

Risk Assessment of Cell Doping in Sports

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OUTLINE

- Stem Cell Biology Overview
- Cell Therapy for Tendon
- Cell Therapy for Muscle
- Clinical Trials
- Safety Issues
- Potential for Doping

Stem Cells Biology

- Function
- Source

Function

Function	Tissue Generated	Examples
Totipotent	TE + germ layers	Fertilized egg
Pluripotent	All germ layers	Embryonic stem cell
Multipotent	Single germ layer	Mesenchymal stem cell
Unipotent	Committed Progenitor	Osteoblast, Myoblast
Cancer Stem Cell	Tumor initiating	AML, Breast CA, ...

Source

- Embryonic stem cells
- Adult stem cells
- Gestational
- Induced pluripotent cells

Embryonic Stem Cells

- Inner cell mass
- Pluripotent
- Ethical issues

Adult Stem Cells

- Mesenchymal stem cells
- Tissue specific stem cells
 - Neuronal
 - Cardiac
 - Skeletal Muscle
- Perivascular stem cells
- Gestational

Mesenchymal Stem Cells

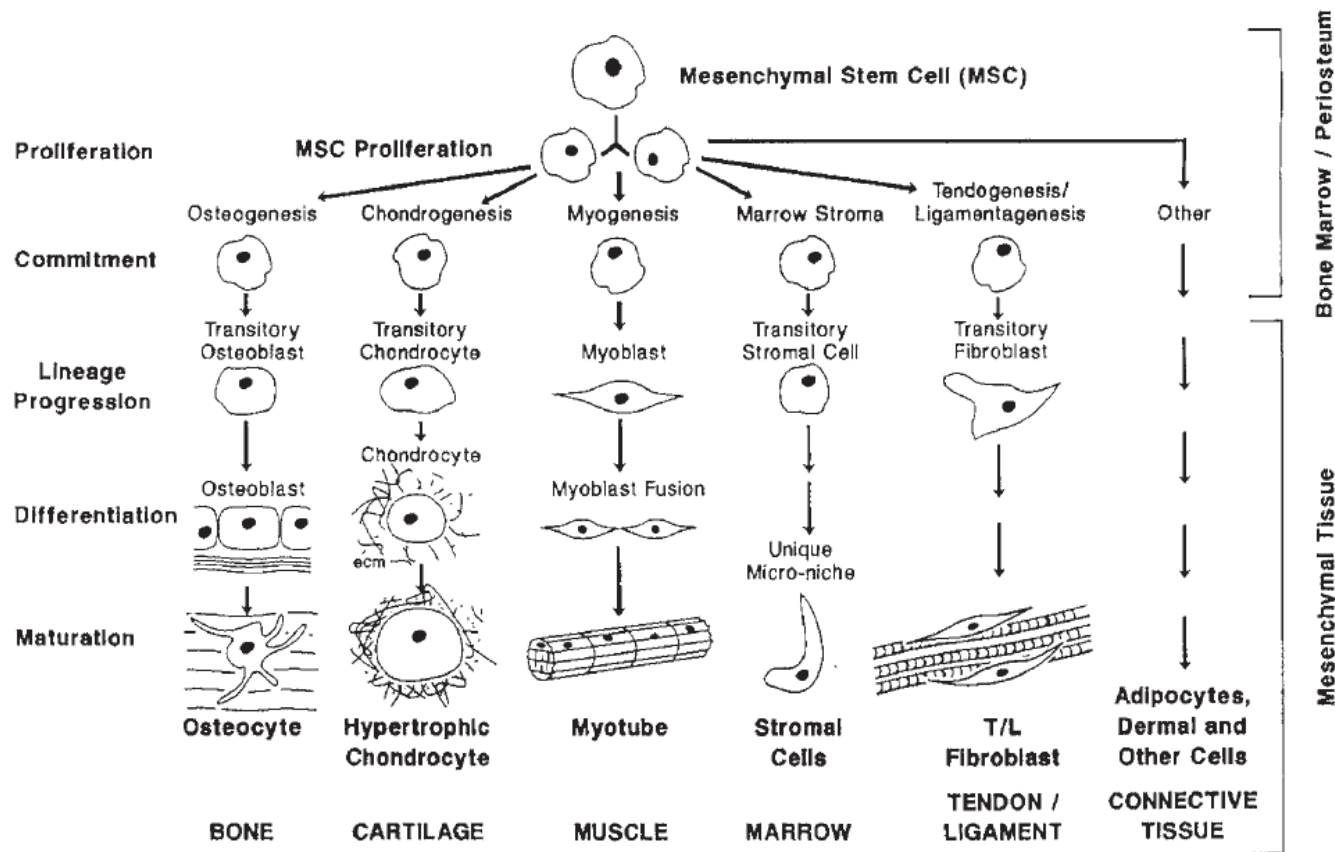


FIG. 1. The mesengenic process. Mesenchymal stem cells (MSCs) have the capacity to differentiate into bone, cartilage, muscle, marrow stroma, tendon/ligament, fat, and other connective tissues.^{1,2} The sequence of this differentiation involves multistep lineages controlled by growth factors and cytokines. This figure is structured in a manner comparable to hematopoietic lineage progression and involves well-described lineages for osteogenic differentiation³⁸ with decreasing information available from left to right.

Source

- Bone marrow
- Blood
- Umbilical cord
- Synovium
- Adipose tissue
- Lung
- Fetal liver
- Dental pulp & deciduous teeth
- ...

Friedenstein, J Embryol Exp Morphol, 1966

Mesenchymal Stem Cells

- Self Renewal
- Differentiation Potential
 - Bone
 - Cartilage
 - Fat
 - Muscle
 - Neuron
 - Hepatocyte

Friedenstein, J Embryol Exp Morphol, 1966

Mesenchymal Stem Cells

- Immunomodulation
 - No MHC class II antigens
 - Disrupt NK, CD8+, CD4+, dendritic cell function
 - Anti-inflammatory
 - IL-10
 - Prostaglandins

Ryan JM, J Inflamm 2005

Function

- MSC
 - VEGF, bFGF, HGF, IGF-1, TGF- β ,...
 - IL-6, IL-10, TNF- α , PGE
 - Angiogenesis
 - Inhibit apoptosis & inflammation
 - Immunoregulation

Mesenchymal Stem Cells

- Paracrine tissue regeneration
 - Cell survival
 - Cell proliferation
 - Angiogenesis
- Osteogenesis Imperfecta
 - Allogeneic MSC
 - Clinical improvement
 - MSC in vivo <2%

Horwitz EM, PNAS 2002

Applications

- MSC
 - Systemic/Local - homing
 - Vehicle for gene therapy
 - Osteogenesis imperfecta
 - Breast CA (Interferon β)
 - Glioma (TNF α - Apoptosis)

Tissue Specific Stem Cells

- Neuronal stem cells
 - Neurons, astrocytes, oligodendrocytes
- Cardiac stem cells
- Muscle stem cells
- Perivascular stem cells

Gestational

- Placental
- Cord blood
- Wharton's jelly
- Amniotic fluid
- Amnion, chorion

Induced Pluripotent Stem Cells

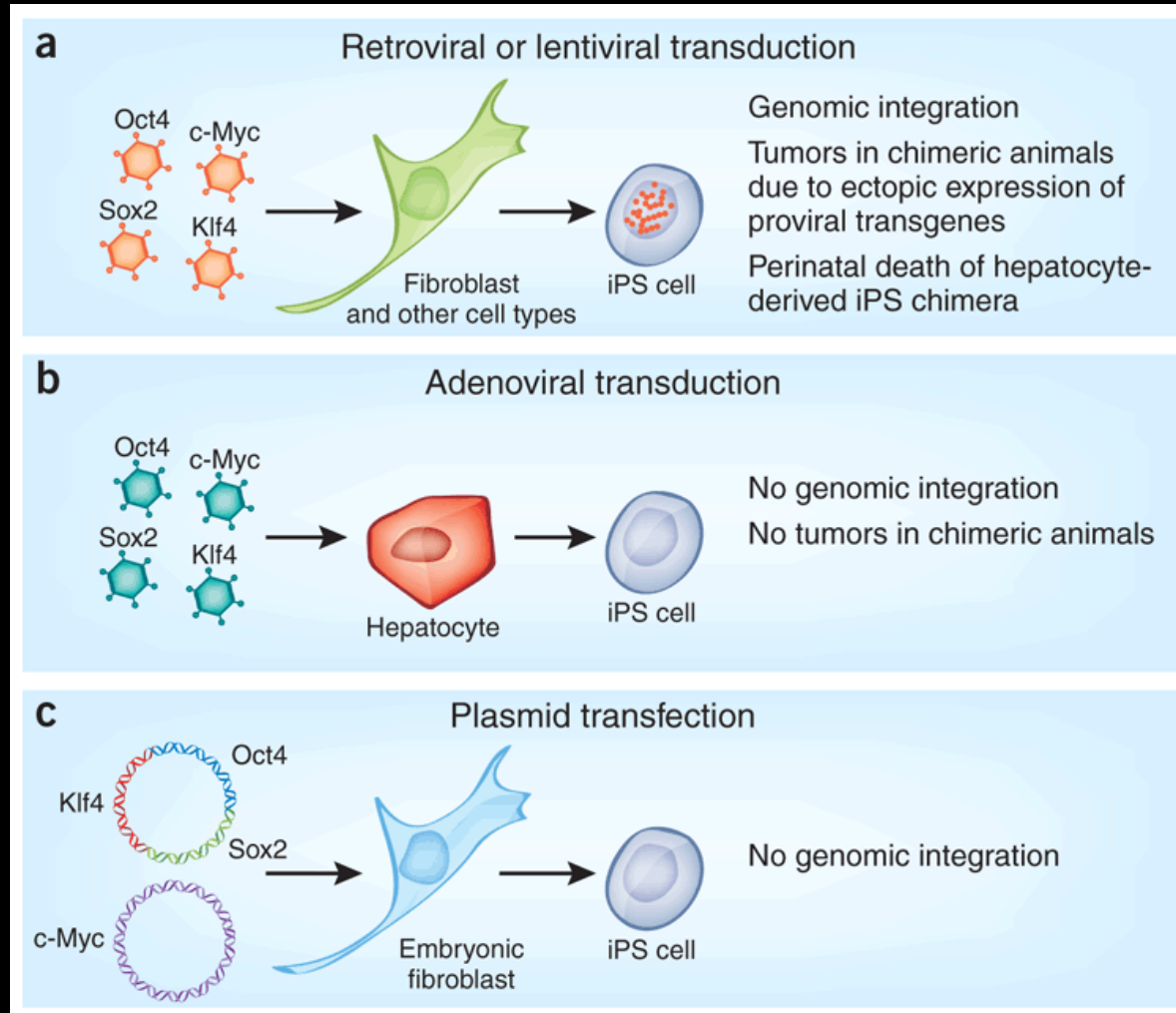
Oct4, Sox2, Klf4, c-myc

Yamanaka, 2006, 2007

Oct4, Sox2, Nanog, Lin28

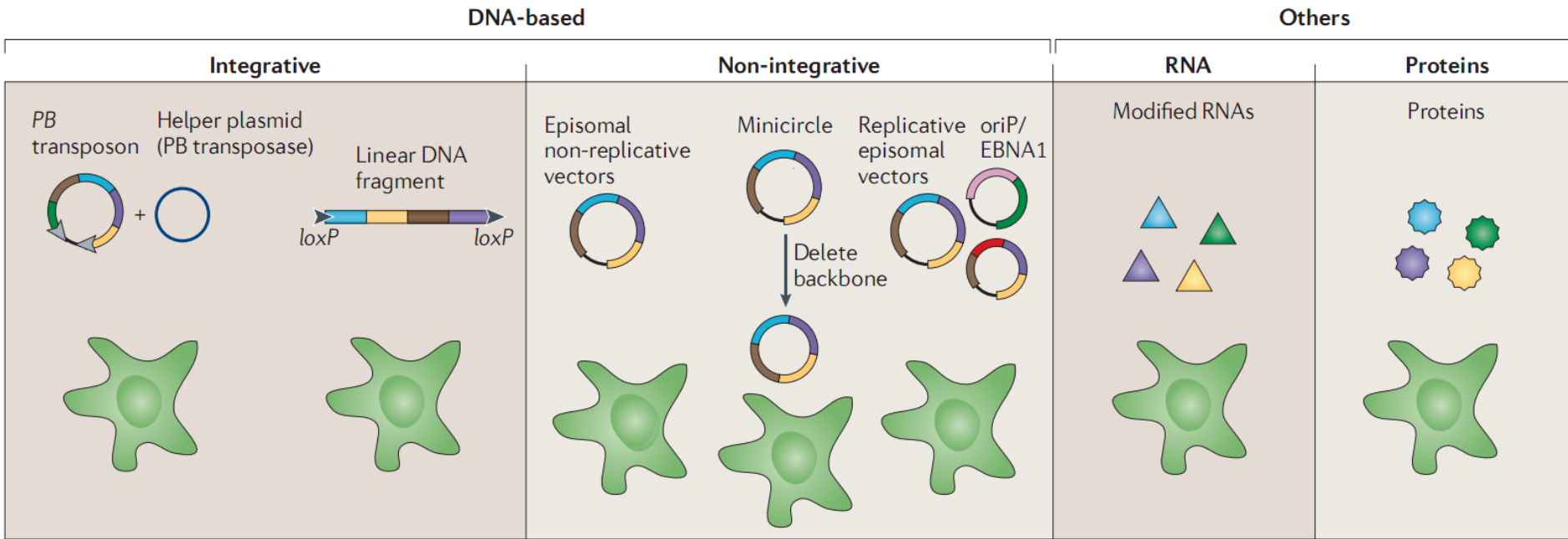
Thompson, 2007

Induced Pluripotent Stem Cells



Lowry WE & Plath K, Nature Biotech 2008

Induced Pluripotent Stem Cells



Gonzalez et al, Nature Reviews 2011

- OCT4 single factor
- Small molecules

Therapeutic Potential

- Rapid “unlimited” proliferation
- Differentiation
- Ready source

Musculoskeletal Therapy

- Bone
- Cartilage
- **Tendon/Ligament**
- **Muscles**

Tendon & Ligament

- 32 Million injuries US annually
- Young: athletic/sports
- Elderly: attenuated tissue
- Acute traumatic
- Chronic repetitive

Tendon & Ligament

- Collagen I (strength)
- Collagen III (crosslinks, repair)
- Tenoblasts (progenitors)
- Tenocytes

Tendon Injury

- >4% Strain microscopic failure
- >8% macroscopic failure/rupture

Acute Tendon Injury

- Surgical Repair
 - Immobilization
-
- Chronic Rupture
 - Tendon defect
 - Autologous graft

Chronic Tendon Injury

- Tendinopathy
 - Tendinosis
 - Tendinitis

Maffulli N, Arthroscopy 1998

Chronic Tendon Injury

- Growth Factors
 - FGF2
 - TGF- β
 - IGF-1
 - VEGF
 - PDGF

Chronic Tendon Injury

- Tissue Engineered
- Scaffolds
 - Natural (collagen, chitosan, silk)
 - Synthetic (PLA, PLGA)
- Cells
- Platelet-Rich Plasma (PRP)

Chronic Tendon Injury

- Platelet-Rich Plasma (PRP)
- Platelets + GF = initial inflammatory response
- ~6000 PubMed reports
- Not on WADA prohibited list

Chronic Tendon Injury

- MSC Therapy
 - Growth factors
 - Cell survival
 - Proliferation of endogenous connective tissue cells
 - Activate tissue progenitors
 - Angiogenesis

Chronic Tendon Injury

- Cell Therapy
 - Allogeneic MSC + fibrin
 - Achilles tendon (rabbit)
 - Histological
 - Biomechanical

Chong AKS, J Bone Joint Surg-A 2007

Chronic Tendon Injury

- ~2,000 Horses
- Autologous MSC
- Intratendinous Injections

www.vetcell.com

Chronic Tendon Injury

- Human
- Autologous skin-derived tenocytes
- Refractory patellar tendinopathy

Chronic Tendon Injury

- Human
- Allogeneic MSC
- Achilles tendon

Clinical Trial, Mark Young, Australia

Muscle

- Athletic Injuries
- 35-45% → Muscle Damage

Andia I, Expert Opin Biol Ther 2011

Muscle

- Satellite cells
 - canonical muscle stem cell
- Numbers == adolescence
- Functional decline in aging
 - Fibrosis rather than regeneration

Muscle

- Division
- Progenitor (myoblast)
- Skeletal muscle cell

Cell Therapy

- MTT: Myoblast Transplant Therapy
 - Poor cell survival
 - Immune rejection
- Combination Therapy
 - Myoblast + Growth Factors (bFGF, HGF)

Tedesco FS, J Clin Invest 2010

Cell Therapy

- MSC
- Intramuscular Injection
- Reduced inflammation
- Activate host progenitor cells

Cell Therapy

- Muscle-derived stem cells
- Slowly adhering
- High level of engraftment
- Angiogenic
- Clinical trials urinary incontinence

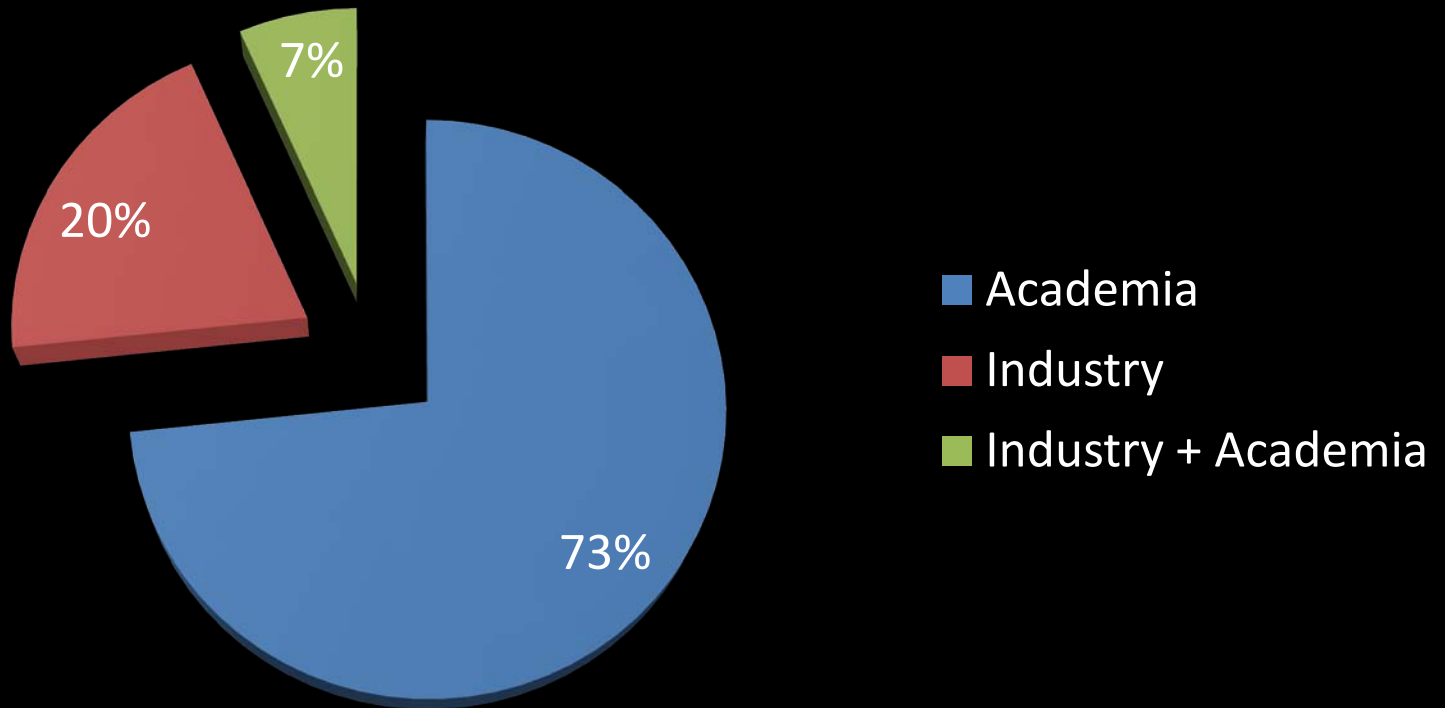
Tedesco FS, J Clin Invest 2010

Cell Therapy

- Myo-endothelial stem cells
- Muscle-derived progenitor cell type
- Higher survival rate
- Superior muscle regeneration

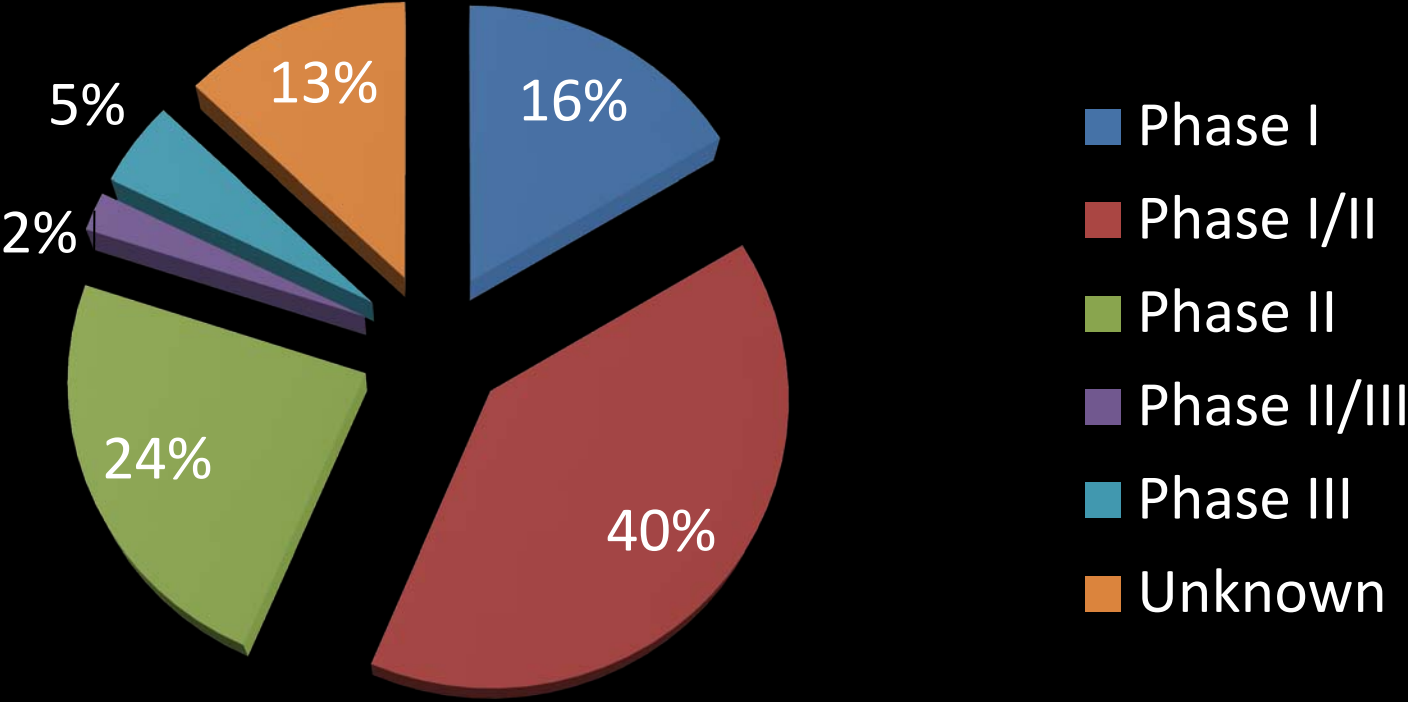
Zheng B, Nat Biotechnol 2007

Clinical Trials



230 Clinical Trial Registered with FDA (2012)

Clinical Trials



230 Clinical Trial Registered with FDA (2012)

Pluripotent Stem Cells

- ESC
- Spinal Cord Injury (Geron)

Frantz S, Nat Biotechnol 2012

- Macular degeneration (ACT)

Schwartz, Lancet 2012

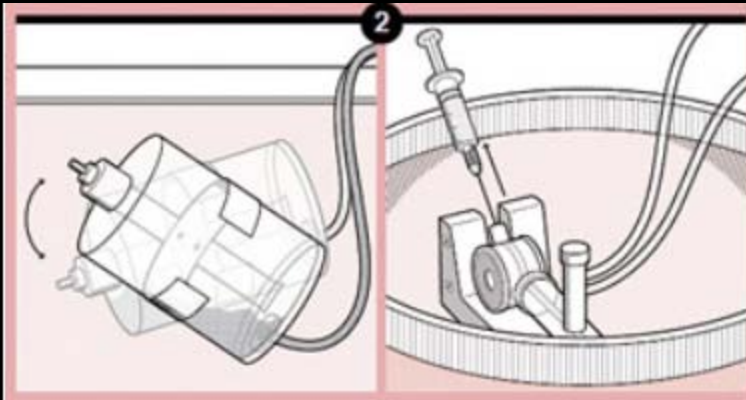
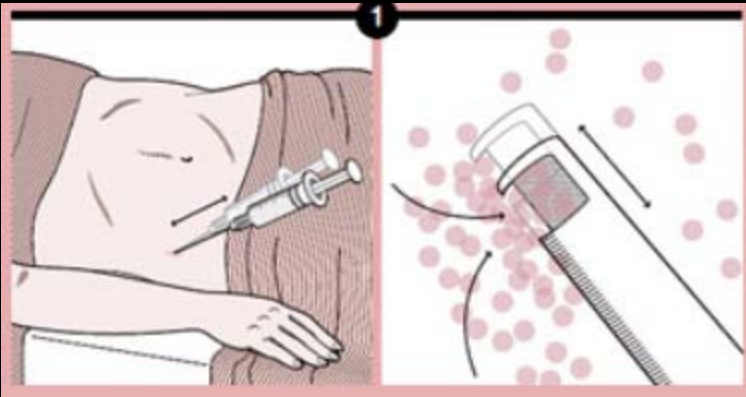
Pluripotent Stem Cells

- ESC
- Inefficient differentiation
- Immunogenicity
- Survival
 - iPSC
 - Disease in a dish
 - Drug screening

Cardiac

- >100 Clinical trials
- MSC
- Myocardial stem cells
- Hematopoietic stem cells
- Skeletal Myoblasts
- Wharton's jelly

Point of Care



- **Cytori**
 - Celution
 - PureGraft



Risks of Stem Cell Therapy

Dangers

- Teratoma formation
- Immune rejection
- Homing
- Instability
- cGMP: unregulated use?

Teratomas

- Teratomas
 - IM (12.5%)
 - SC (25% → 100% with Matrigel)
 - Intra testicular (60%)
 - Below kidney capsule (100%)

Immune Rejection

- Loss of graft
- Adverse response to immunosuppression
- HLA-matching?

Systemic Migration

- Systemic migration
 - Hypoxia
 - Tissue damage
- Systemic allogeneic MSC
 - No adverse effects
 - Immunomodulatory effects

Transformation

- Chromosomal abnormalities
 - HUES1, H1, H9, BG01, HES-1, WA15
 - Recurrent gain: 12, 17, X
- Similar to tumors
 - Testicular tumors
 - HSRs
 - iPSC instability

Safety of Adult MSC

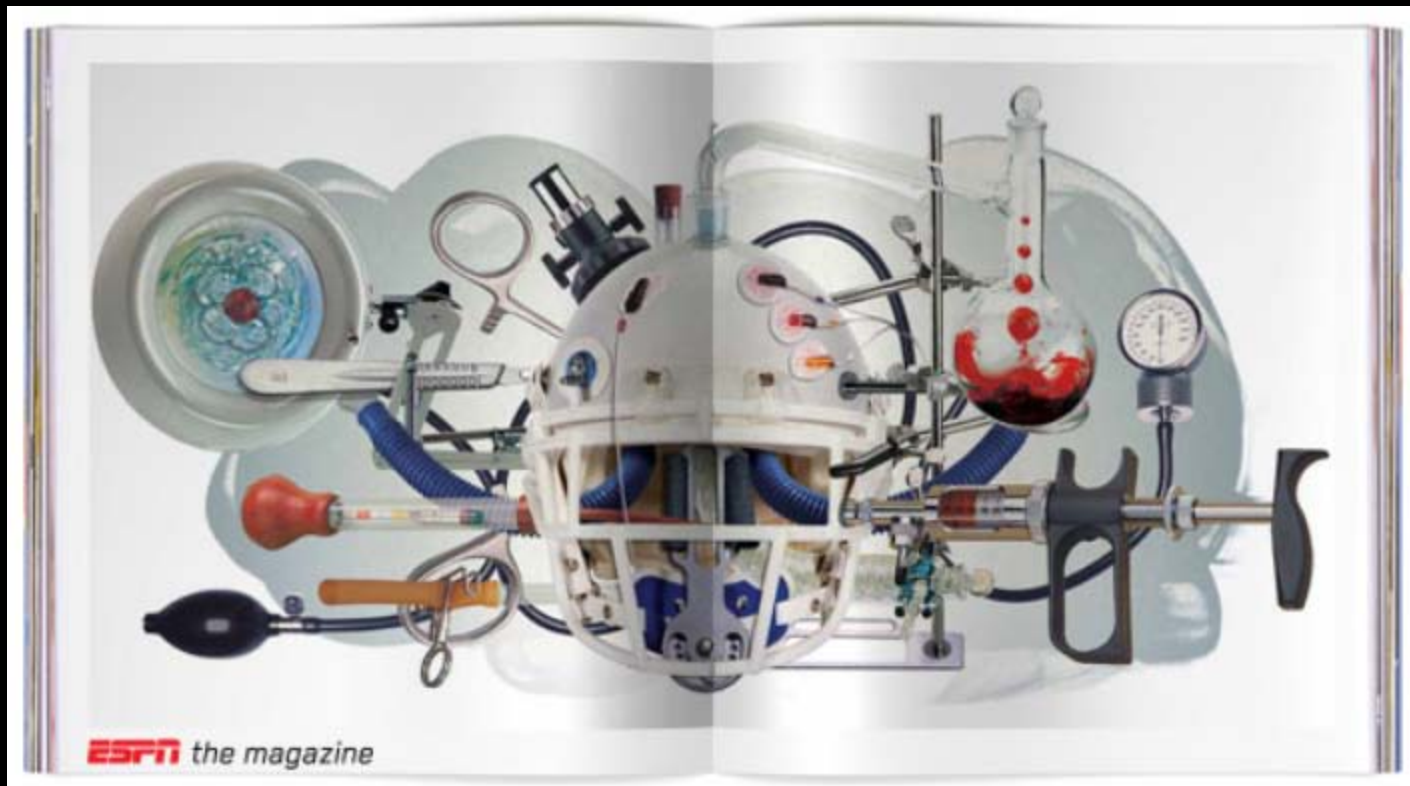
- Culture-expanded mesenchymal stem cells
Re-injected into **peripheral joints** (n=213) or
into **intervertebral discs** (n=13)
- No neoplastic complications were detected at
any stem cell re-implantation site (MRI)

Centeno CJ, Curr Stem Cell Res Ther 2010

DOPING

Stem Cells & the Athlete

Chasing the miracle cure



ESPN The Magazine Oct 2011



NFL

NBA

MLB

Stem Cell Therapy Clinics



Advanced Stem Cell Procedures

Stem cells are the repairmen of the body...

- ▶ Regenexx-SD: Same Day Stem Cell Procedure
- ▶ Regenexx-AD: Adipose Derived Stem Cell Procedure
- ▶ Regenexx-SCP: Stem Cell Plasma Procedure
- ▶ Regenexx-C: Advanced Cultured Stem Cell Procedure

Stem Cells & the Athlete

Bartolo Colon

- Shoulder & Elbow
- Dr. Sergio Guzman
- 4% of future earnings

Kobe Bryant

- Knee injection

Peyton Manning

- Cervical spine
- Neuro

LaRon Landry

- Achilles Tendon

Amniomatrix

- AmnioMatrix
- Cryopreserved human amniotic tissue
- Contains living cells
- Claim that amniotic cells are not stem cells

Performance Enhancing

- Indirect cell doping:
 - Stem cells secrete “naturally” occurring performance enhancing proteins
- Mechanogrowth factor
- Released naturally
 - muscle stretching and exercise
- Rapid increases in muscle mass and strength
- Mechanogrowth factor is on WADA's prohibited list
- Illegal without a prescription

Performance Enhancing

- Indirect
 - Overtraining and treating repair or regeneration with cell therapy
 - Analogous to local anesthetic injection or corticosteroids

Indirect Cell Doping

Supplements to stimulate endogenous stem cells



“The Stemtech HealthSciences, Inc product ingredient list is found on the product labels. After review of the **WADA (World Anti-Doping Association) banned substance list**, to the best of our knowledge Stemtech products do **not contain ingredients that are on the banned list**. We encourage all athletes subject to anti-doping testing to become fully informed of all products that they consume as well as thoroughly know and understand their organization’s banned substance regulations”



Focus Performance Recovery



What People are Saying

"I recently shattered the U.S. Outdoor record in the M65 mile, beating the old time by a whopping ten seconds. That old record had stood for 37 years! There are days on the track when I feel as good as I felt running for Villanova University - 45 years ago! My recovery time during training has been cut in half... and I hear the same thing from all of the competitors on the Senior circuit who are taking it. They wouldn't train without it!"

- **Frank Condon**
 (World Class Runner)

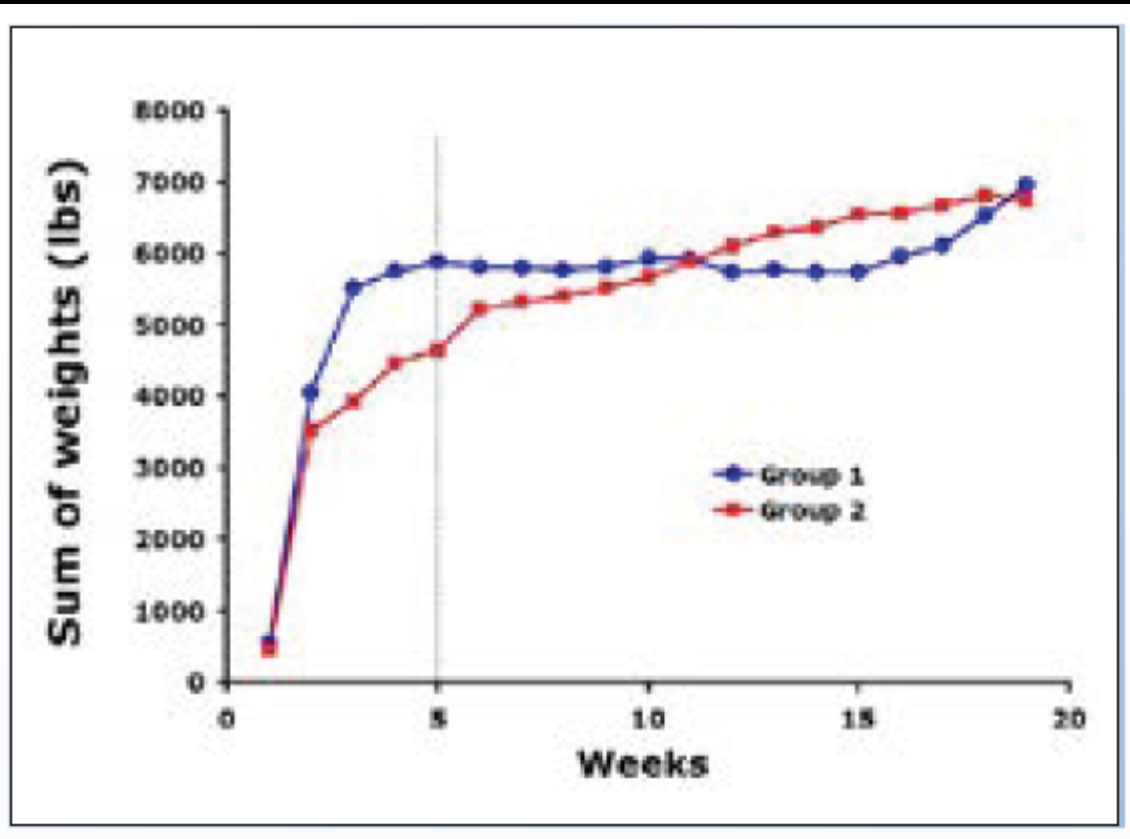


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Fitness & Recovery
 Using the power of your adult stem cells

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StemEnhance



Double Blind Placebo Controlled
Cross Over Study

StemSport Advance

- Aphanizomenon flos-aquae Concentrate
- Gotu Kola Extract
- Cordyceps sinensis
- D-Ribose
- Indian Gooseberry Extract
- Enzyme Blend
- Grape Pomace Extract
- Undaria pinnatifida Extract
- Turmeric Extract
- Lemon Fruit Extract
- Blackcurrant Extract

StemSport Advance

- Aphanizomenon flos-aquae Concentrate

Mobilization of human CD34+ CD133+ and CD34+ CD133(-) stem cells in vivo by consumption of an extract from Aphanizomenon flos-aquae

Jensen GS, Cardiovasc Revasc Med. 2007

StemSport Advance

- Fucoidan, galactofucan sulfate (a putative hematopoietic progenitor stem cells mobilizing agent)

Fucoidan ingestion increases the expression of CXCR4 on human CD34+ cells.

Irhimeh MR, Exp Hematol. 2007

Performance Enhancement

Abuse

Detection

Science progresses to fill an unmet need

Humans will find a way to manipulate advances for their own gain

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