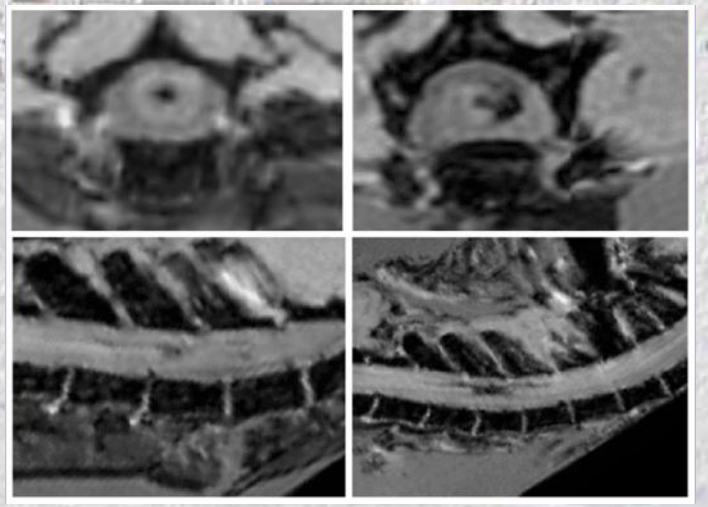
Non-invasive tracing of MSCs

Fe_3O_4 nanoparticles

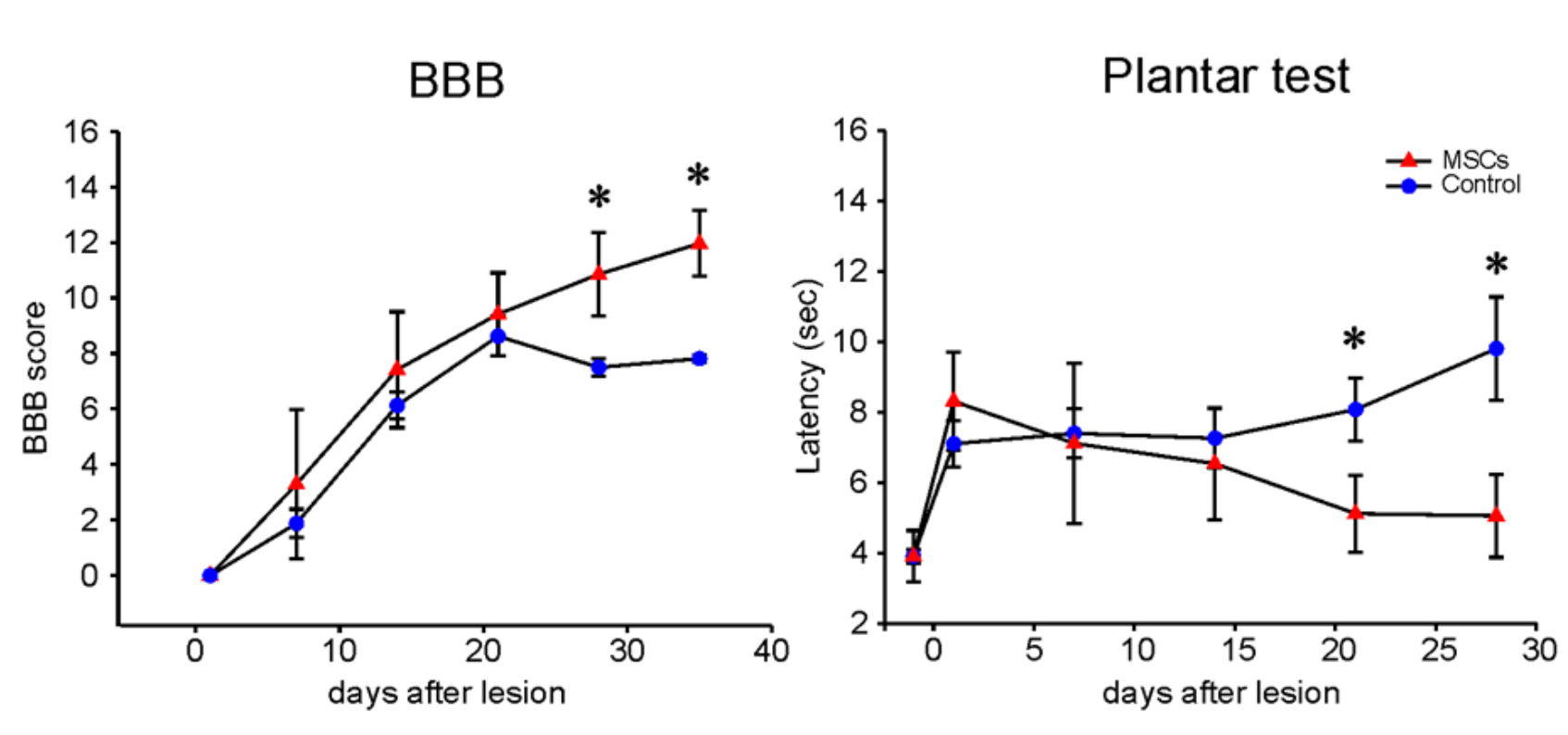


Nanoparticle coated with a surfactant monolayer shell (stabilised and soluble)





Functional effect of i.v. inj. of BMSC to rats with SCI



Syková, Jendelová, Urdzíková (2004)

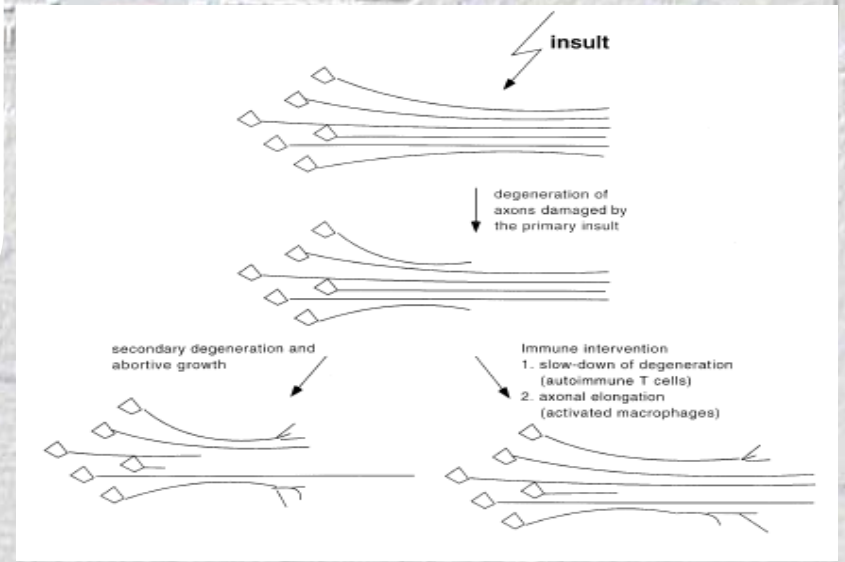
II. Immunity (activated macrophages and T cells)

The concept of „protective autoimmunity“

M. Schwartz

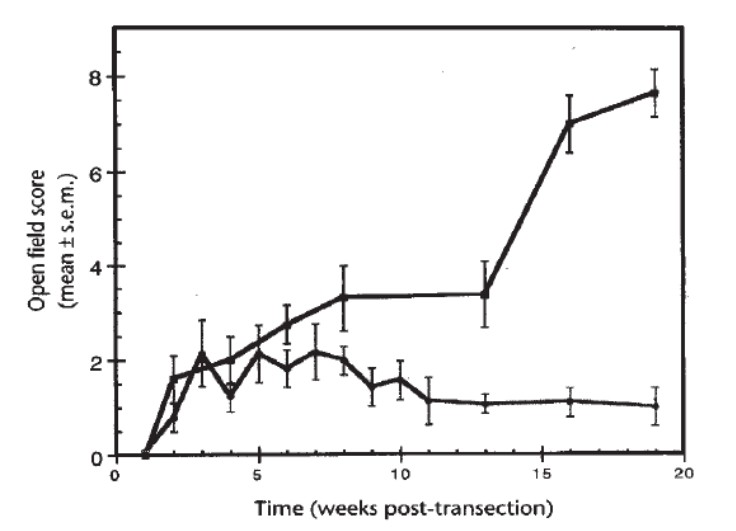
Weizmann Inst of Sciences, Izrael

- the immune system serves tissue protection and repair after injury in most tissues (not in the CNS)
- T cells and macrophages are mostly involved in this process
- how to do it:
  - withdraw individual‘s blood
  - isolate the monocytes
  - “educate” them on peripheral nerve tissue
  - replace them in the natural site of activity (the site of injury)
  - this way it is possible to induce a self-propagating process of healing

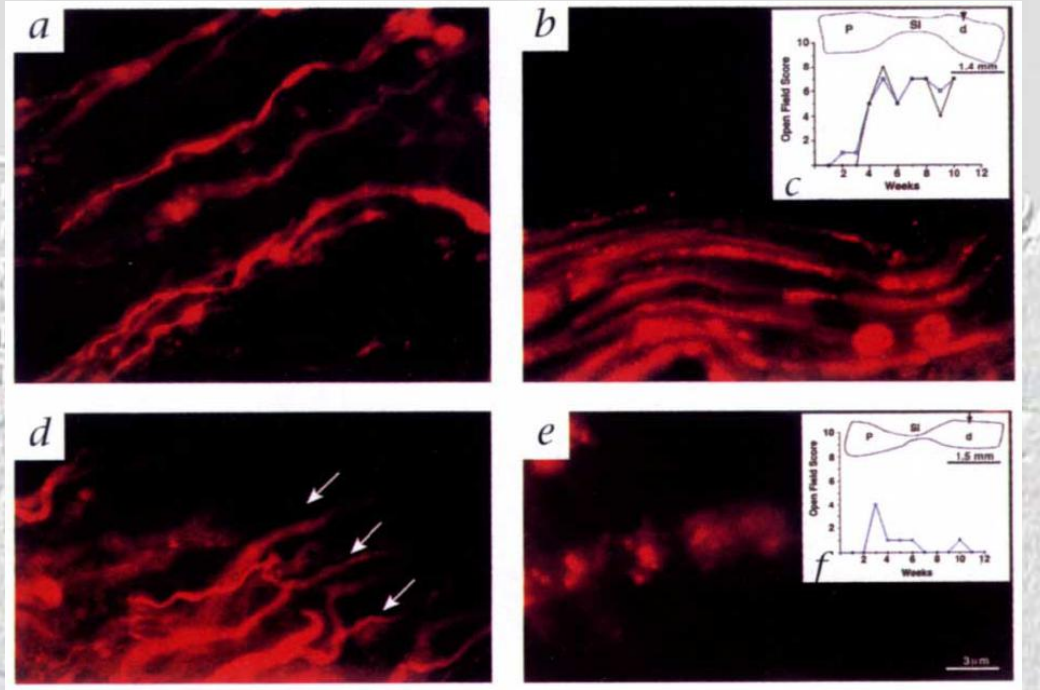


Protective immunity and complete SCI

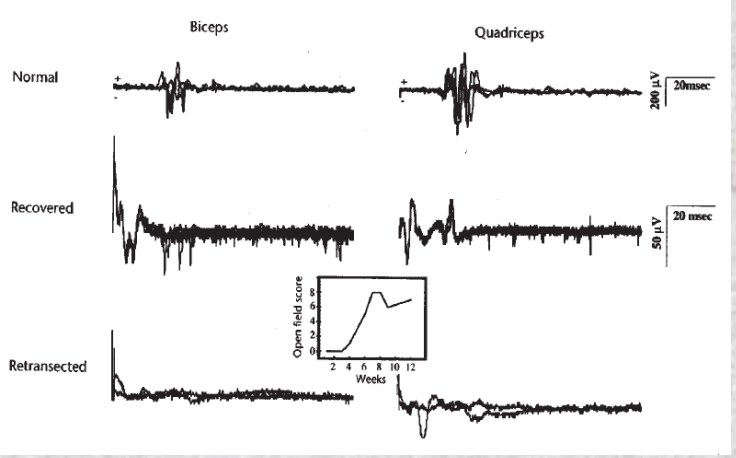
motor score improvement



anterograde tracing



MEP recordings recovery



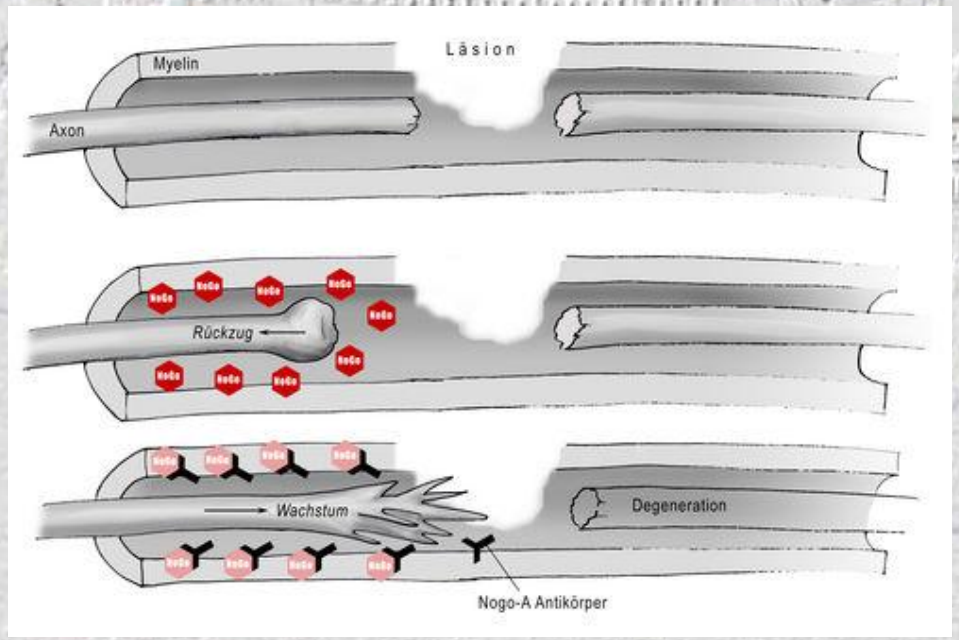
III. Neurotrophic factors in SCI repair

Neural response promoted	Neurotrophic factors
Motor neuron survival	BDNF, NT-3, NT-4/5, CNTF, GDNF
Motor neuron outgrowth	BDNF, NT-3, NT-4/5, CNTF, GDNF
Sensory neuron survival	NGF, NT-4/5, GDNF
Sensory neuron outgrowth	NGF, BDNF, NT-3
Spinal cord regeneration	NGF, NT-3, CNTF, FGFs
Peripheral nerve regeneration	NGF, NT-3, NT-4/5, CNTF, GDNF, FGFs
Sensory nerve growth across the PNS-CNS transition zone	NGF, NT-3, GDNF, FGFs

Schmidt and Leach, Annu Rev Biomed, 2003

IV. Overcoming glial scarring

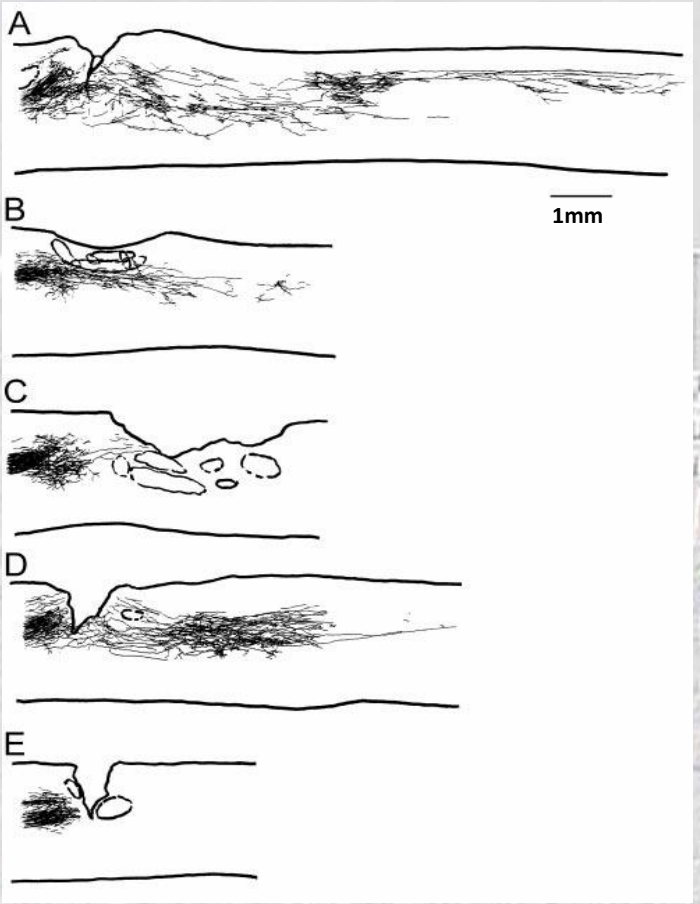
- **the limited plastic and regenerative capabilities of axons in the adult mammalian is due toe myelin-associated neurite growth inhibitors, such as Nogo-A**
- **the growth of neurites in the CNS can be can be enhanced by the application of a monoclonal antibody (mAb), IN-1, raised against Nogo-A**



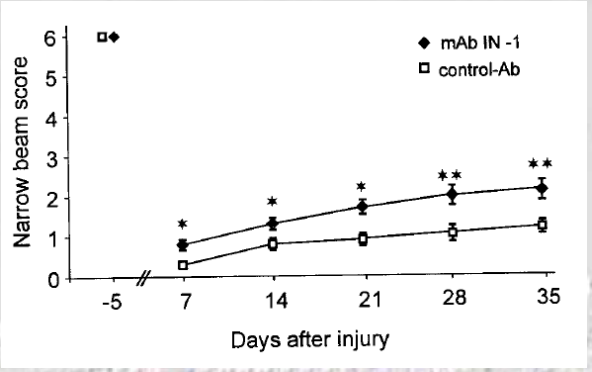
Martin E. Schwab
Brain Research Institute, Zurich, Switzerland

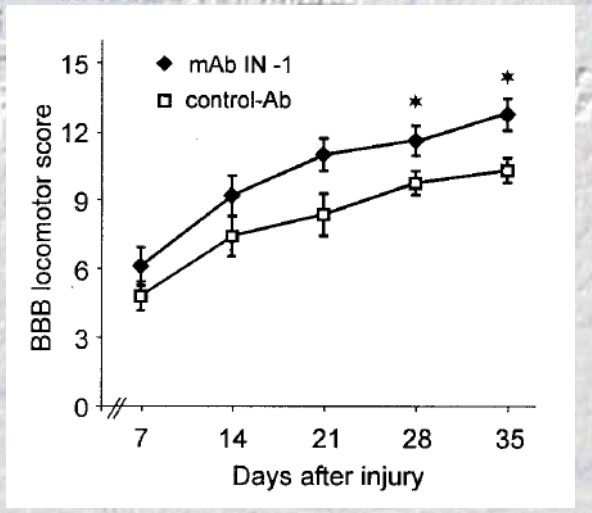
IV. Overcoming glial scarring

CST regrowth after IN-1 application



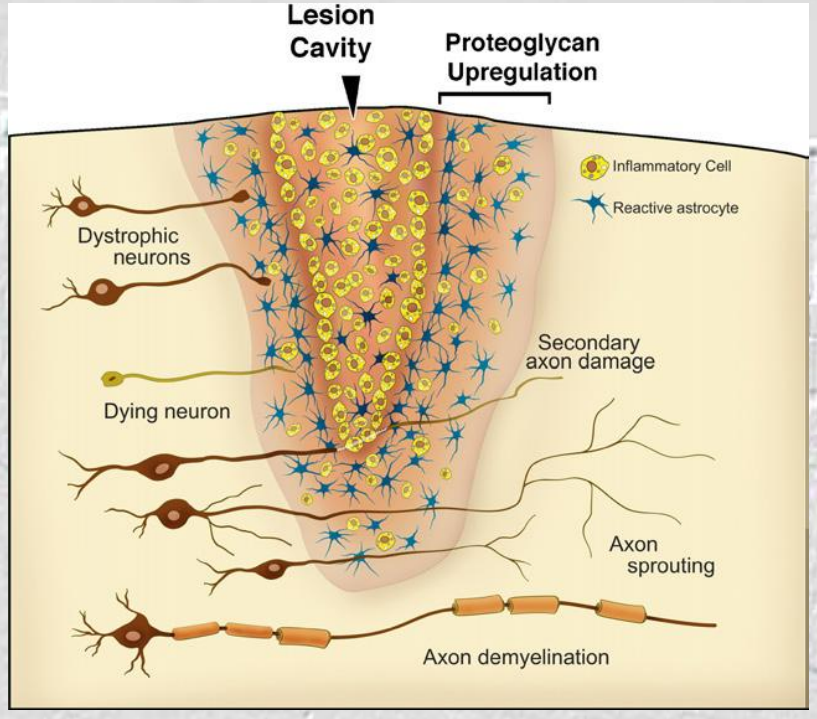
Functional improvement after IN-1 application





IV. Overcoming glial scarring

chondroitin sulphate proteoglycans
structural macromolecules
of the ECM in the CNS

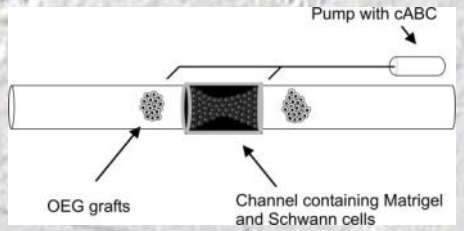


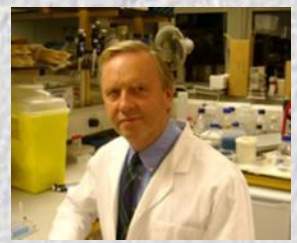
from Fitch, Silver, 2007

chondroitinase ABC
bacterial enzyme

- **treatment of SCI with chABC leads to degradation of CSPG (effect lasts for at least several weeks)**
- **neuritic outgrowth both in white and grey matter**
- **leads to functional recovery**

intrathecal delivery

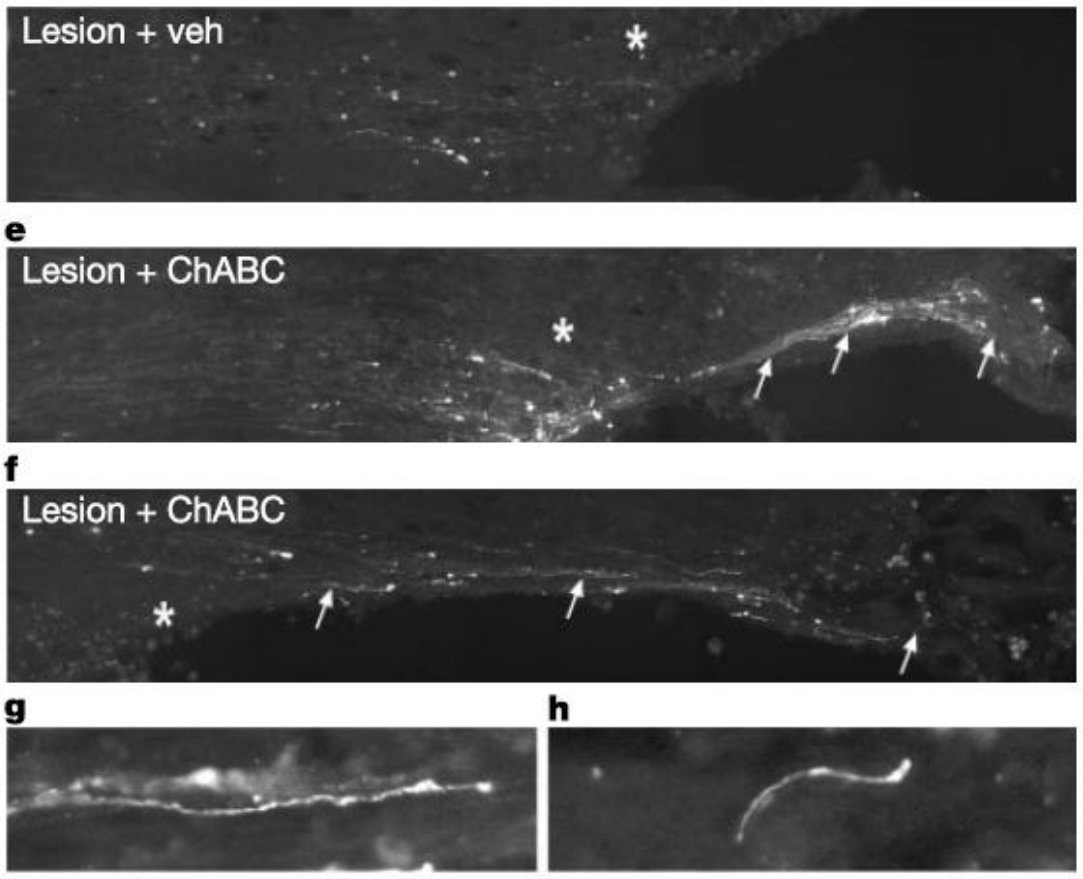




James W. Fawcett
Cambridge University Centre for Brain Repair, UK

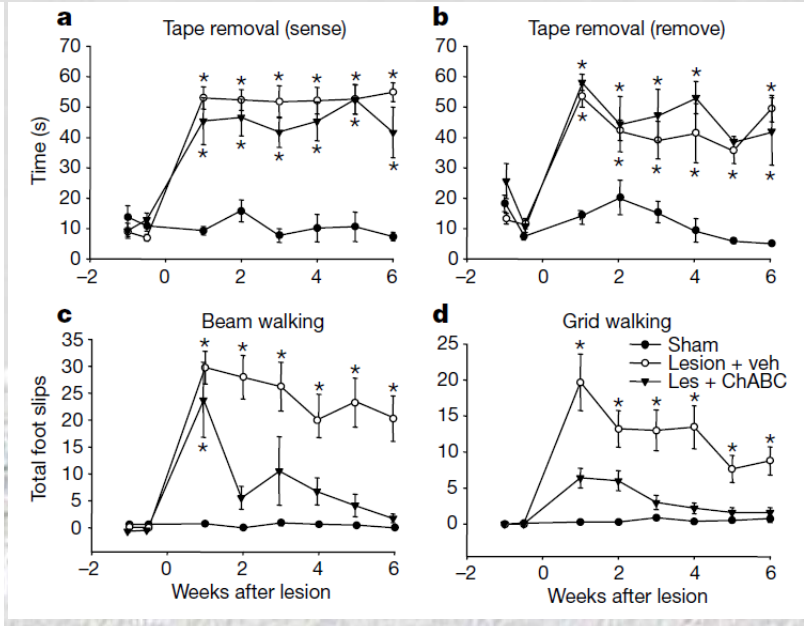
IV. Overcoming glial scarring

anterograde tracing

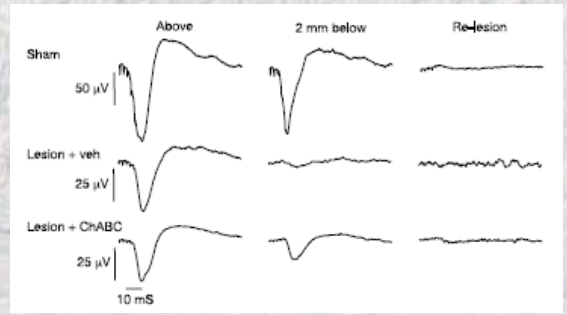


Bradbury *et al*., Nature 2002

functional improvement



MEP



V. Guidance therapies

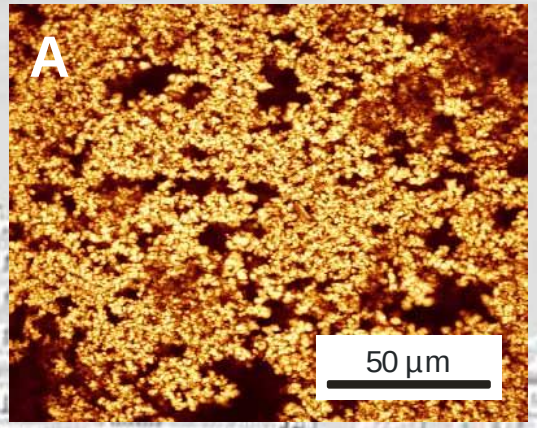
1. Natural polymers

2. Synthetic polymers

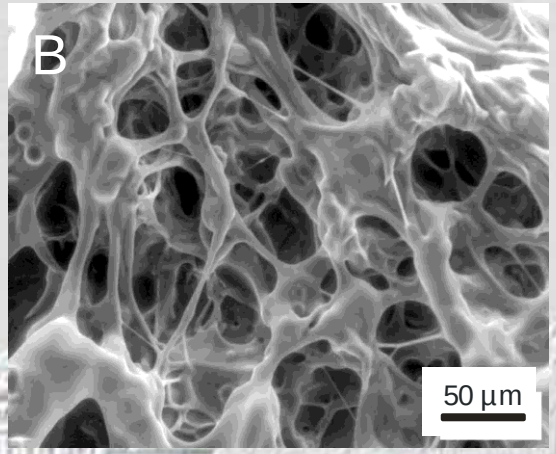
hydrogels

- biocompatible
- high water content
- macroporous (50-90 µm)
- large surface area
- physical properties similar to extracellular space of CNS
- chemical properties widely adjustable
- allow diffusion of neurotrophic factors
- provide scaffold

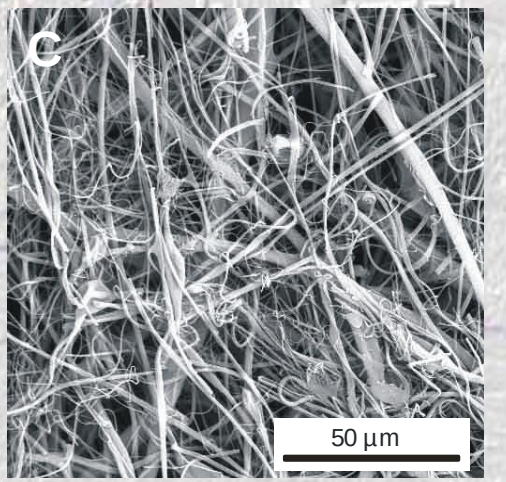
HPMA-RGD



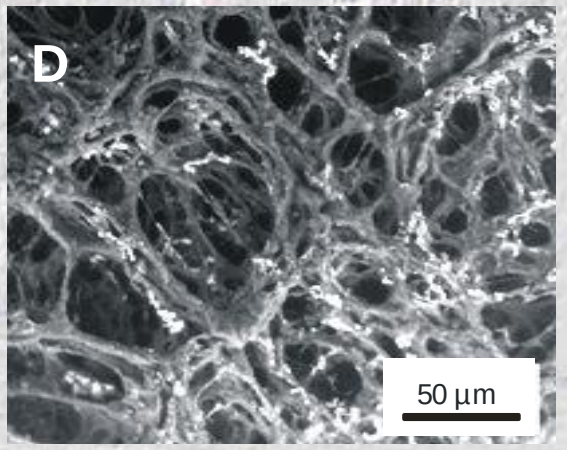
HEMA



nanofibres



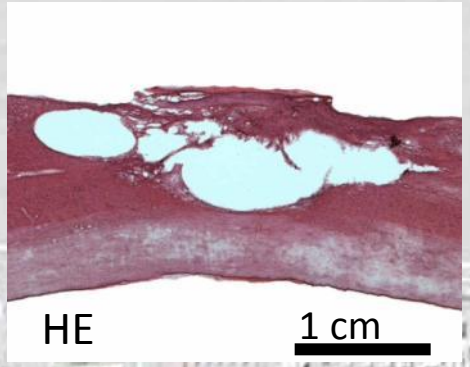
resorbable HEMA



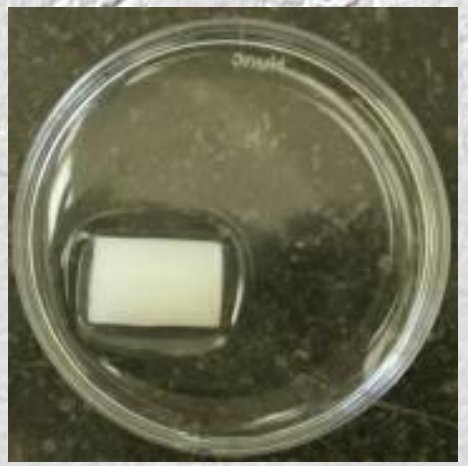
Hejčl *et al*., Phys Res, 2008

V. Bridging the cavity

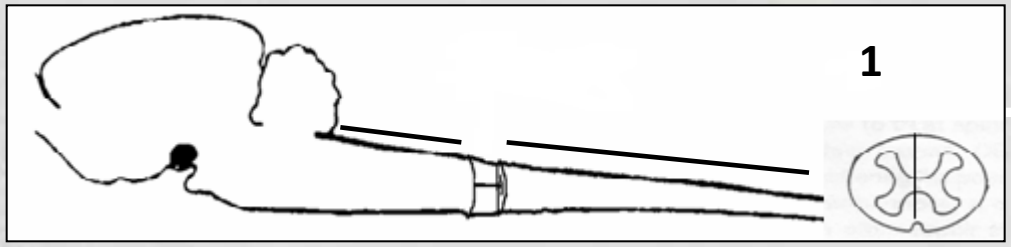
SCI - cavity



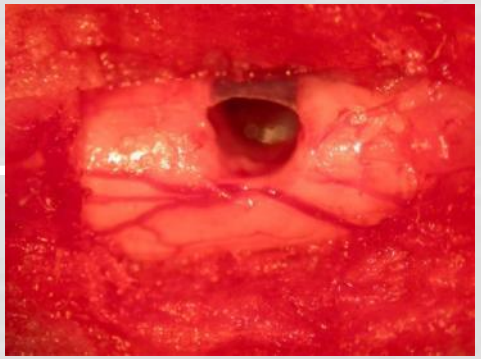
hydrogel - bridge

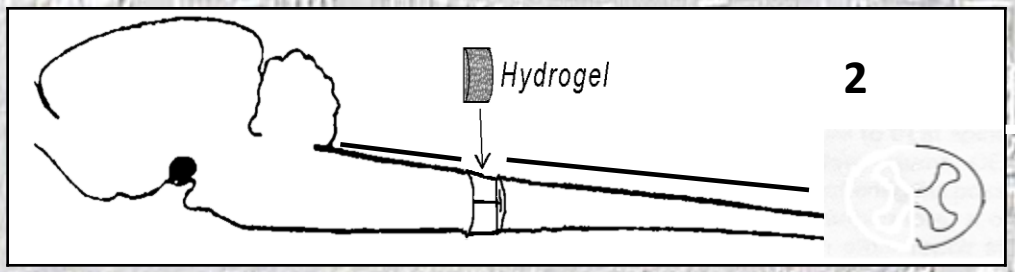


V. Typical bridging protocol

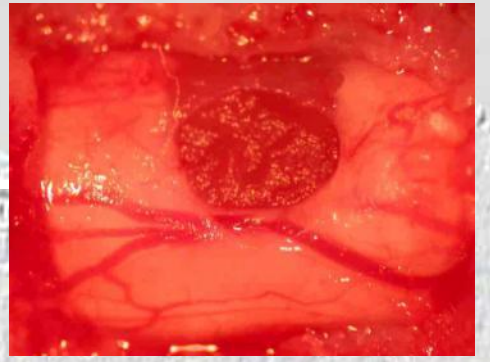


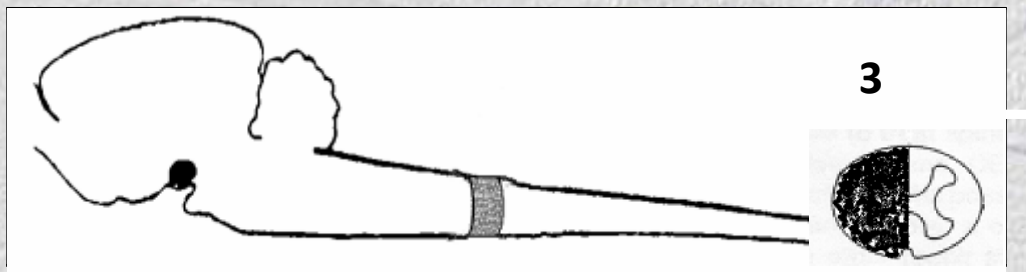
spinal cord injury



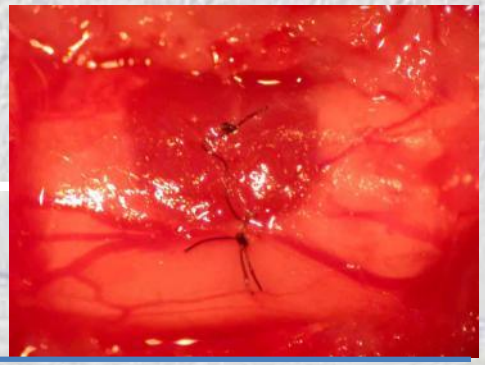


bridging the lesion



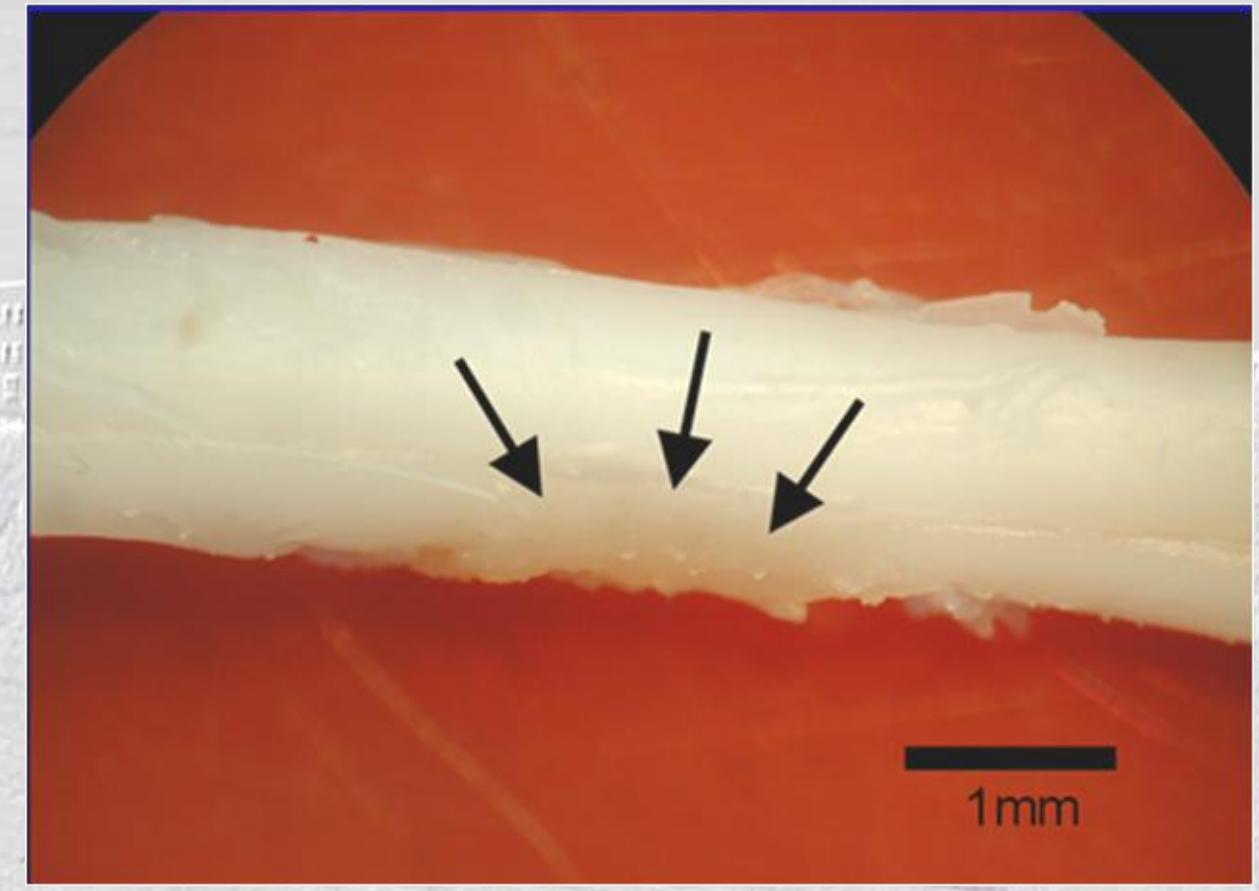


suturing the dura

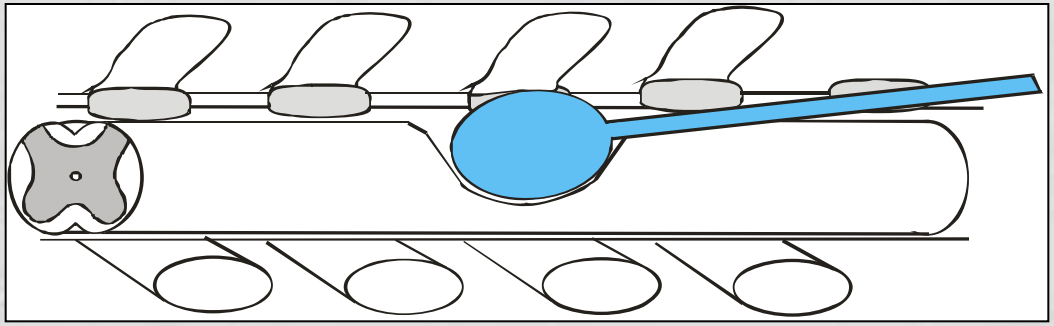


Syková, Hejčl, *et a*l., 2006

1 month after hydrogel implantation



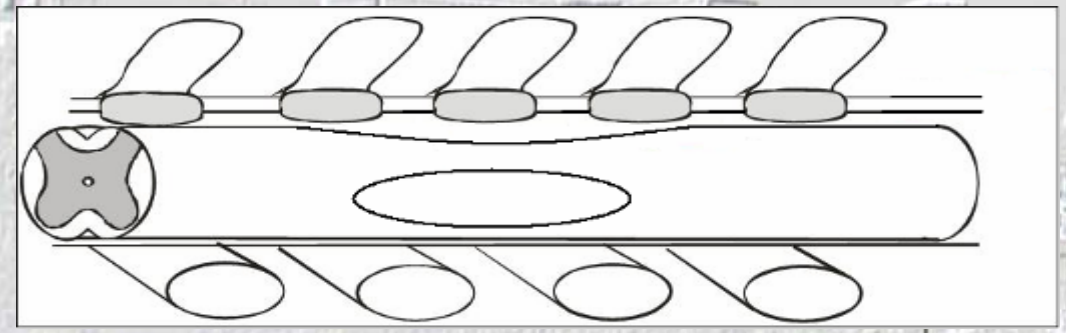
V. Hydrogel bridging a chronic SCI



balloon compression

lesion

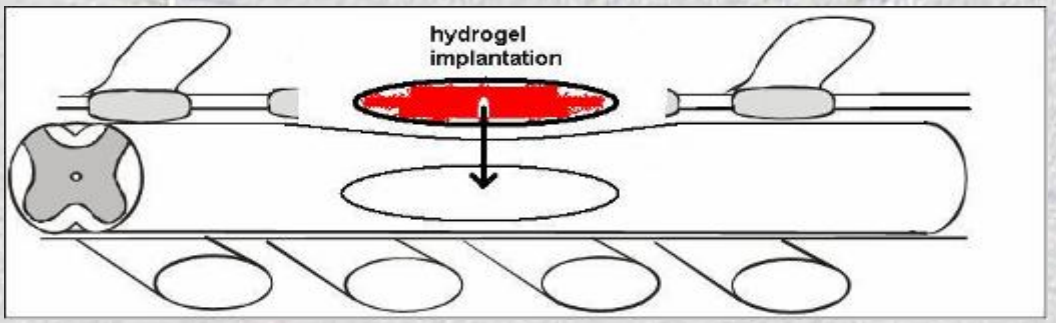
Fogarty catheter inflated in the epidural space for 5 minutes



posttraumatic pseudocystic

cavity

5 weeks after the SCI

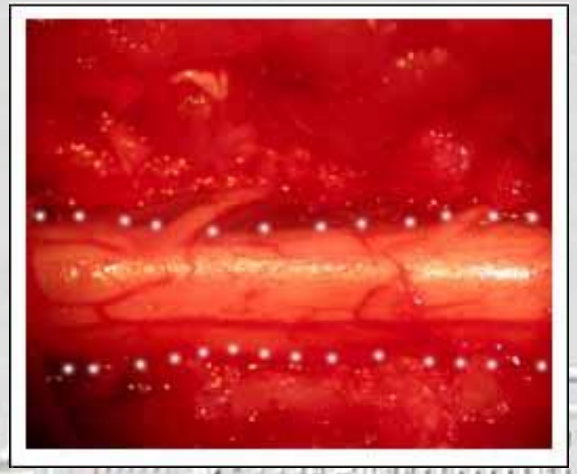


hydrogel implantation

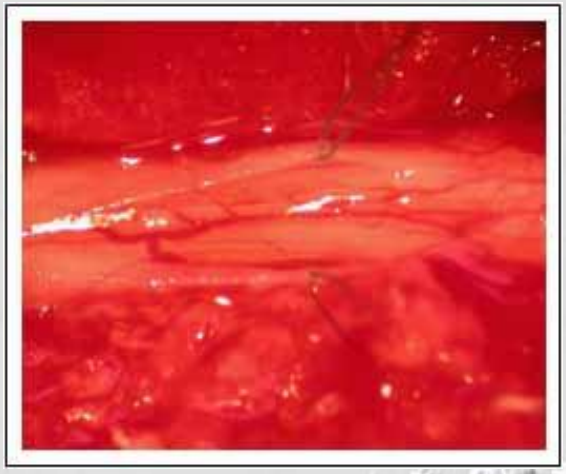
inside the cavity

hydrogel bridges the lesion

Hydrogel implantation in chronic SCI



1. spinal cord atrophy 5 weeks after a BCL



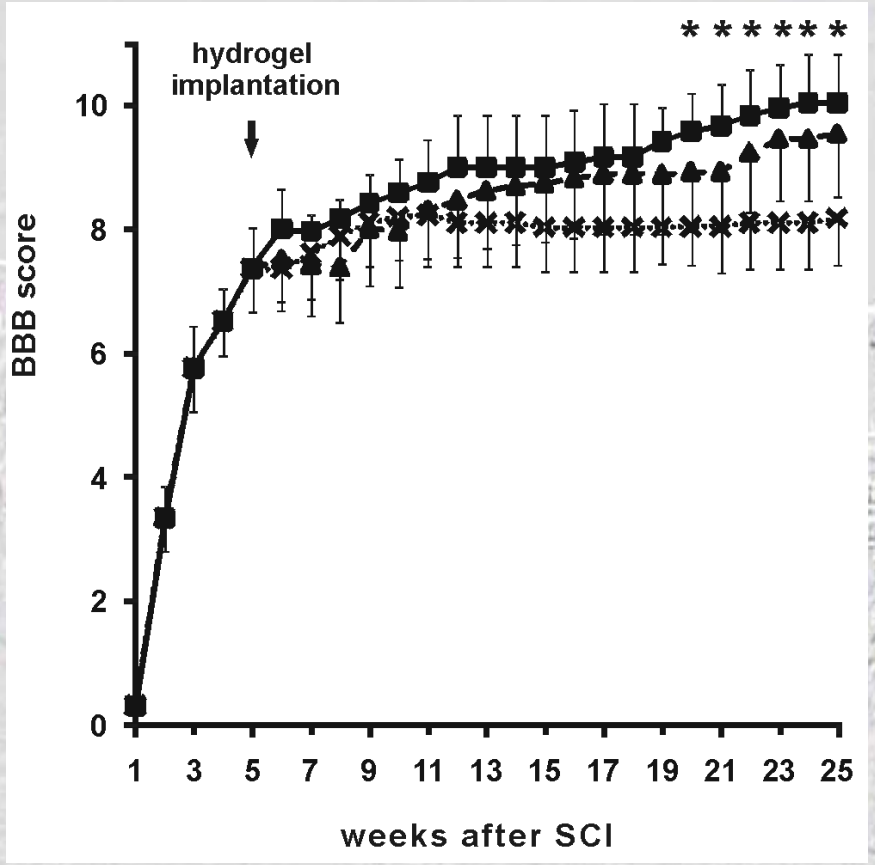
2. dural opening

3. pseudocyst bridged with hydrogel scaffold

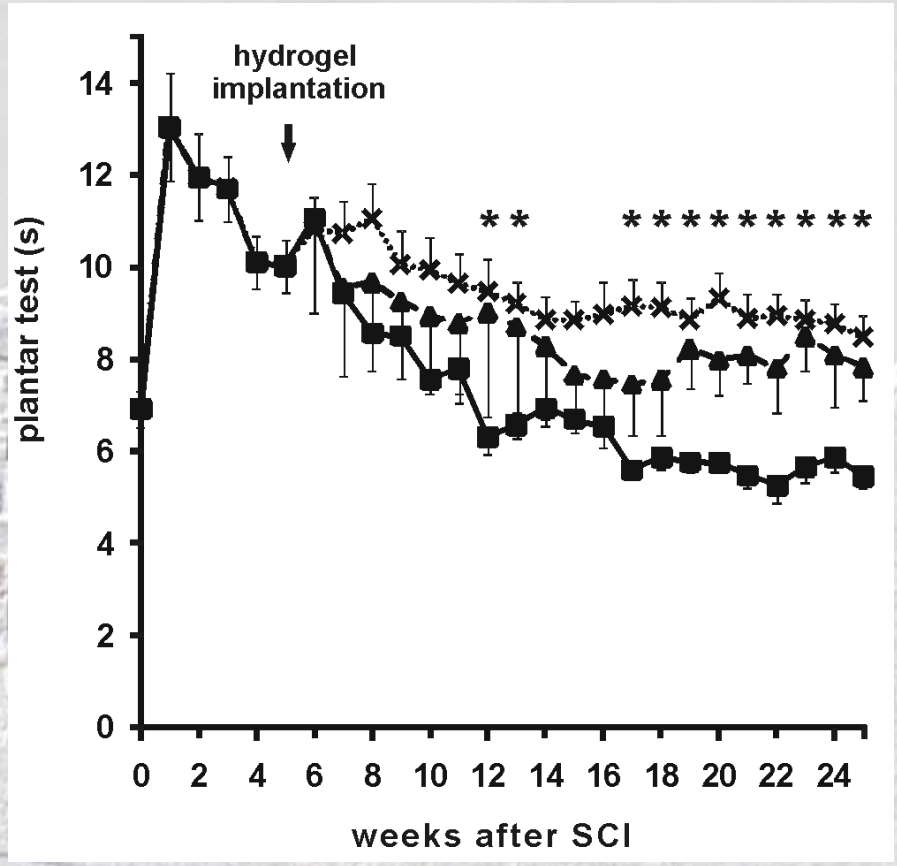
4. dural closure

Hydrogel implantation in chronic SCI

motor function



sensory function

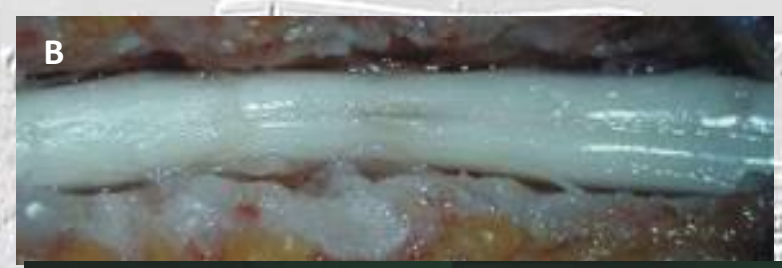


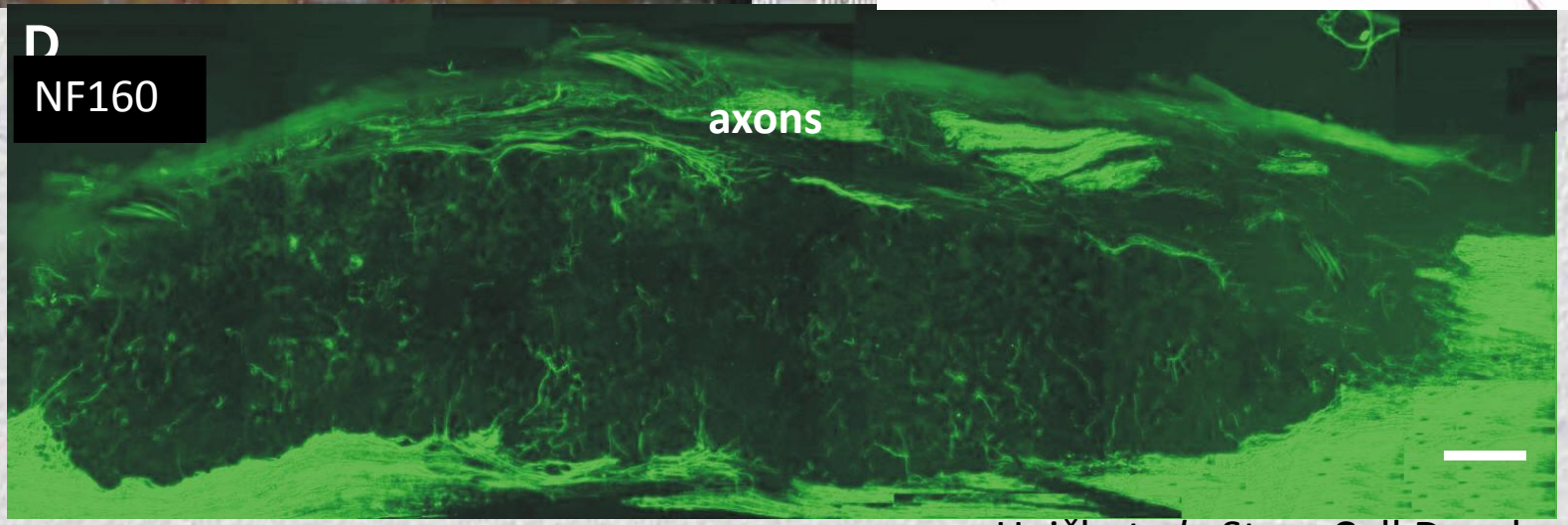
Hejčl *et al.*, Stem Cell Develop., 2010

Hydrogel implantation in chronic SCI

SCI

SCI + hydrogel

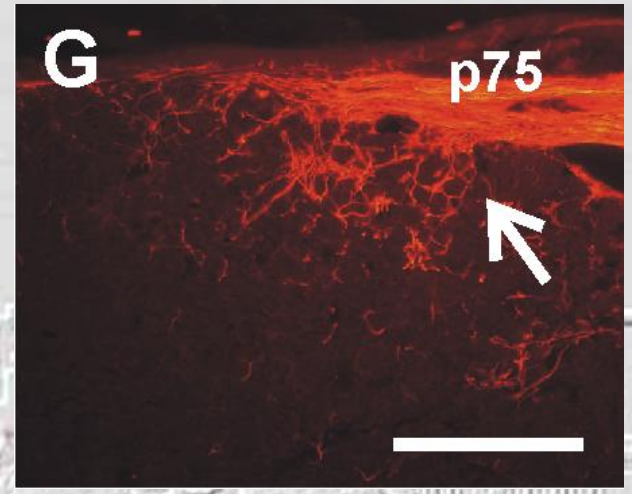


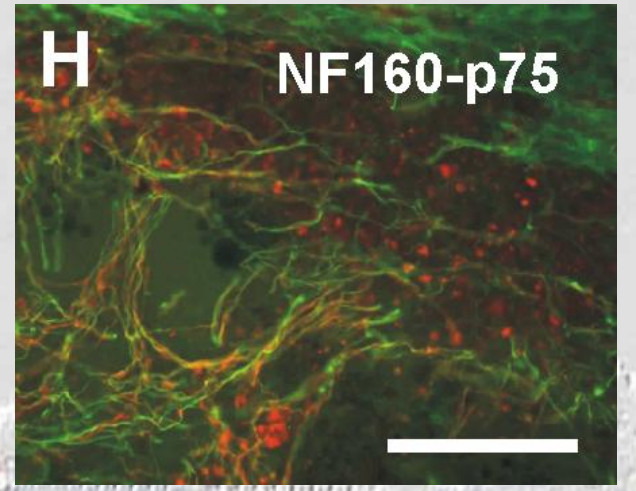


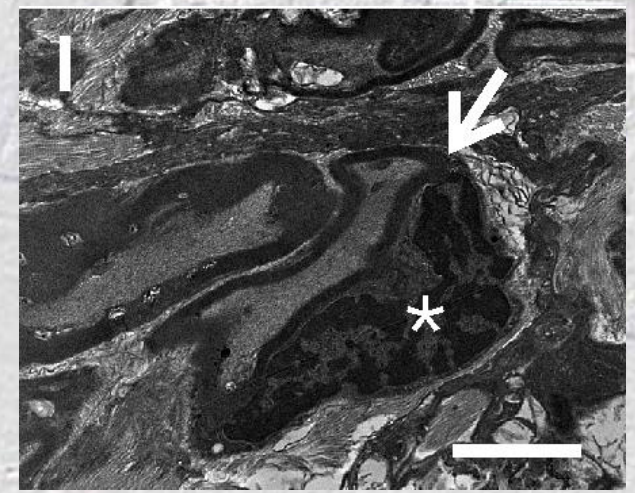
Hejčl *et al*., Stem Cell Develop., 2010

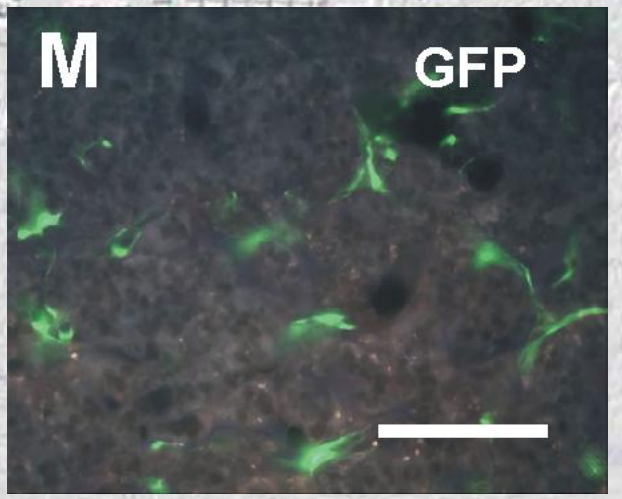
Hydrogel implantation in chronic SCI

Schwann cells and BMSCs



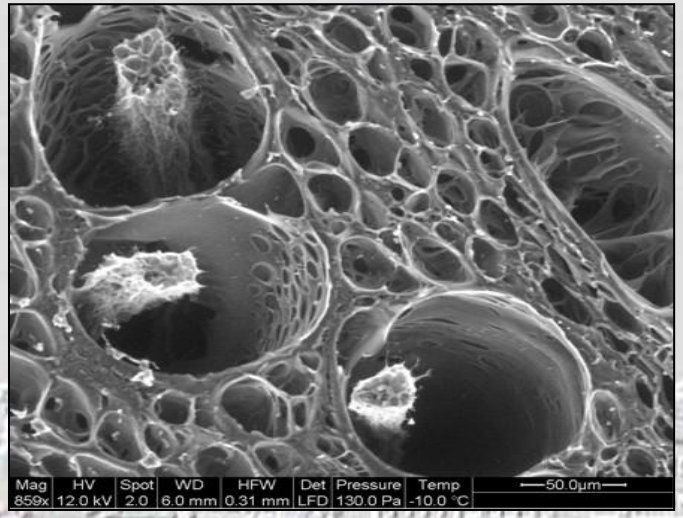


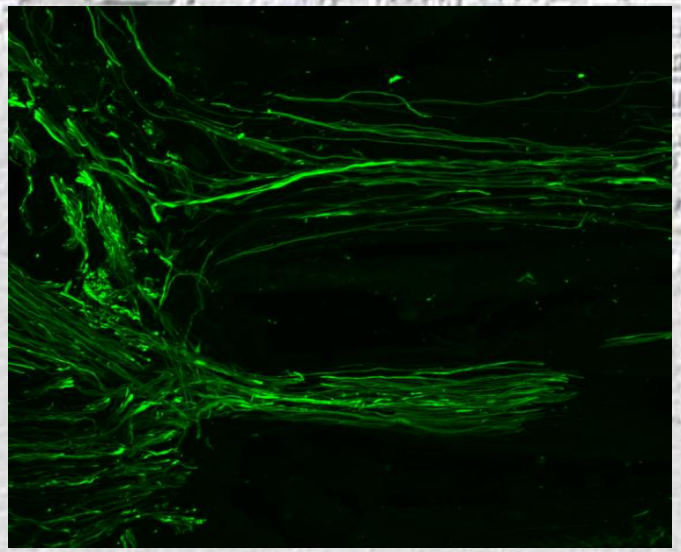


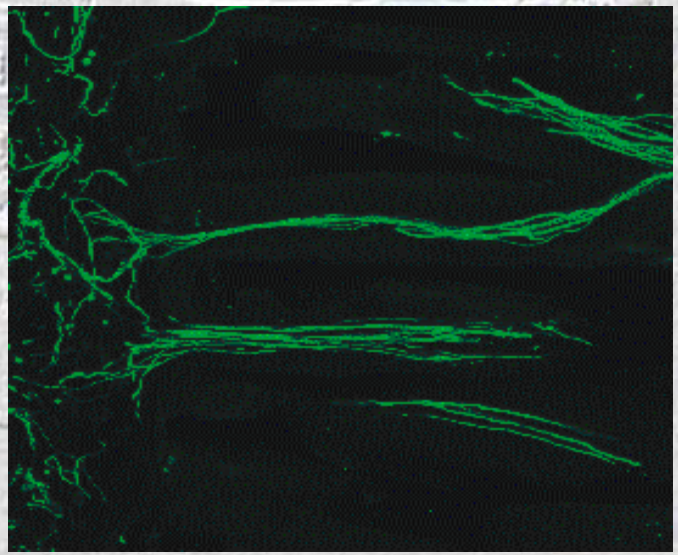


Hejčl *et al*., Stem Cell Develop., 2010

Hydrogel with oriented pores







Clinical studies

	ASIA	Time	No.	Route	Report
BMSC					
Czech Republic, Prague	A	acute and chronic	20	i.v., i.a.	Syková et al., Cell Transplantation 2006
Korea	A	acute and chronic	35	lesional	Yoon et al., Stem Cells, 2007
Russia, Siberian Acad Sci	A	chronic	18	lesion+i.v.	Chernykh et al., Cell Tech Biol Med, 2007
Brazil, Sao Paulo	A	chronic	39	i.v.	Cristante et al., Spinal Cord 2009
Turkey, Ankara	A	chronic	9	?	Deda et al., Cytotherapy, 2008
OEG					
Portugal, Lisbon	A	chronic	7	lesion	Lima et al., J Spinal Cord Med, 2006
Australia, Brisbane	A	chronic	6	lesion	Ferón et al., Brain 2005, 2008
China	?	?	1255 (656)	?	Huang et al., Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi, 2009
autologous macrophages					
Israel, Tel Hashomer	A	acute	8	lesion	Knoller et al., J Neurosurg Spine, 2005
Schwann cells					
Iran, Teheran	A-C	chronic	4	lesion	Saberi et al., Neurosci Lett, 2008

Conclusions and further perspectives

1. **No definite treatment for SCI is available yet (experimental or clinical).**

2. **Several approaches have been shown promising for partial recovery after SCI.**

3. **Better understanding the pathophysiology of SCI can lead to improvement of application of experimental therapies (timing, dose, route of application, etc.)**

4. **SCI is a complex problem requiring complex solutions – combination of therapies.**

5. **Transferring our results from laboratories to clinics.**

Thank you for your attention