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Stem cell biology and applications in pre-clinical experiments

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BIOMATCELL
VINN Excellence Center

Presentation outline

Part I

Introduction

Common features of stem cells

Stem cell niches

Part IIA-B

In vivo xenotransplantation and cell tracing experiments

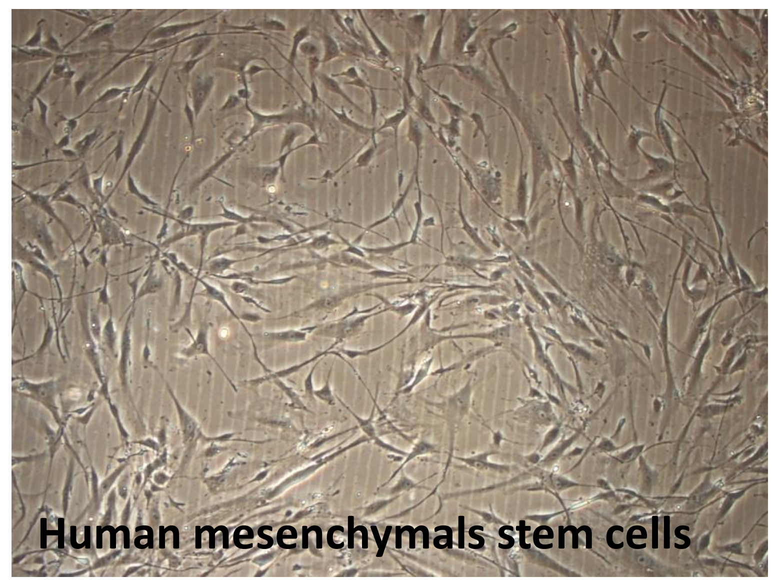
Stem cell therapy for the human degenerated disc?

PART I

Regenerative medicine and tissue engineering is a rapidly increasing research field....

Stem cell therapy - important issues

- cell viability; prior and after transplantation
- distribution of cells, migration; cell tracer (animal studies)
- differentation of mesenchymals stem cells (MSCs) e.g. chondrogenic lineage
- microenvironment



Human mesenchymals stem cells

Clinical applications using mesenchymal stem cell therapy in use today..

relatively common treatments

uncommon treatments

Hematological disorders

Leukemia

(allogenic hemapoetic stem cells)

- Fouillard L et al. Leukemia. 2003
- Wayne A et al. Pediatr.Clin.North Am.2010

Osteogenesis imperfecta

(allogenic bone marrow mesenchymal stem cells)

- Horwitz et al. Proc Natl Acad Sci USA. 2002
- Le Blanc K et al. Transplantation. 2005

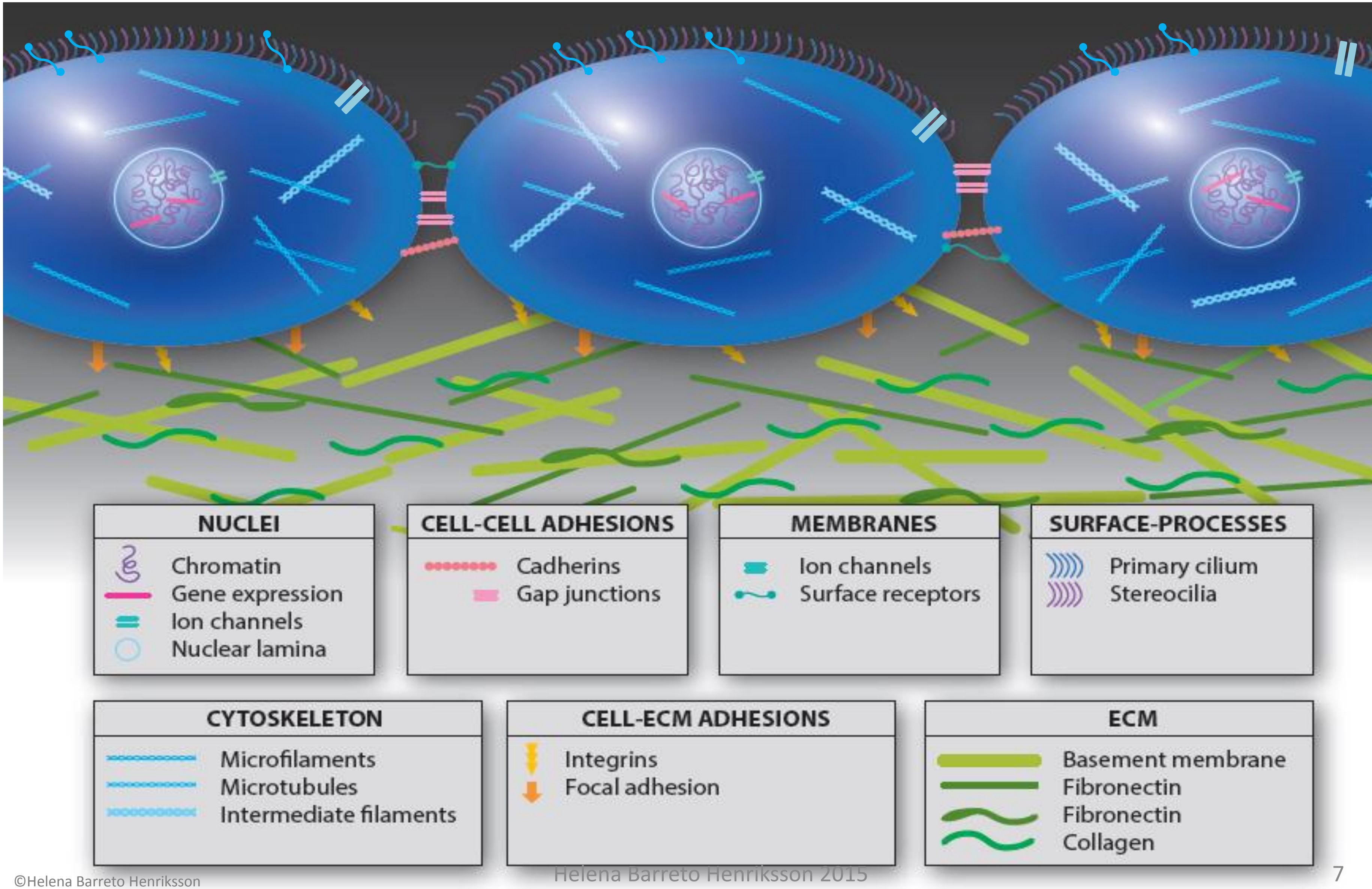
Hurler syndrome,

and MLD (metachromatic leukodystrophy)(allogenic bone marrow mesenchymal stem cells)

- Koç ON et al,Bone Marrow Transplant.2002

and more…

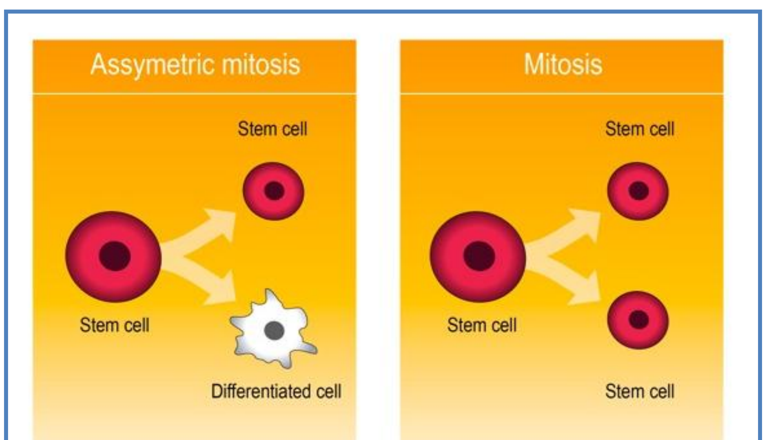
The micro environment for cells is complex and needs consideration when working with biomaterials……..

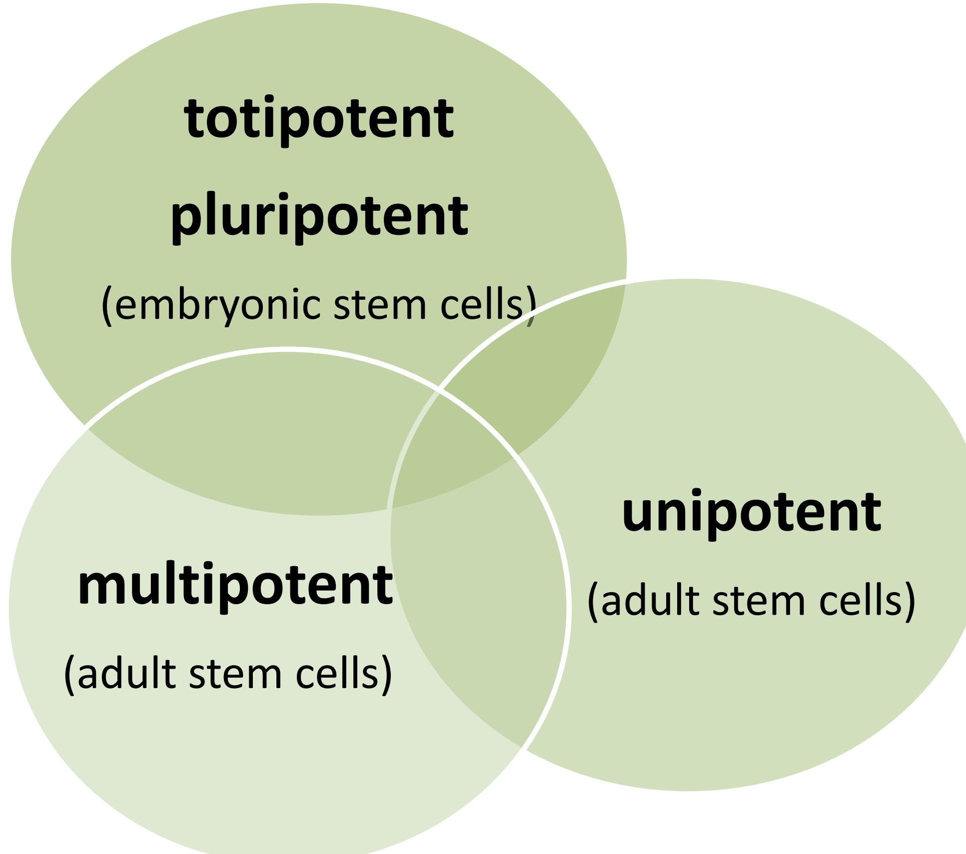


Stem cells

Stem cells have special features....

- ”self renewal”
- asymmetric cell division
- migration ability
- ”slow cycling cells”
- react to distant signals





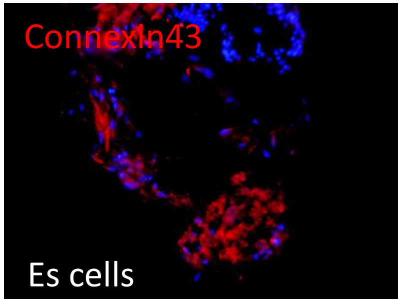
Embryonic stem cells (Es)

Ectoderm
Mesoderm
Endoderm

- Pluripotent
- High growth rate
- Needs feeder layer for growth *in vitro* (other cell type) in monolayer
- Hanging drop culture

Drawback:

- risk for teratoma *in vivo*



Stem cells is undifferented (immature) cells

Embryonic stem cells
-the embryo

No clinical application
-risk for teratoma

Umbilical cord stem cells
-Whartons gelly
-umbilical cord blood

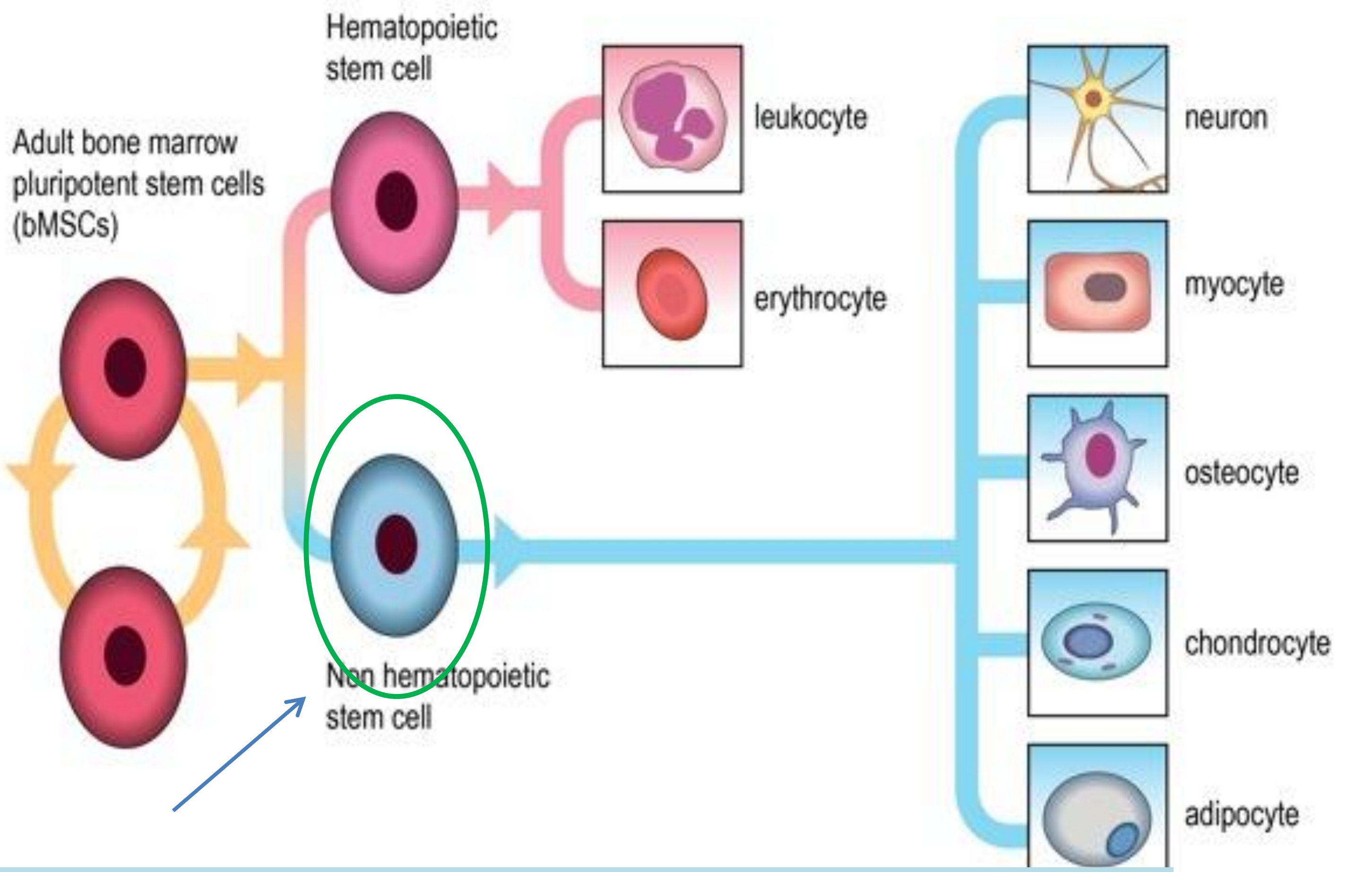
Umbilical coed blood is collected at present time
-national blood bank for future use
- needs to be tested for hereditary diseases

Adult stem cells
-mesenchymal stem cells* (bone marrow)
-hemapoetic stem cells
-tissue specific stem cells

In clinical use today
-osteogenesis imperfecta
-leucemia

* MSCs

Differentiation capability of bone marrow derived mesenchymal stem cells (MSCs)



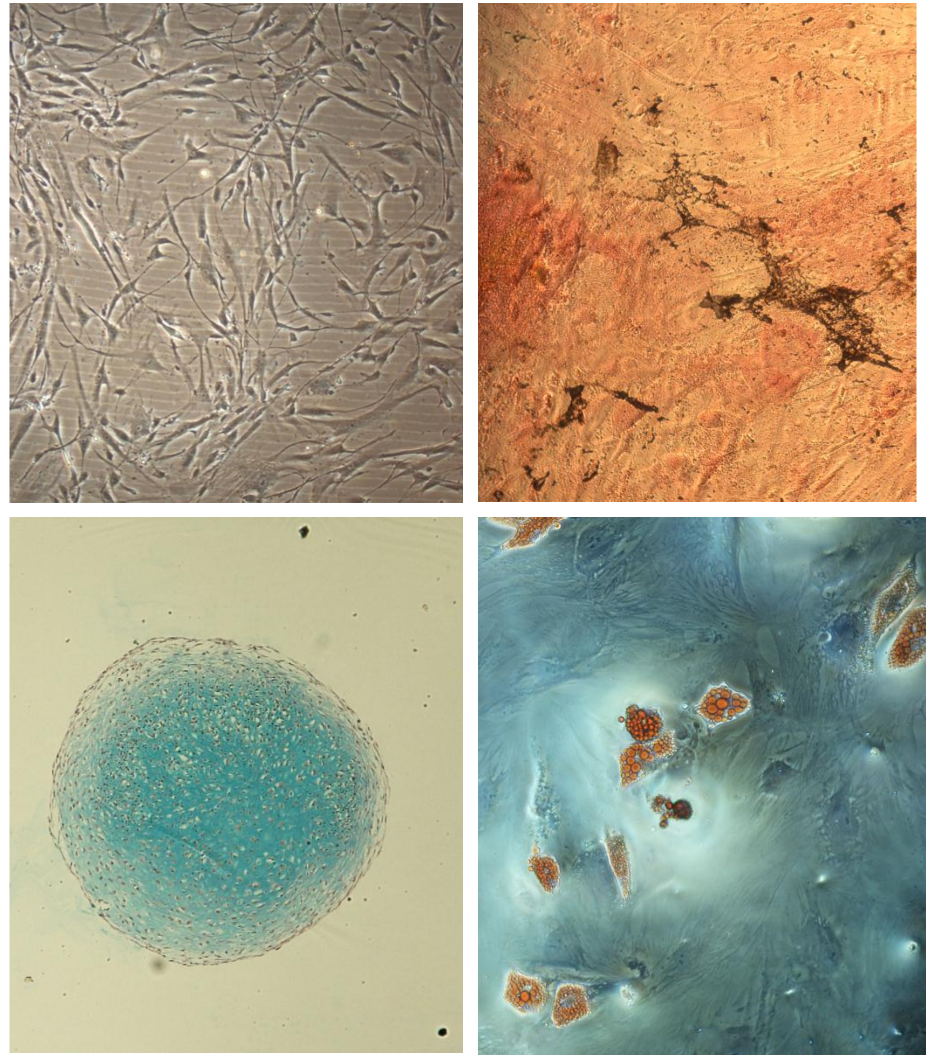
hMSC: CD105+, CD166+, STRO1+, CD34-, CD45-

Differentiate = maturation process (cells)

Mesenchymal stem cells can be influenced *in vitro*

- adipogenesis
- chondrogenesis
- osteogenesis

- growth factors



Where can adult stem cells be found?

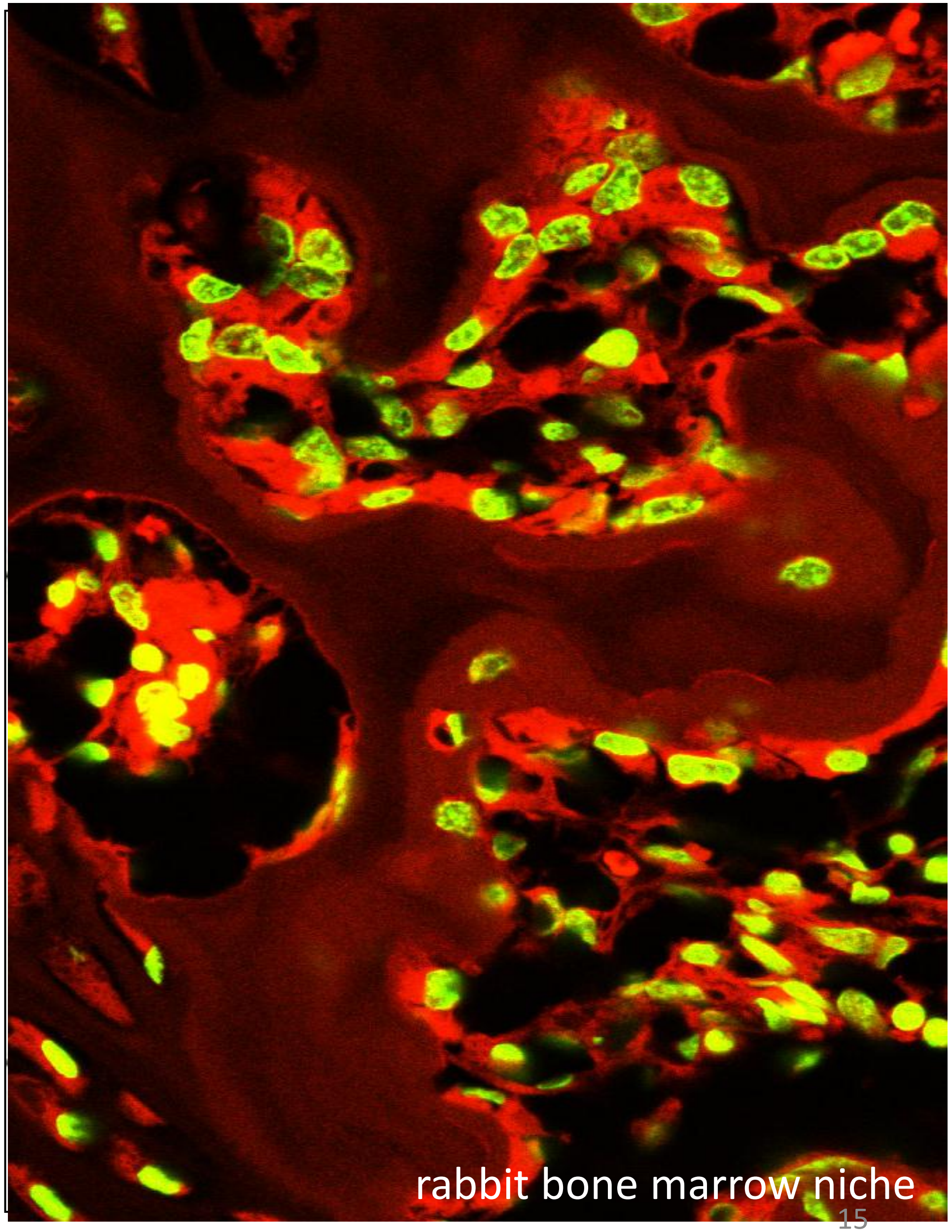
Adult stem cells can be found.....

The stem cell niche: a microenvironment that harbours stem cells and **a specific anatomical localisation** where stem cells is present, in senescence or migrating in/out

Stem cell niches (mammalia)

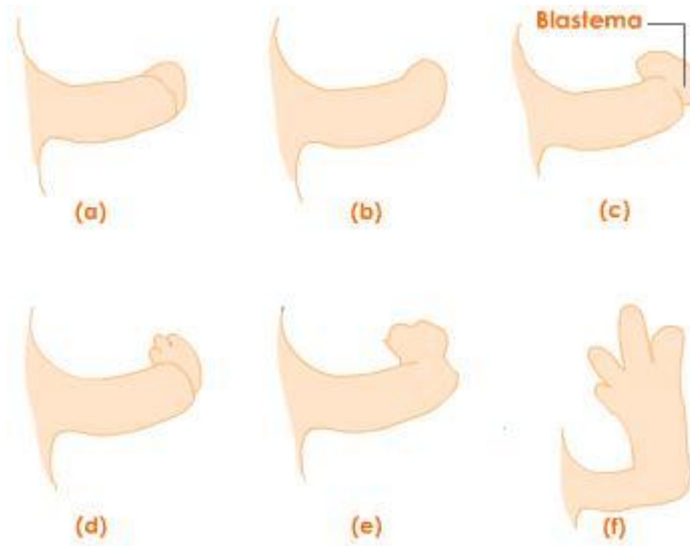
- bone marrow
- skin
- intestine
- brain
- and more..

Epimorphosis

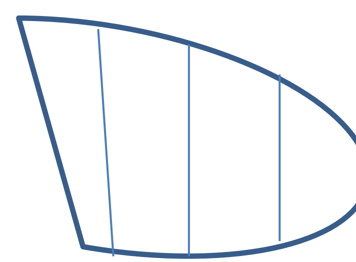


Vogt.G, Hidden treasures in stem cells of indeterminately bilateral invertebrates, 2011
Mitsiadis. T.A et al. Stem cell niches in mammals, 2007
Poulsom et al. Adult stem cell plasticity, 2002
Ohlstein, B. et al. The stem cell niche theme and variations, 2004

Epimorphosis



•Regeneration, cellular mechanisms
•Blastema formation
•Regeneration/growth of front leg 35-40 days

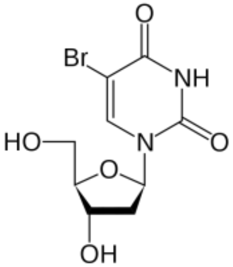


http://www.tutorvista.com/content/biology/biology-iv/growth-regeneration-ageing/regeneration.php#

The stem cell niche

- A microenvironment that harbours stem cells
  A specific anatomical localisation where stem cells are in a resting state, proliferating or migrating in/out

- **Simple nisches-** one type of stem cells, partner cells
- **Complex nisches-** different types of stem cells present, partner cells
- **Storage nisches**- different stem cells, partnerceller, ”slow cycling cells” (can be detected with BrdU* *in vivo* labelling of DNA)



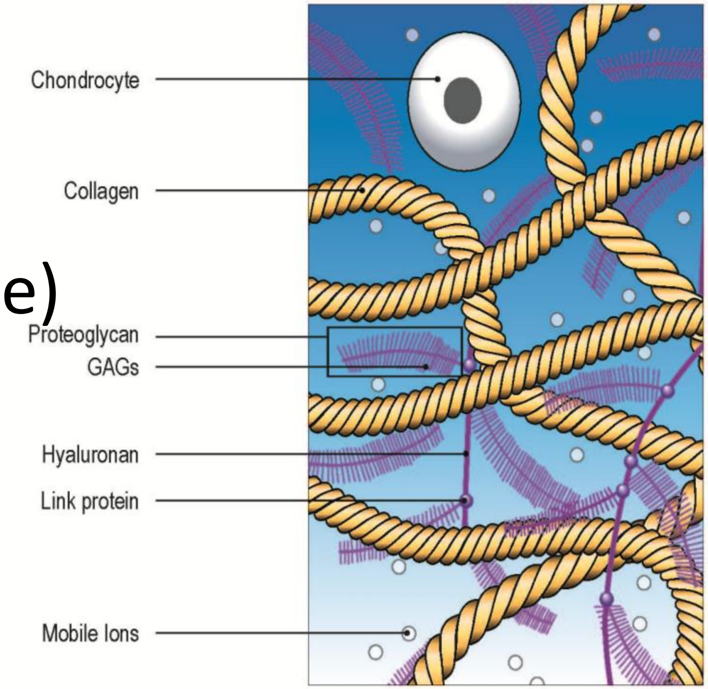
*5-bromo-2'-deoxyuridine
thymine analogue

Transit amplyfing cells = progenitor cells

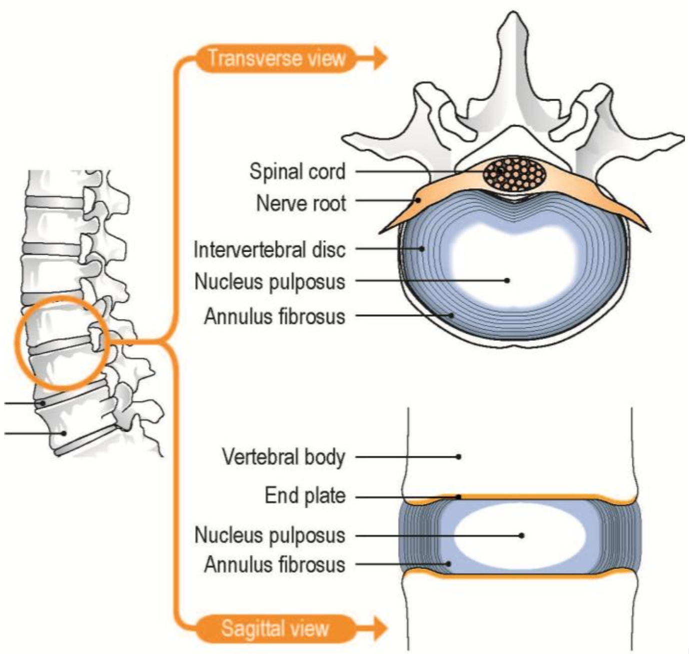
Label retaining /slow cycling cells

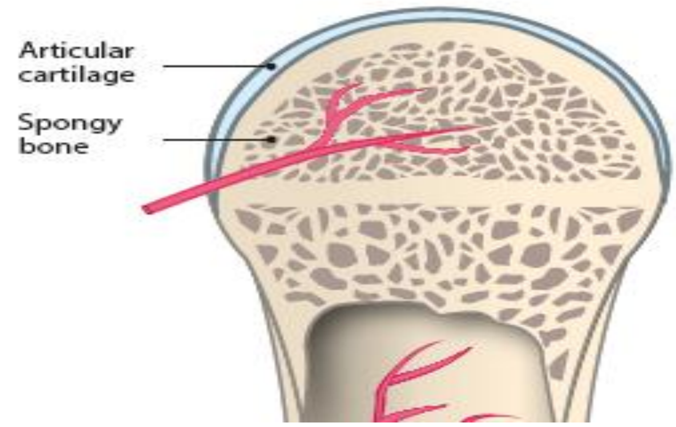
Cartilage

- fibrocartilage
- articular cartilage (hyaline cartilage)
- elastic cartilage

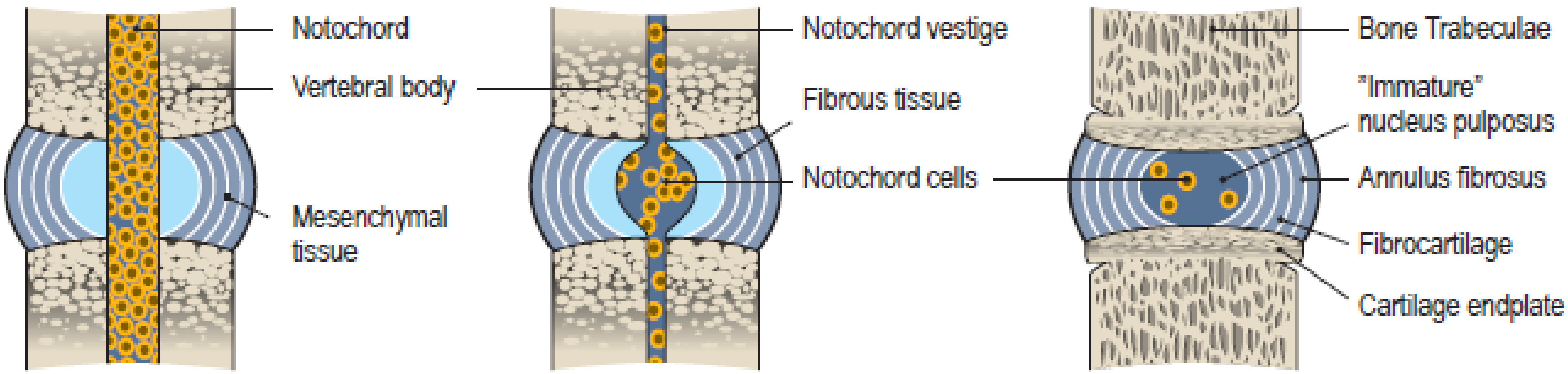


epiphyseal cartilage

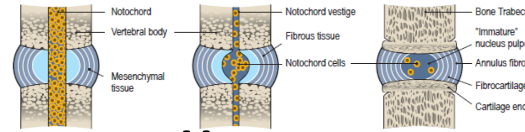




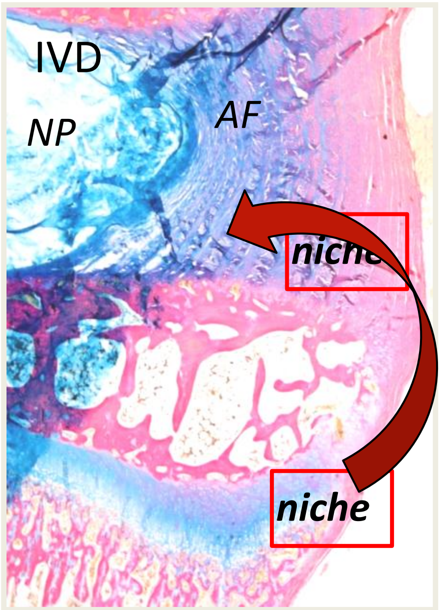
The intervertebral disc development
- cell migration

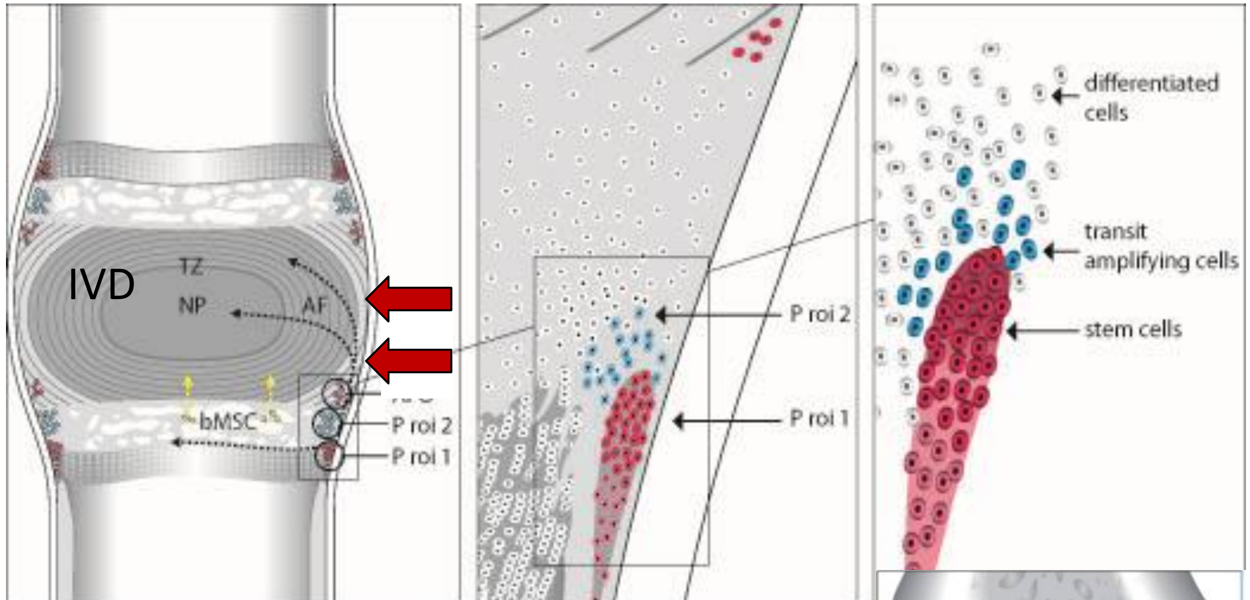


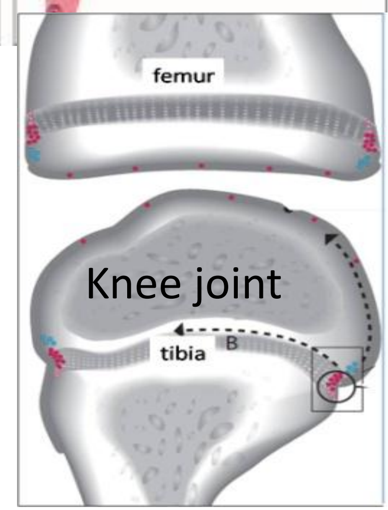
Local stem cells in cartilage?



Hypothetical migration route- progenitor cells



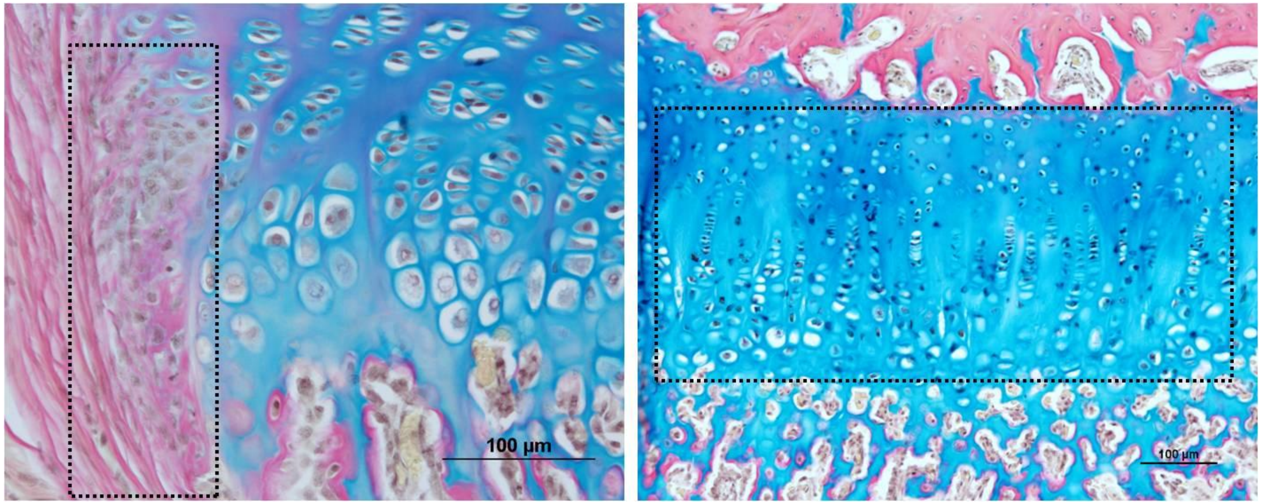




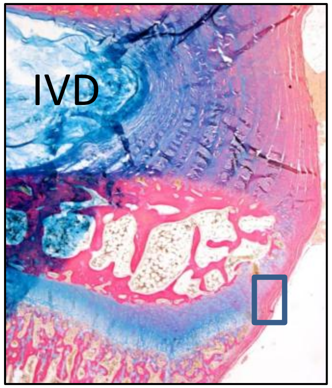
- ❑ label retaining cells (progenitor cells –niches)
- ❑ cell proliferation-AF and NP
- ❑ stem-/progenitor cells (STRO-1, Notch1, Jagged1)

1. Intervertebral disc: Henriksson H et al. Spine, 2009
2. Knee joint: Thornemo M et al. Journal of Anatomy, 2009
   and Dowthwaite G P et al, J Cell Sci, 2004

Stem cells niche/chondroprogenitors?

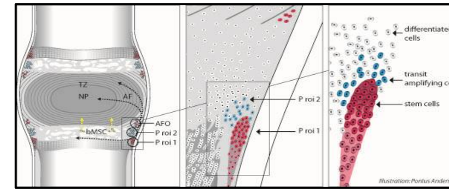


Images of the zone of Ranvier´s groove (knee joint) to the left with densely packed cells and the epihyseal plate with cells in characteristic columnar formation to the right, in a rabbit IVD, age 3 months. The locations are indicated by squares.

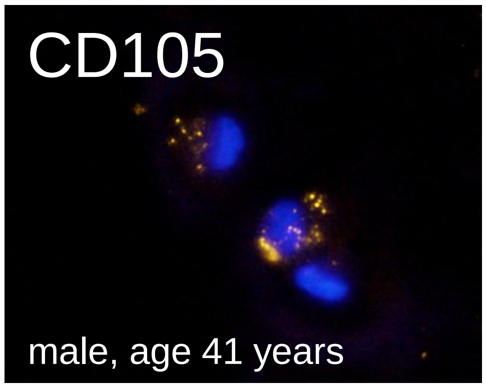


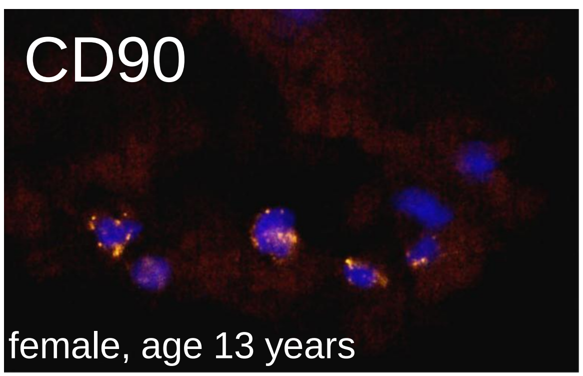
Ref: (thesis Barreto Henriksson H, 2010).

Local stem- and progenitor cells in human degenerated disc tissue



Can they be stimulated *in situ* with growth factors?





Images above from human degenerative intervertebral disc samples

Henriksson HB et. al. SPINE, 2009

In vitro/in vivo:

- IL-1, OP-1, TGF-β, FGF2, PDGF, β-FGF and IGF-I stimulation
- TIMP1 - transfected cells
- Stimulation by notochordal cells, chondrocytes, MSCs

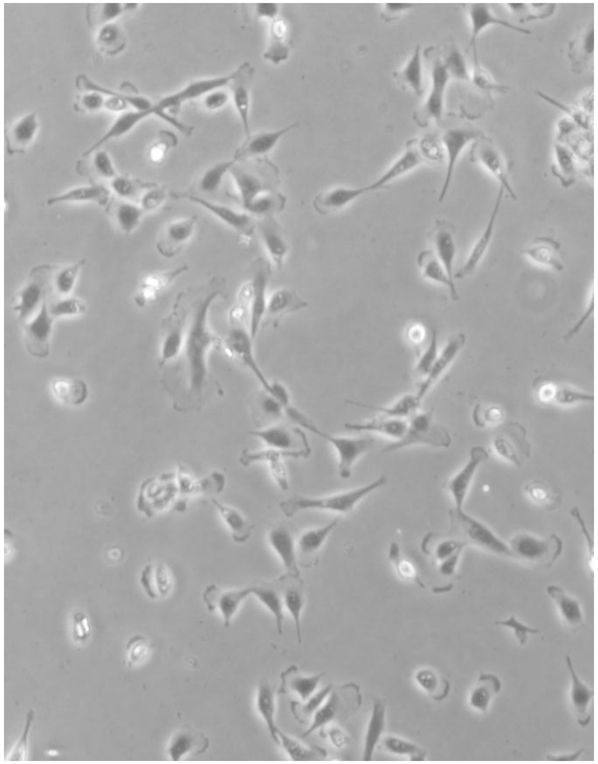
Clinical studies: **?**

Takegami et al Spine 2002, Masuda et al Spine 2006, Wei et al 2008, Pratsinis et al Eur Spine J. 2007, Li et al Arthritis Res Ther. 2008, Haberstroh et al Tissue Cell., 2009,Liu et al Chin Med J (Engl). 2011

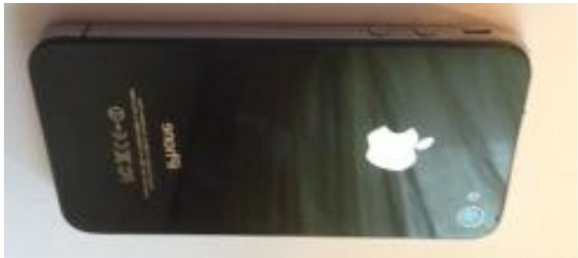
Can we influence stem cells *in vivo*?

Cells can react to different stimulus…

Nutrients
Pressure
Patogens
Light
Hormones
Pain
Tissue injury
Temperatur
Environment/surface structures



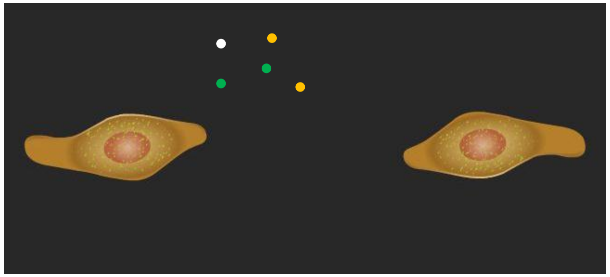
How do cells communicate?



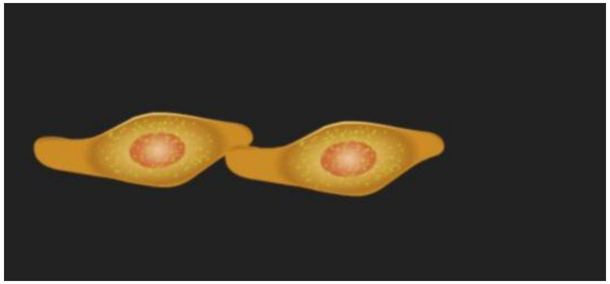
- Secretion of soluble molecules

-Extracellular signalling

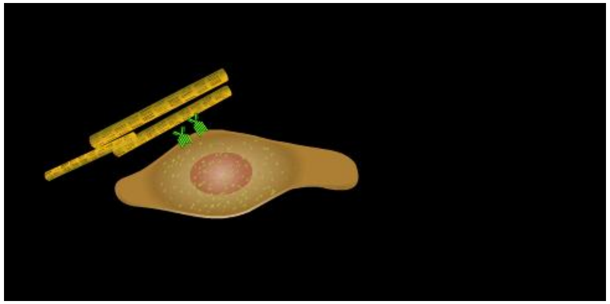
-Intracellular signalling



- Cell- to cell contact



- Extracellular matrix modification



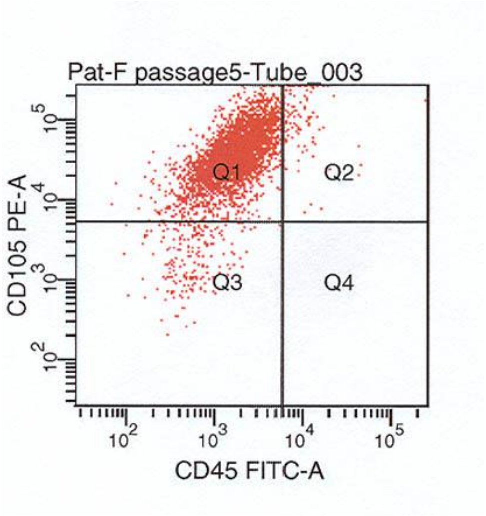
Part IIA
Xenotransplantation and cell tracing pre-clinical experiments

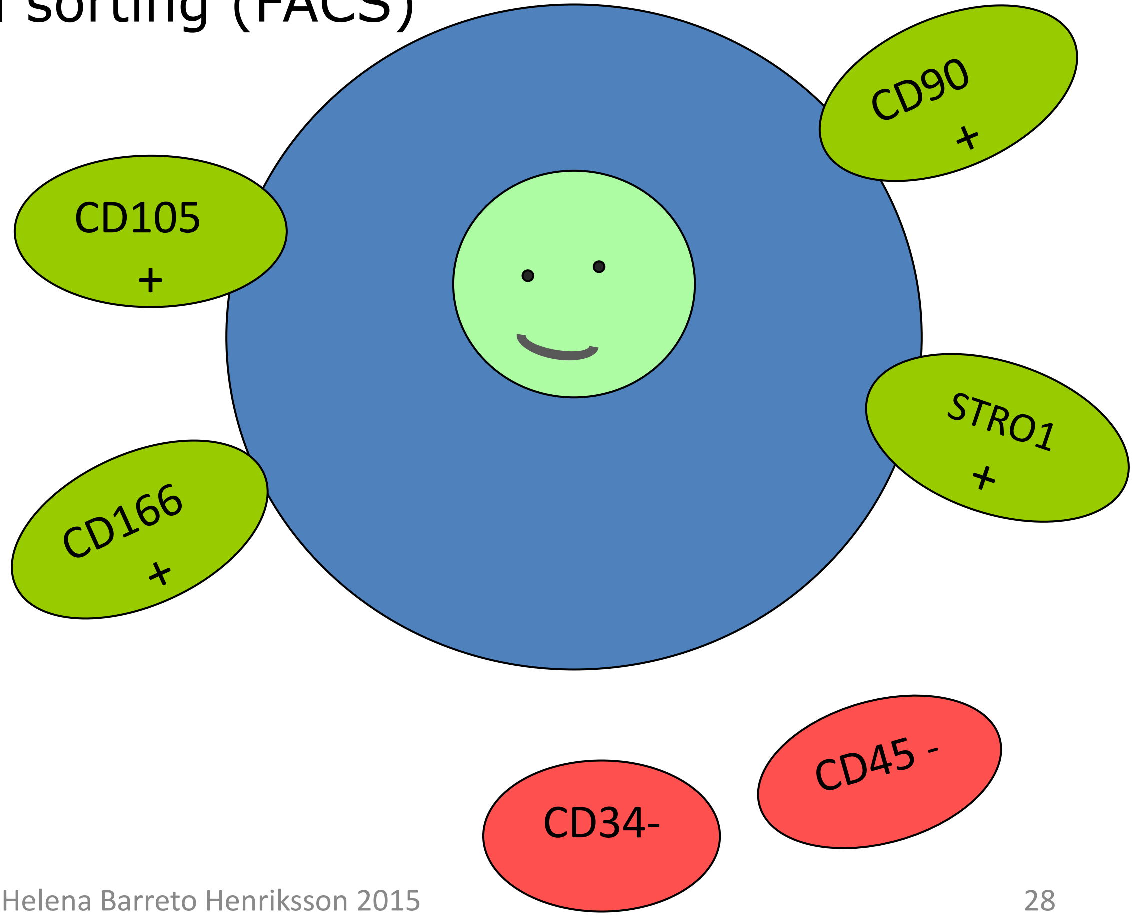
Criteria - cell tracer

- Non-toxic
- Maintain a detectable signal over a long time period
- No leakage from the cytoplasm
- Minimum of interference - cellular processes e.g differentiation

Prior to experiments the purity of the mesenchymal stem cell population needs to be tested

- after 3-5 passages in cell culture:
- control of cellular surface markers
- fluorocence activated cell sorting (FACS)

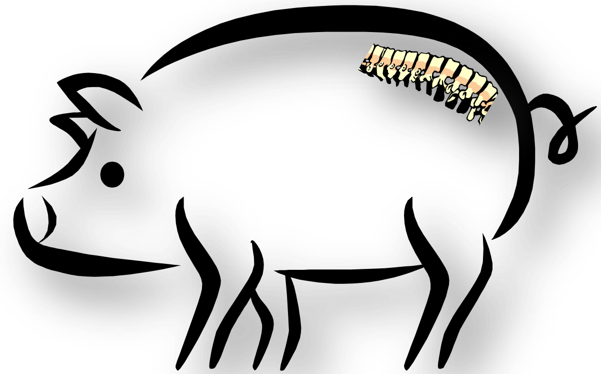


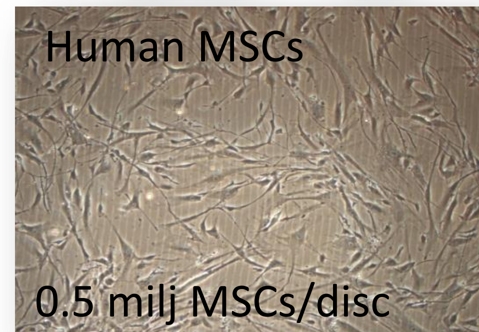


International society of cellular therapy (ISCT)

Xenotransplantation of human MSCs
Cell survival? Differentiation?

- injury model - intervertebral disc (L1-L5)
- after 2 weeks: transplantation of MSCs
  hydrogel or hyaluronan gel
- evaluation after 1, 3, 6 months
- cell viability (human transplanted cells)
- disc morphology
- function of cells





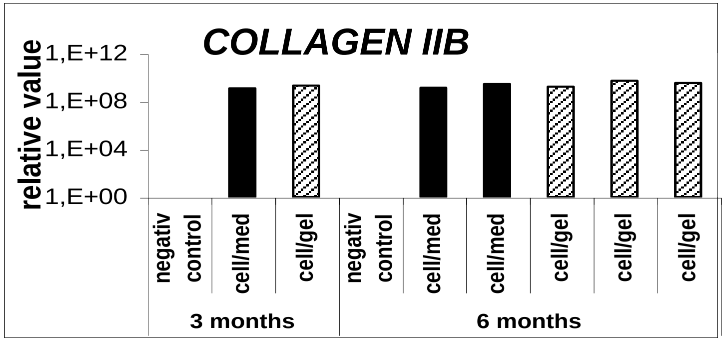
2 series of animal experiments
serie 1 n=10 hydrogel carrier
serie 2 n=10 hyaluronan carrier

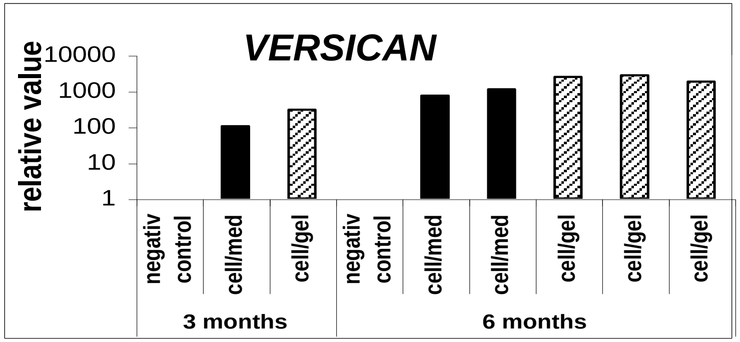
Henriksson HB et al, SPINE, 2009

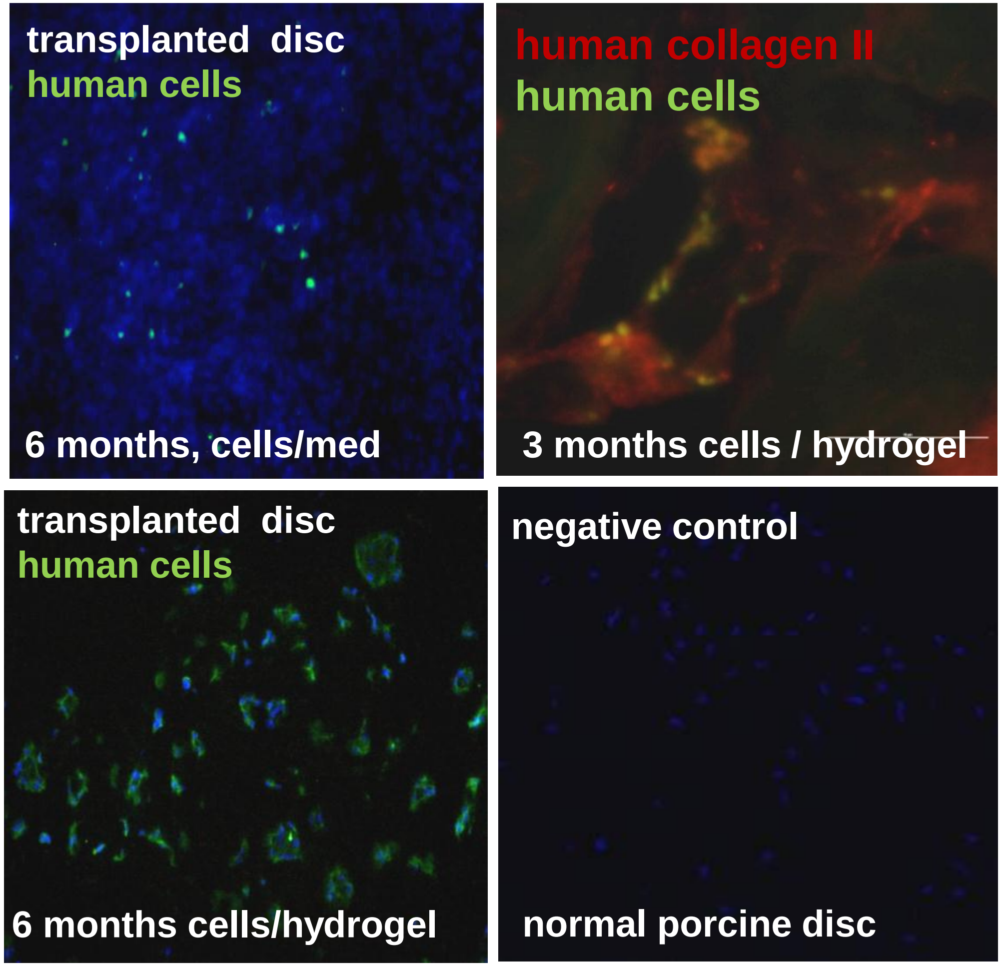
No immunosupplimental drugs were given to the animals

Functional and viable transplanted human cells detected in porcine discs

- human cells detected - all time points
- human collagen II
- chondrocyte - like cell type
- chondrogenic markers detected on gene - and protein level







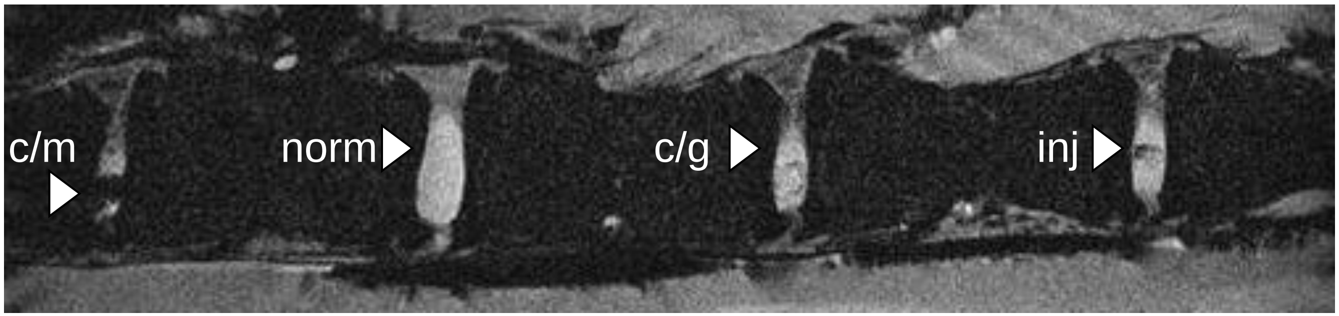
human cells (green)
collagen II (red)
nuclei (blue)

Henriksson et al, SPINE,2009

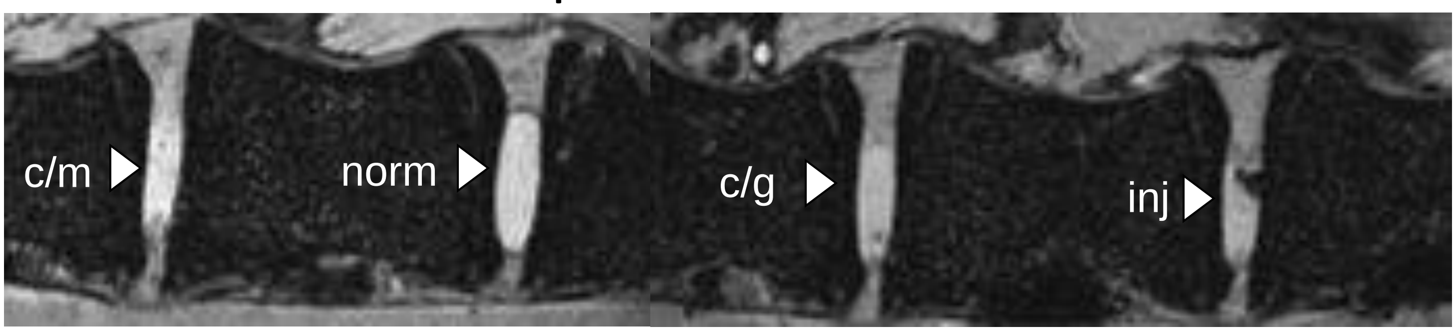
Hydrogel cell carrier -

positive disc appearance on MRI after stem cell transplantation

3 months after transplantation



6 months after transplantation



cells/ medium (c/m)

normal (norm)

cells/ hydrogel (c/g)

injured disc (inj)

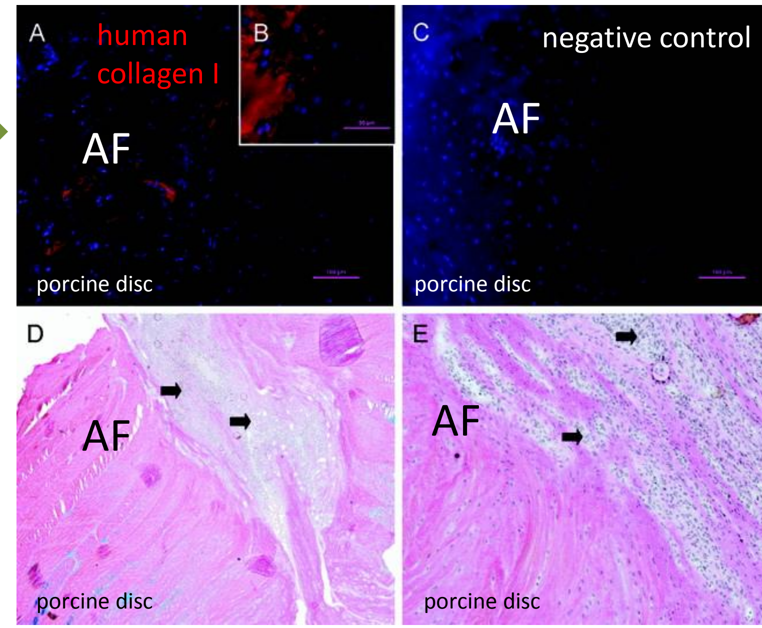
Henriksson et al, SPINE,2009

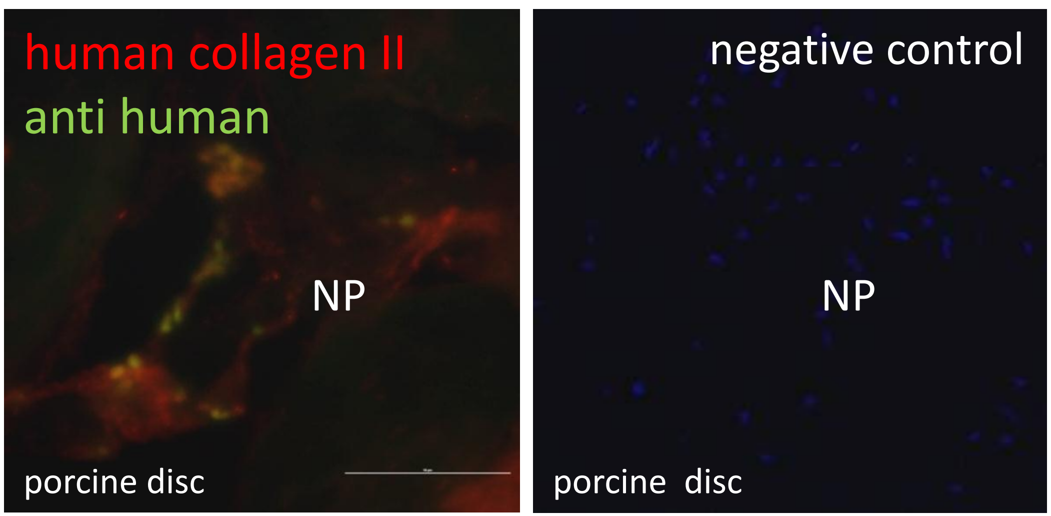
The transplanted MSCs can respond to local signals from the microenvironment..

- MSCs can differentiate into a chondrocyte - like cell type
- produce different types of extracellular matrix

Collagen II
NP

Collagen I
AF





NP = nucleus pulposus
AF = annulus fibrosus

Hyaluronan based cell carrier - variating degenerative signs observed in transplanted porcine discs on MRI

A= normal disc

B= injured untreated disc

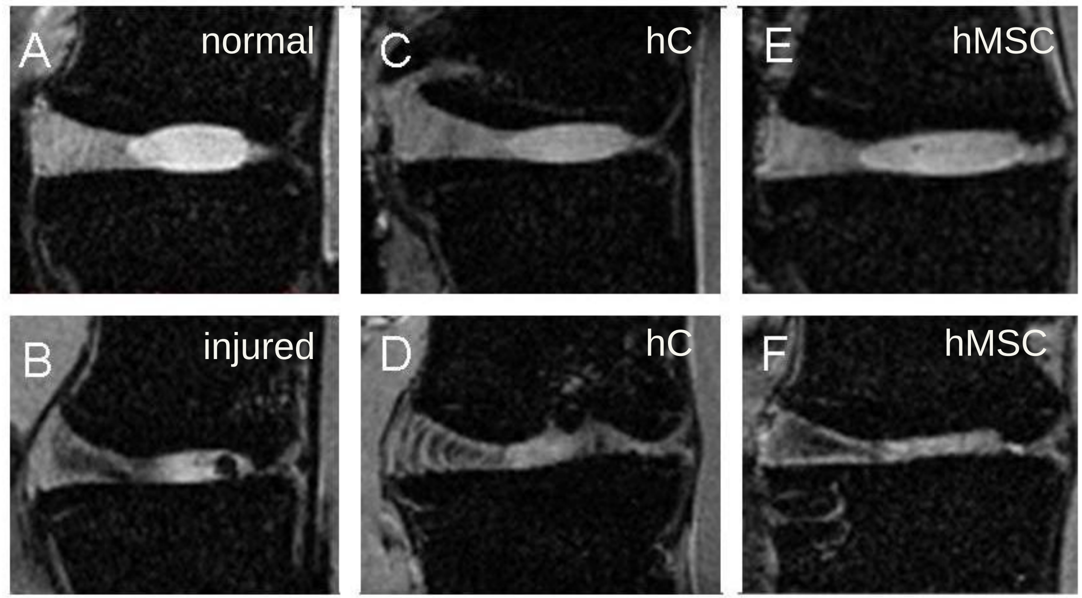
C= hCs /gel injected disc resembling the normal disc

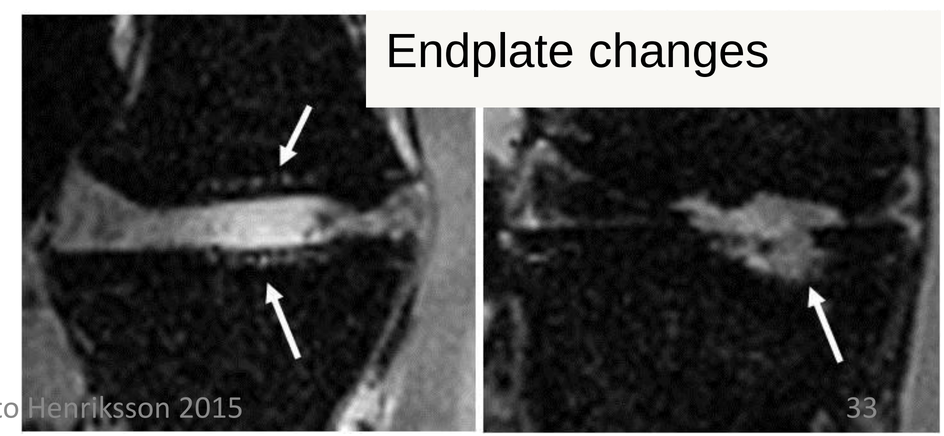
D= hCs/gel injected disc with signs of degeneration

E= hMSCs /gel injected disc resembling the native disc

F= hMSCs /gel injected disc with signs of degeneration

6 months after transplantation



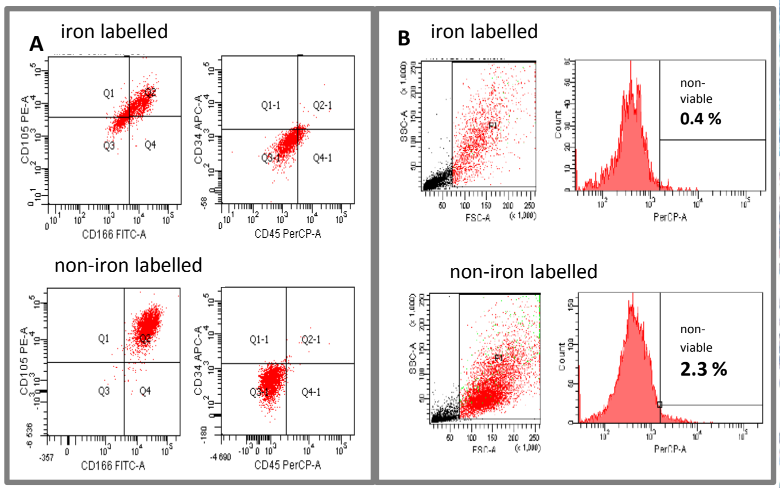


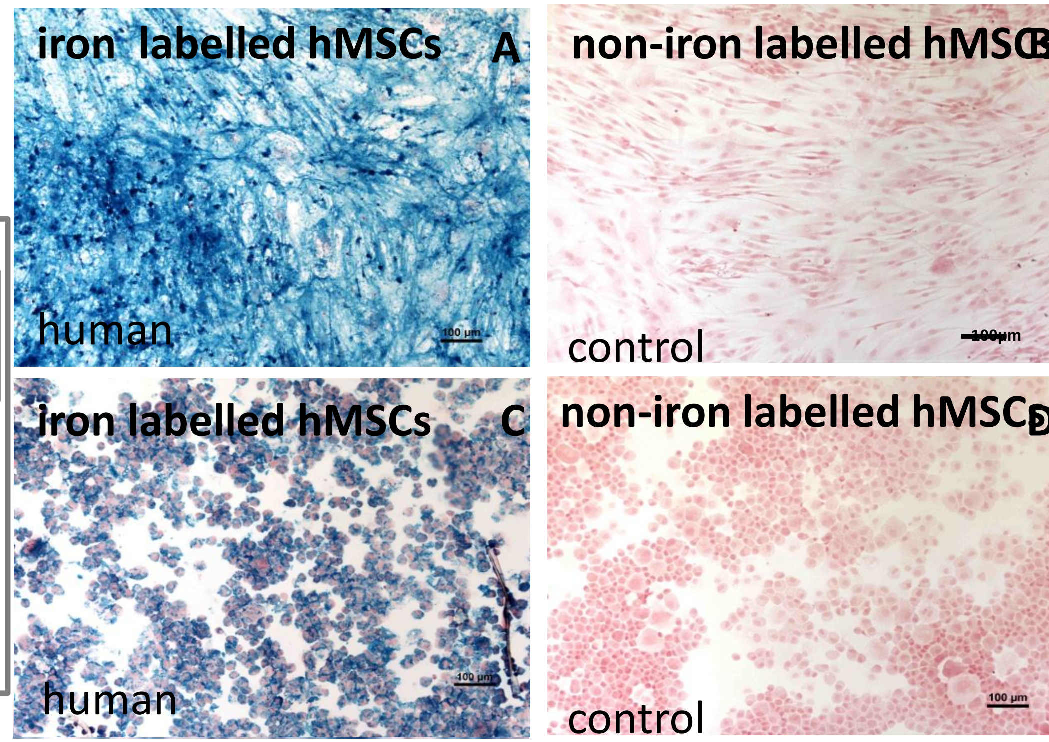
Cell tracking experiments using iron compounds as cell tracers

Iron sucrose labeling of human MSCs

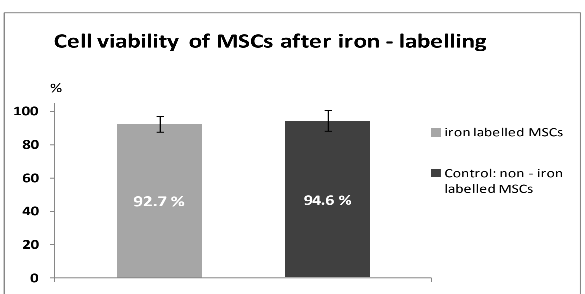
16 h

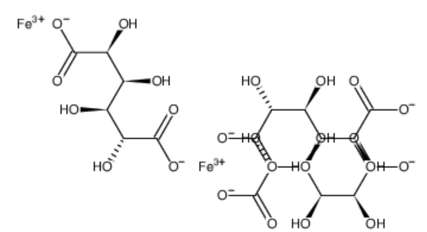
1 mg/ml iron sucrose





Staining: Preussian blue

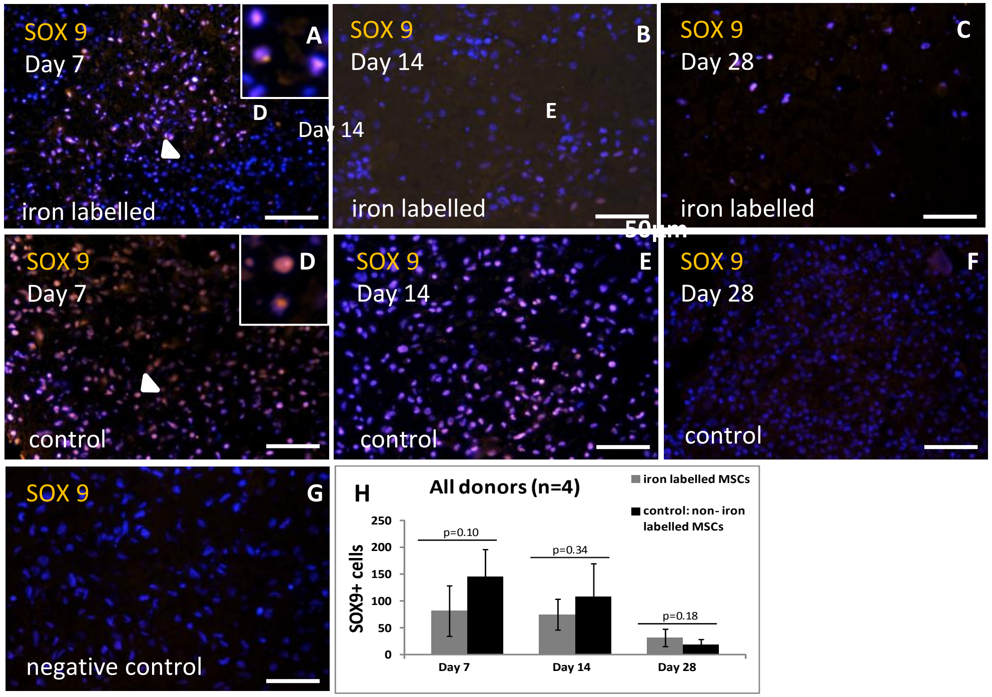


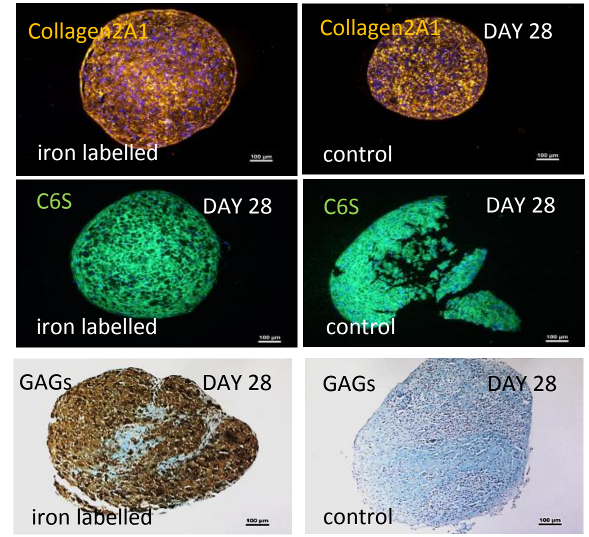


$[Na^{2}Fe^{5}O^{8}(OH)\cdot 3\cdot(H2O)]^{n})\cdot m(C^{12}H^{22}O^{11})$

Does the iron sucrose labeling affect the multilineage capability of human MSCs ?

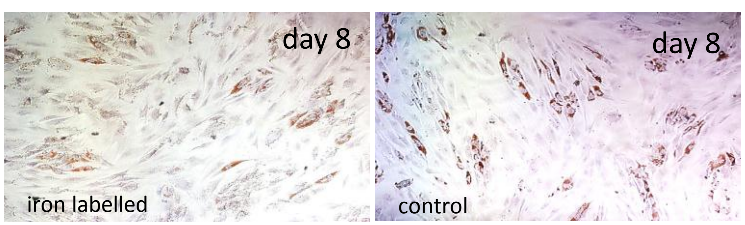
chondrogenic lineage





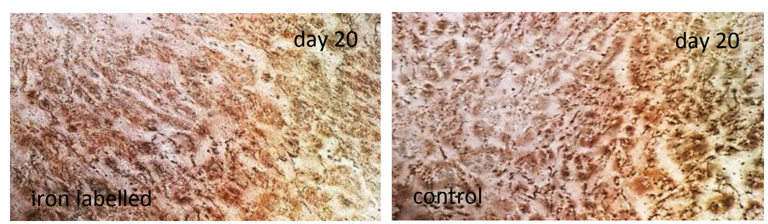
Staining: alcian blue van Gieson (lower row)

adipogenic linerage



Staining: Oil red

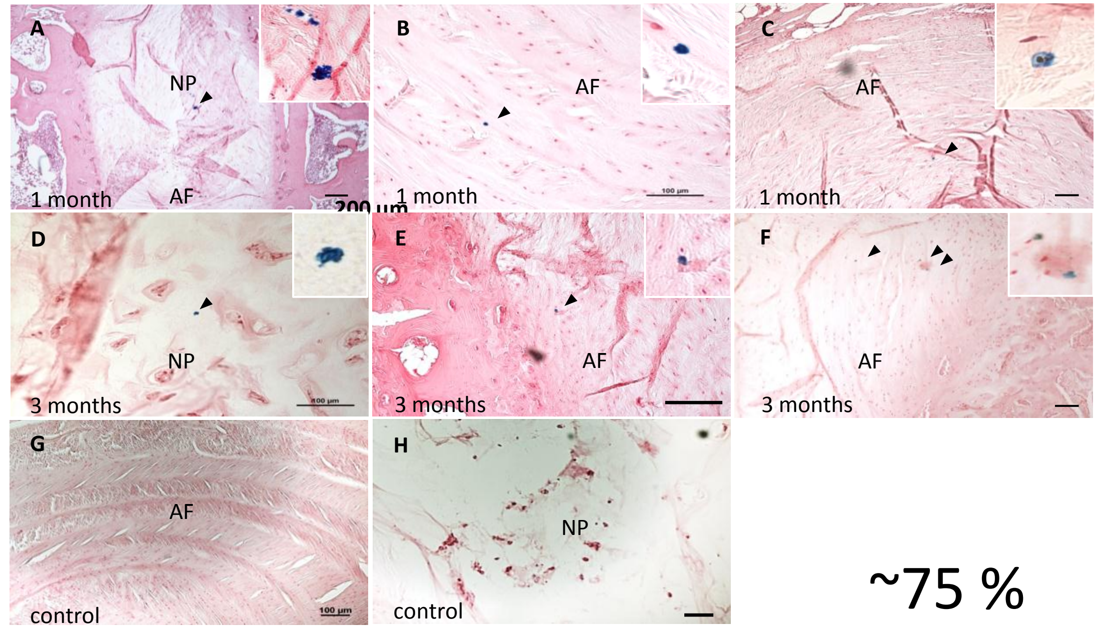
osteogenic lineage



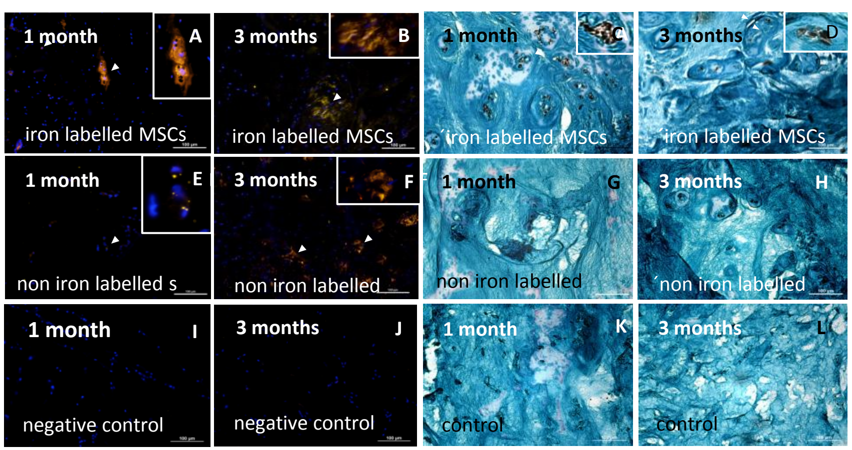
Staining: Von Kossa

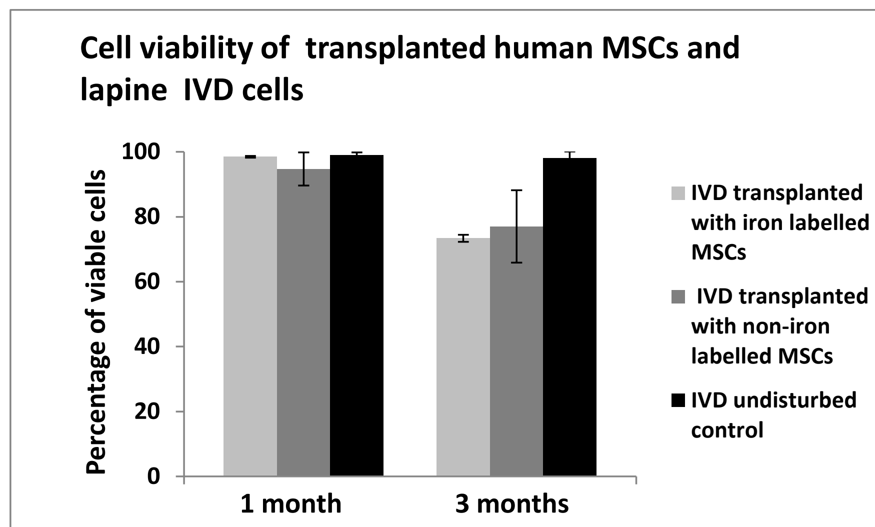
Xenotransplantation of human iron labeled MSCs into lapine IVDs

- **human MSCs injected in lapine IVDs**
- **IVDs collected at 1 and 3 months**
- **cells traced by IHC, histolgy**
- **cell viability, FACS**



~75 %

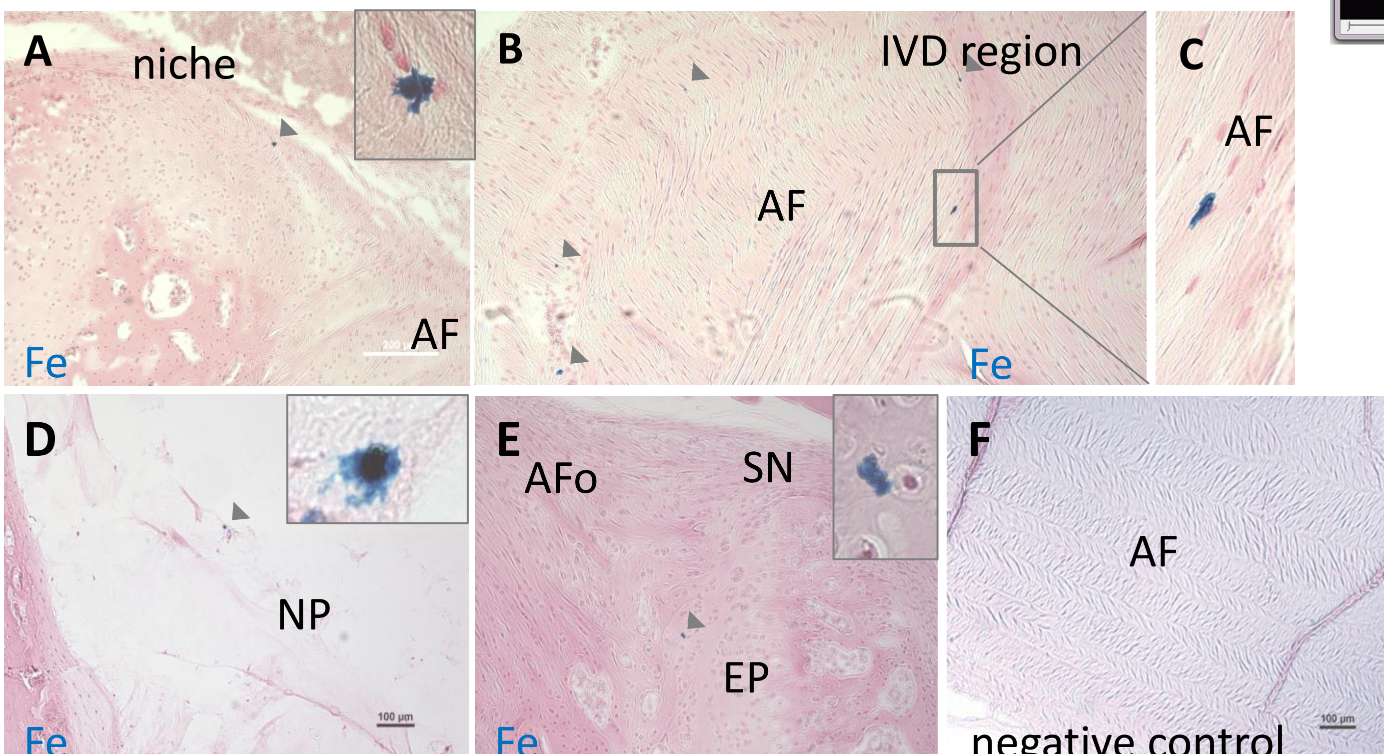




Studies of cellular migration/ distribution with cell tracer (SPIOs*) in the intervertebral disc region

- cells labeled *in situ* in niche area with iron- compund
- 6 weeks after injection, migrating iron- labeled cells were traced in intervertebral disc region tissue

Lapine model
n=12

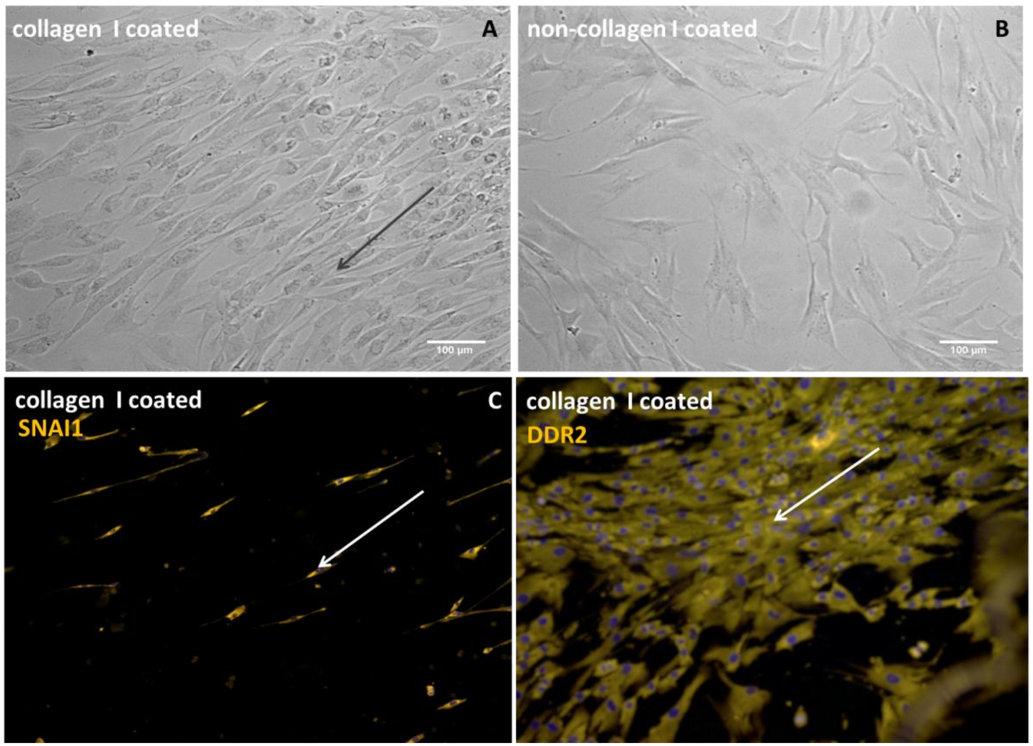


Staining: Preussian blue

* Endorem, superparamagnetic nanoparticles

Henriksson HB et.al.
Stem cell Research & Therapy, 2013

The arhitecture of biomaterials is important for cellular migration

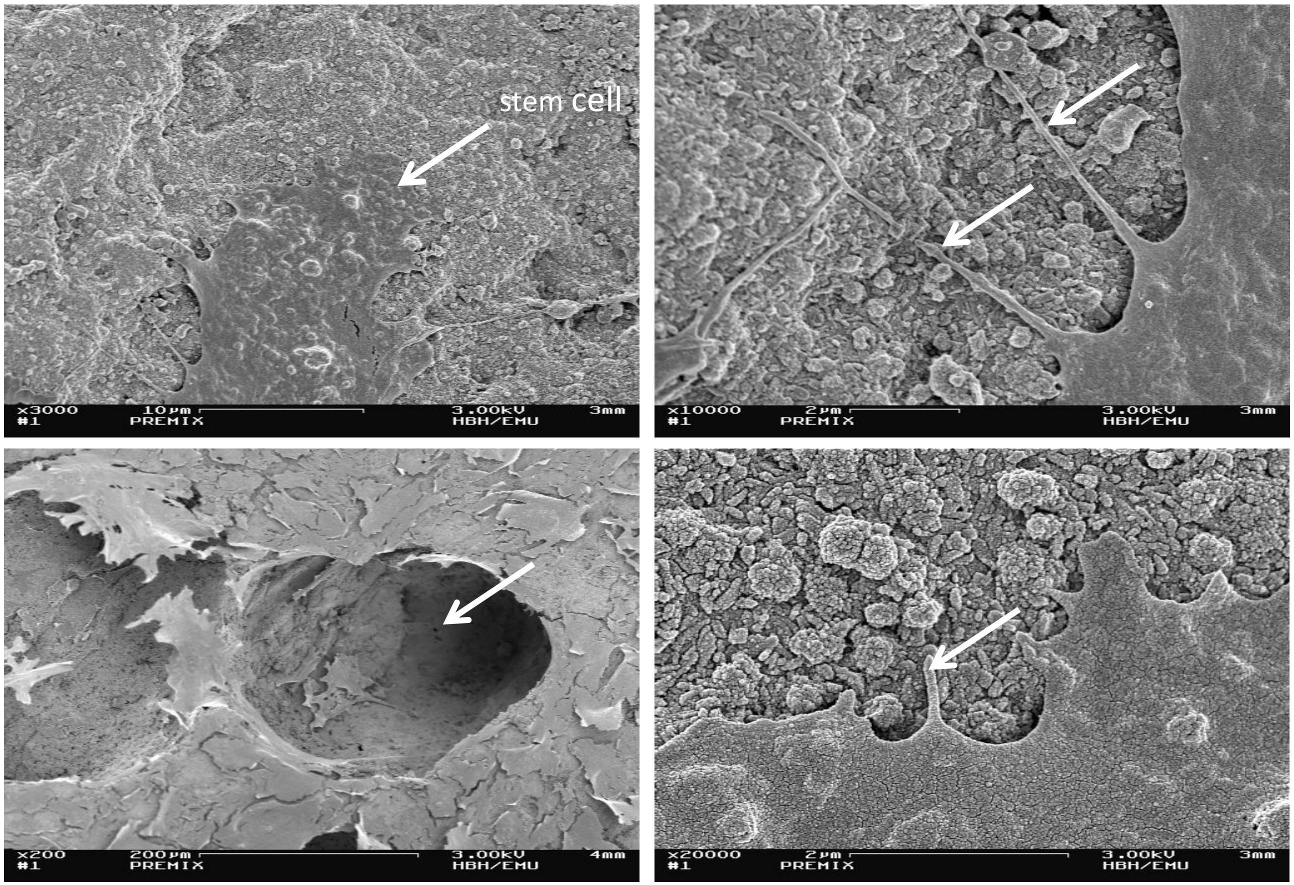


Supplementary material 1_.avi

Supplementary material 2_.avi

Cellular compability with biomaterials is important

adhesion | migration | pores



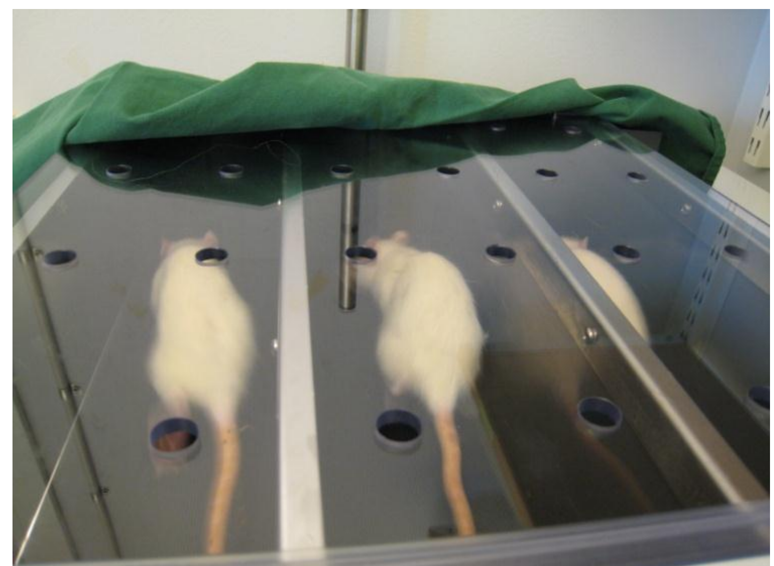
Aberg J et.al.
Journal of Nano and Biomaterials, 2011

Other potential biological tissue regeneration strategies

for IVD , knee joint, tendon, vascular grafts are under investigation

- gene therapy
  (Leckie S et.al. Spine J. 2012, Nishida K et.al. *SPINE*. 1999)
- scaffolds (cells or no cells, decellurasized)
  (Sakai et. al. Biomaterials. 2006, Whitlock PW et. al. J Surg Orthop Adv. 2013, Olausson M et. al. Lancet, 2012), Zhang H et. al. Biomaterials, 2013,
- marine biomaterials as scaffolds (Wysokowski M et al, Int J Biol Macromol, 2013, Li X et. al, Curr Med Chem. 2013)
- physical exercise
  (Sasaki N et. al., SPINE, 2012, Kiviranta et. al., 1992)
- 3D printing - scaffolds?

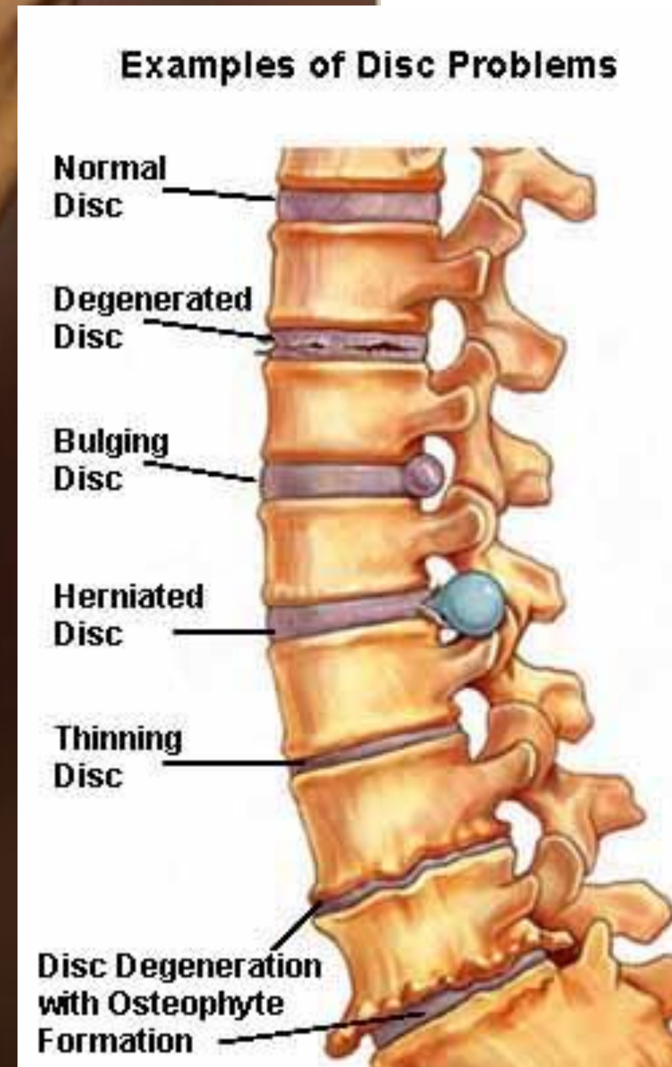
and more……..



Part IIb
Stem cell therapy for the human degenerated disc?

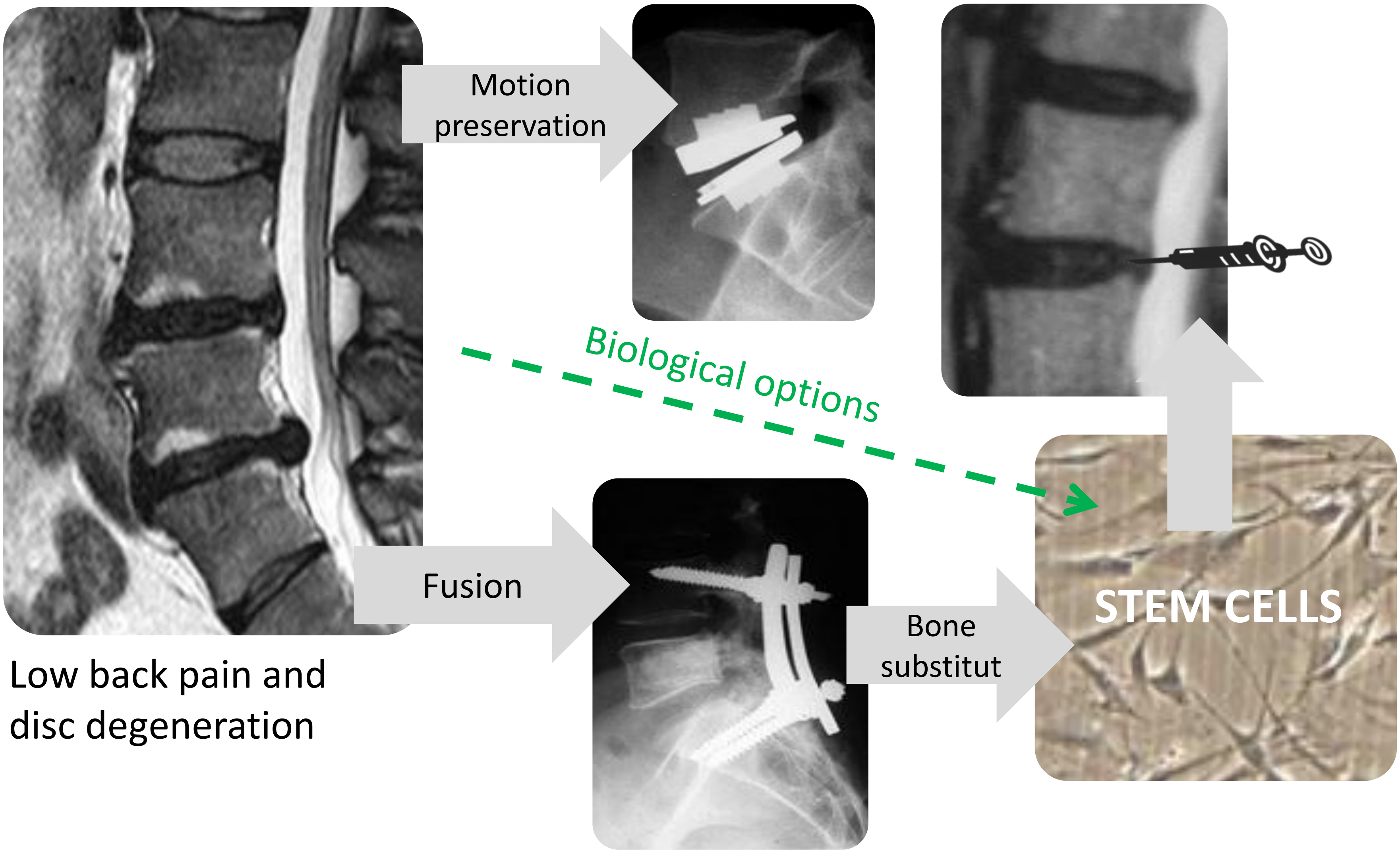
Low back pain

- common in the western world
- a large number of sick leave for chronic back pain/year
- costly for the society and painful for the individual



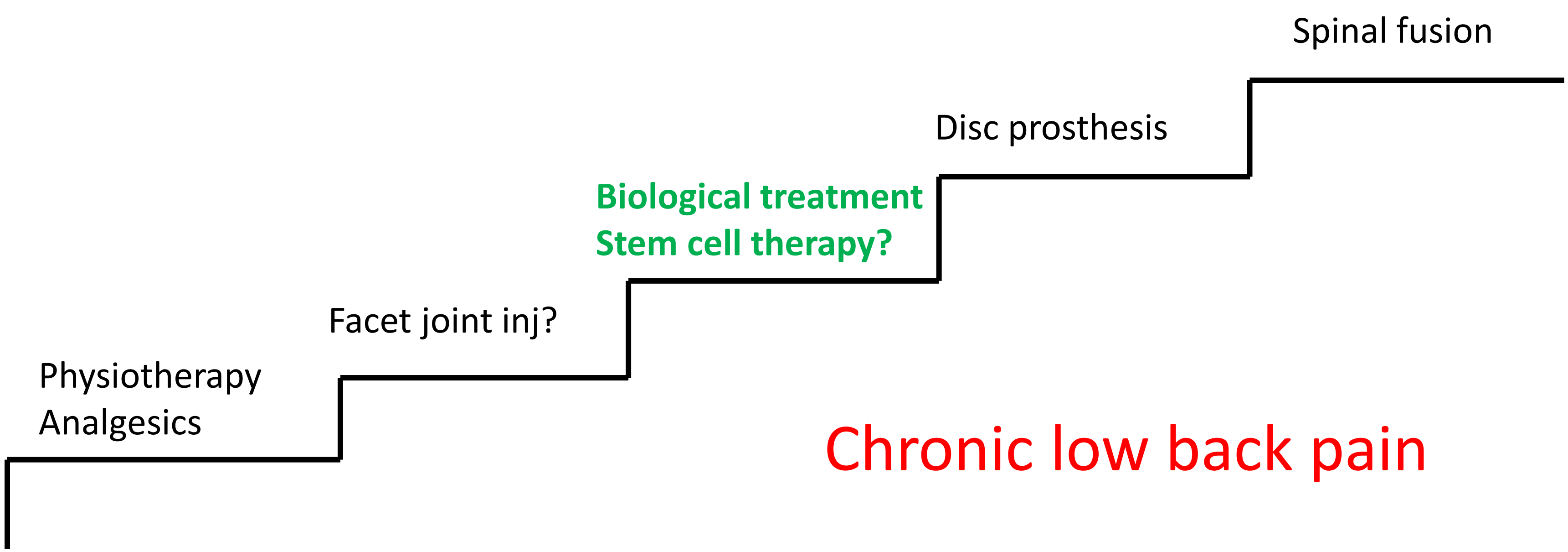
Right image: http://images.jupiterimages.com/common/detail/
left image http://www.augustaortho.com/images/spine

Options for treatment of degenerated discs?

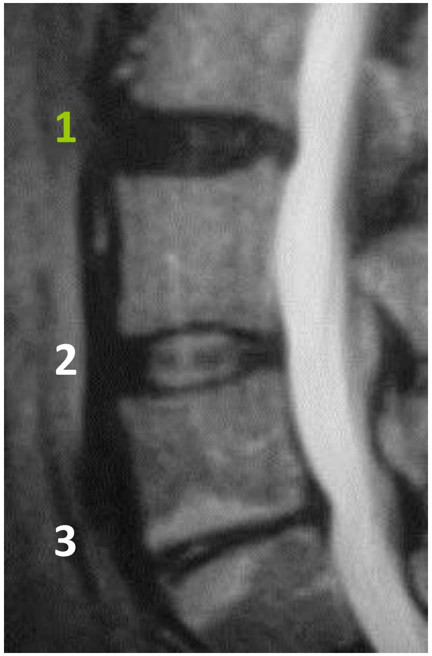


Regenerate the disc- a biological option?

- Biological repair
- Mini - invasive, small procedure
- Delay fusions, other implants
- Costs?



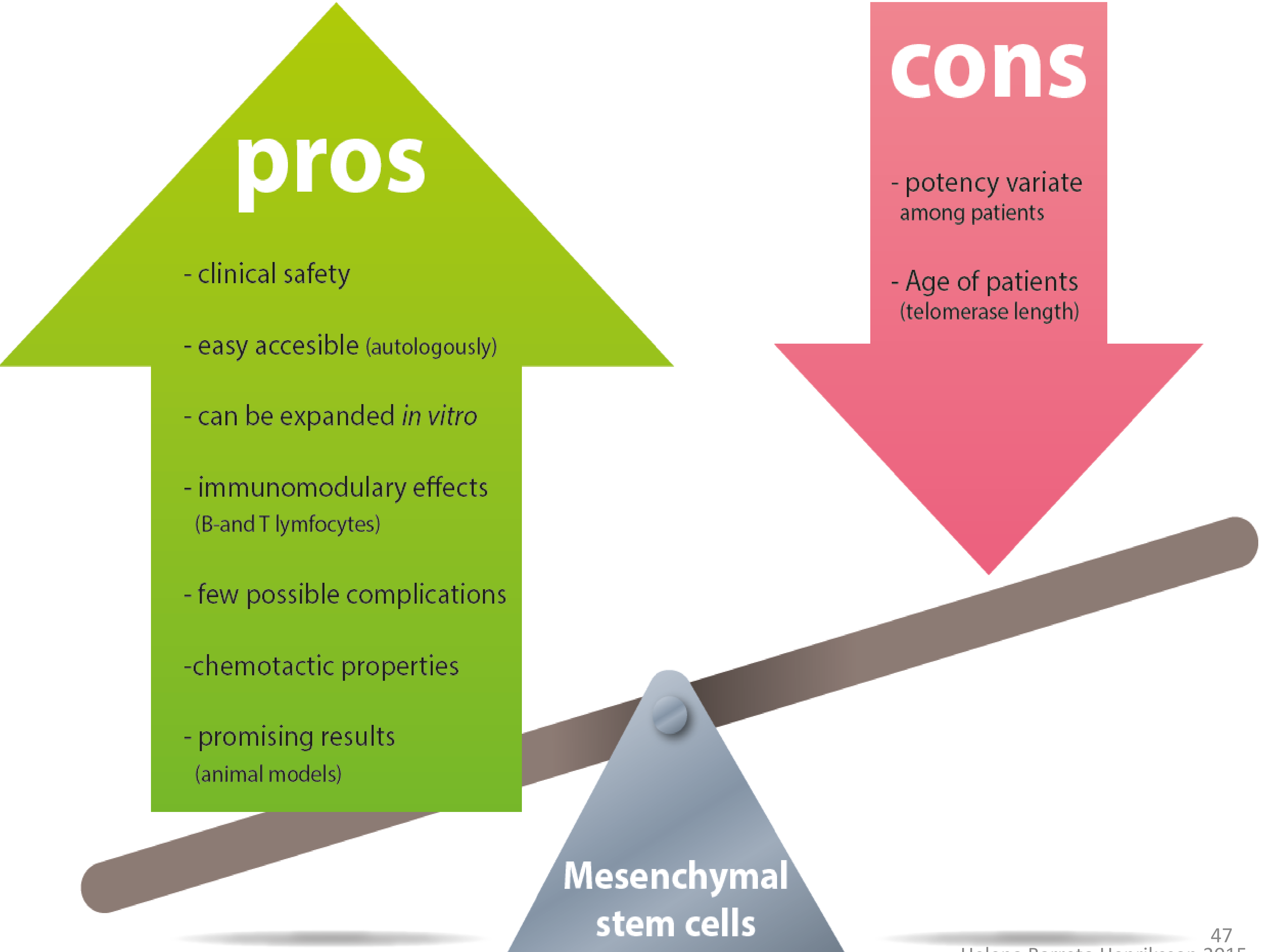
Expanded arsenal of treatment options for disc degeneration in the future?



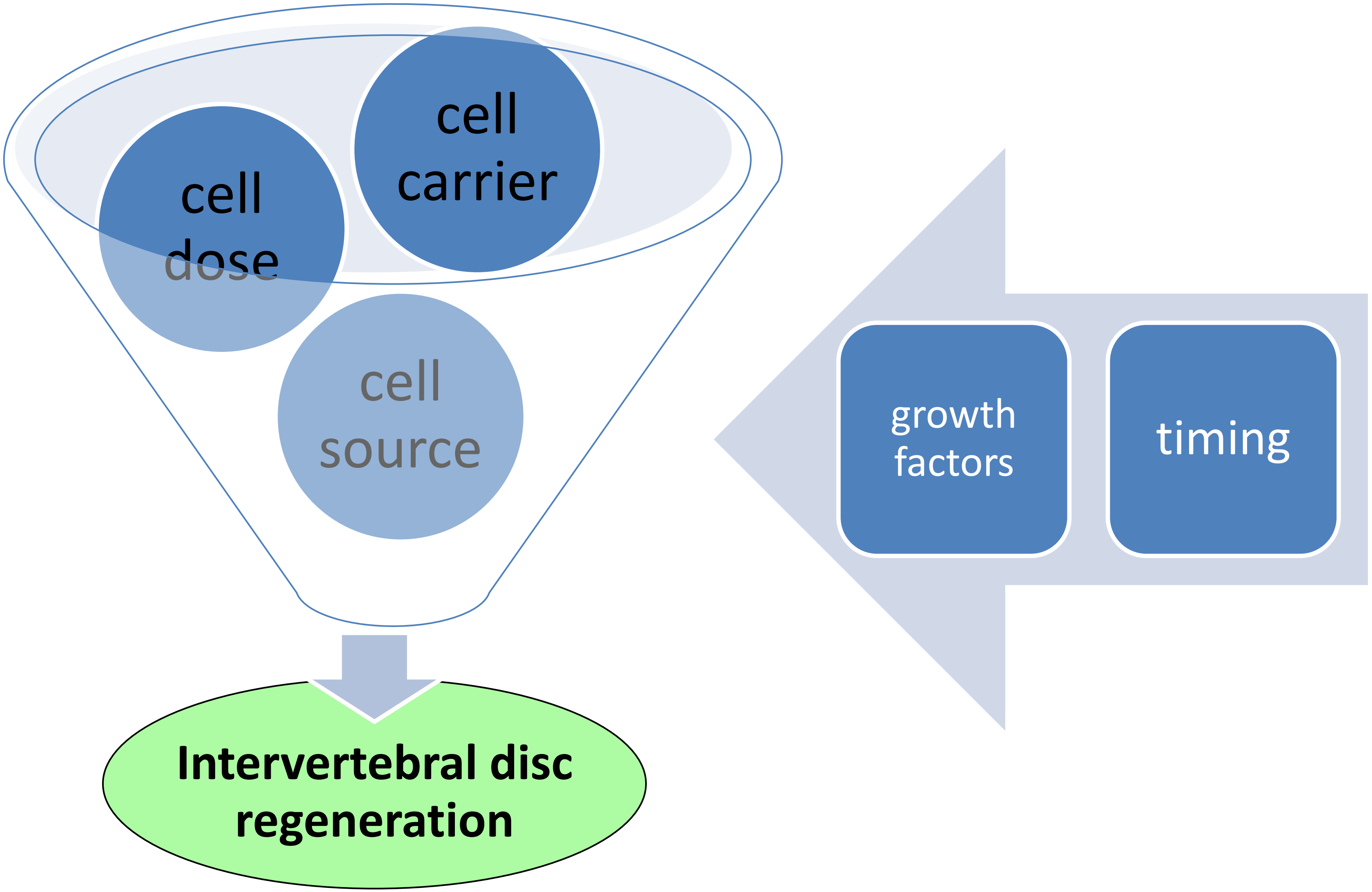
Three discs within the lower lumbar spine in a patient with severe back pain where both the L-34 and the L5-S1 are considered to be painful.

Possible future discussion if biological treatments options become available:

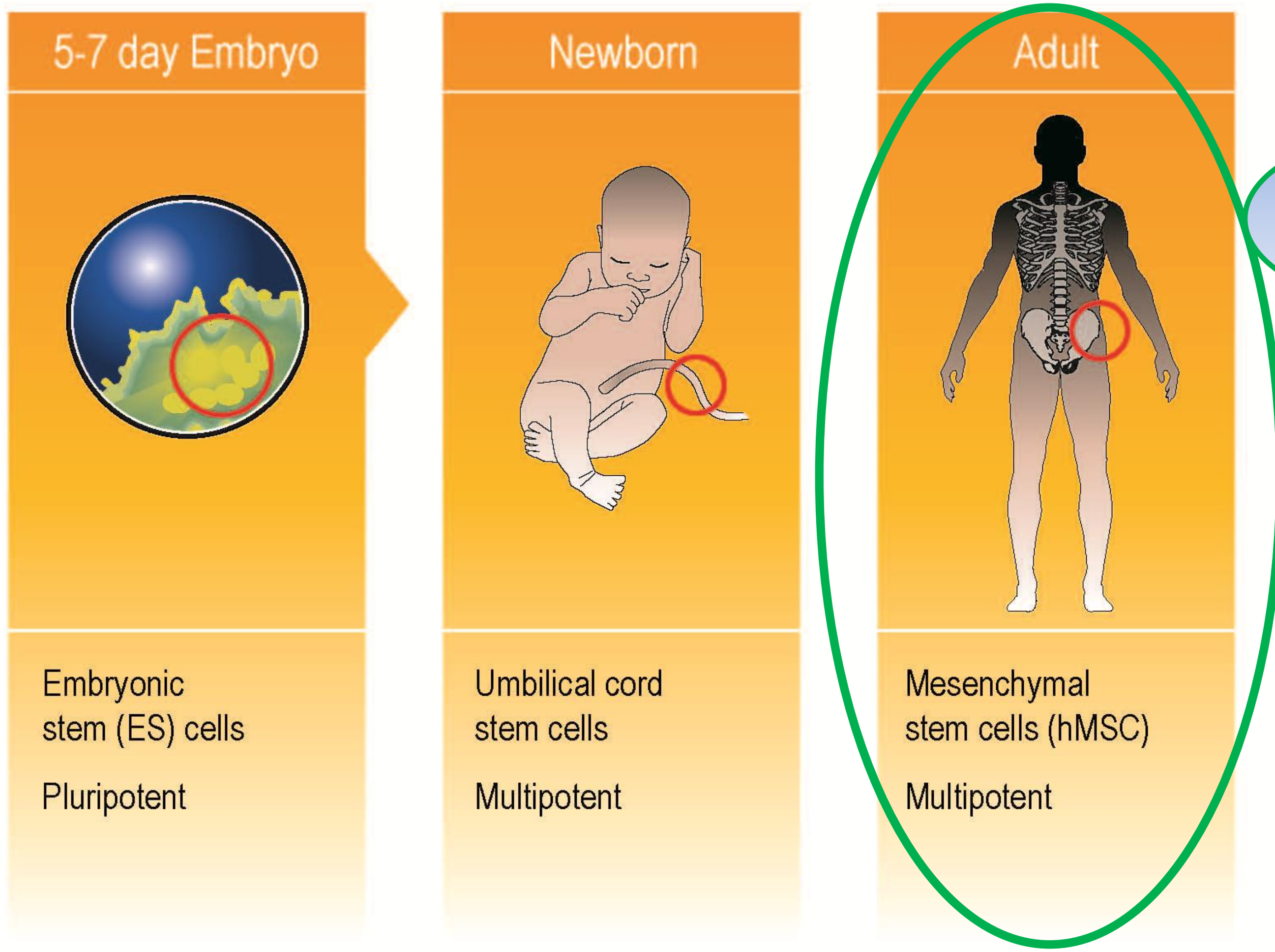
1. L3-4. Mild degree of degeneration, might be discussed for biological treatment options
2. L4-5. Normal or close to normal disc
3. L5-S1. Severe disc degeneration, not suitable for biological treatment options


pros
- clinical safety
- easy accesible (autologously)
- can be expanded *in vitro*
- immunomodulary effects
(B-and T lymfocytes)
- few possible complications
-chemotactic properties
- promising results
(animal models)
cons
- potency variate
among patients
- Age of patients
(telomerase length)
Mesenchymal
stem cells

Key factors- stem cell transplantation



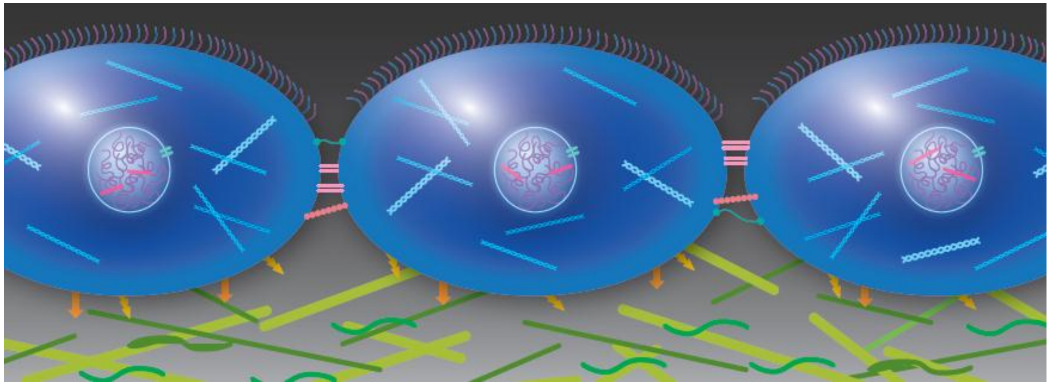
Possible cell sources- stem cell transplantation



SAFEST ALTERNATIVE

AUTOLOGOUS
ALLOGENIC

Challenges for stem cell therapy research

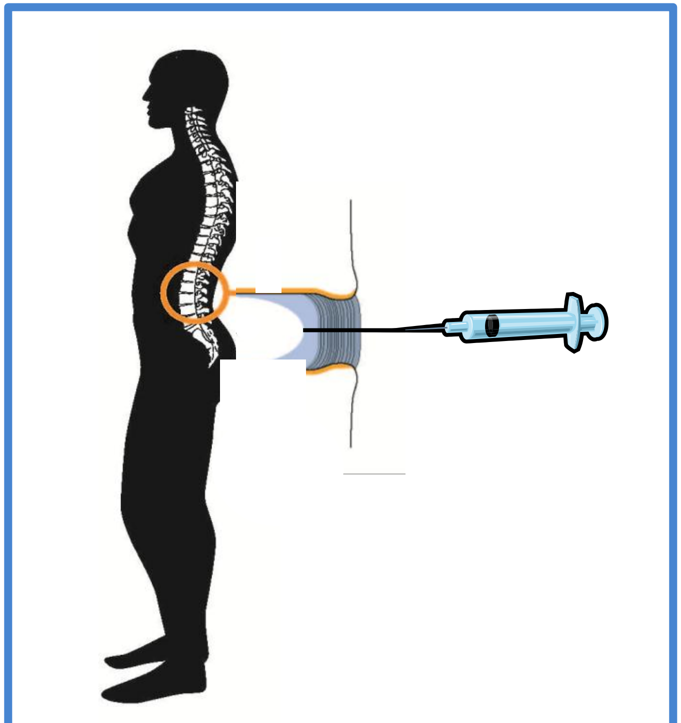


- Cell survival?
- Nutrition?
- Why should a transplantated cell population do better than the local original cells?
- Distribution of the transplanted cells? Cell migration?
- Cell signaling?

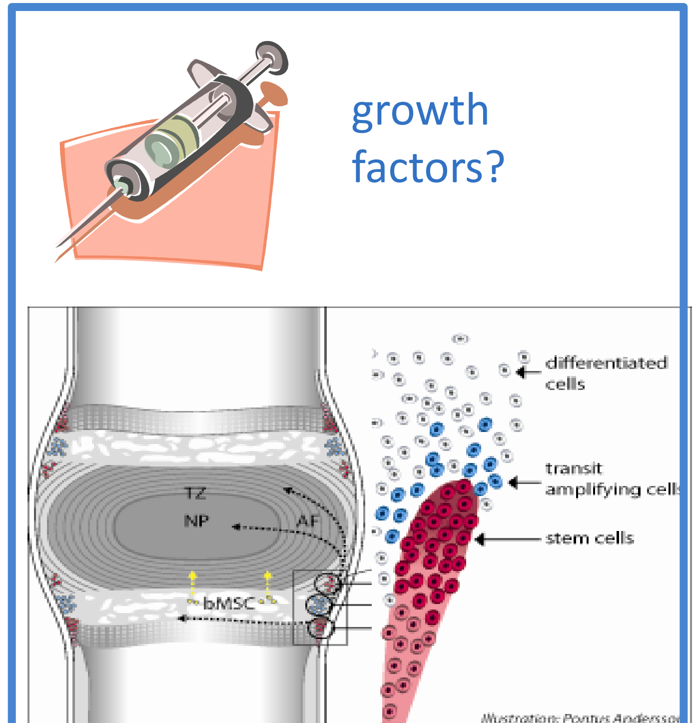
Potential future clinical relevance

Biological treatment options for degenerated discs?

Transplantation of autologous mesenchymal stem cells



Stimulation of local stem -/progenitor cells



Thank you for Your attention!

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