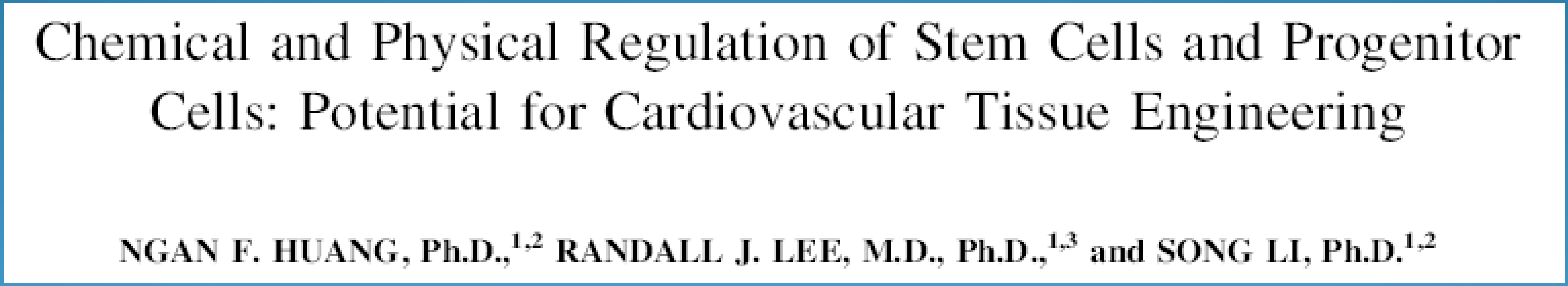
Stem Cells and Tissue Engineering Part 1

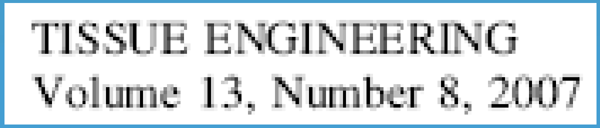
Aaron Maki

April 24, 2008



Chemical and Physical Regulation of Stem Cells and Progenitor Cells: Potential for Cardiovascular Tissue Engineering

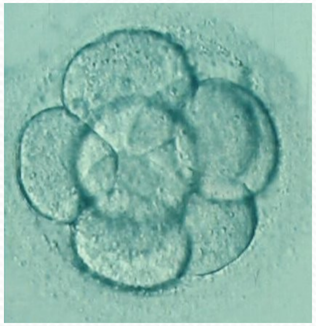
NGAN F. HUANG, Ph.D.,[1,2] RANDALL J. LEE, M.D., Ph.D.,[1,3] and SONG LI, Ph.D.[1,2]



TISSUE ENGINEERING
Volume 13, Number 8, 2007

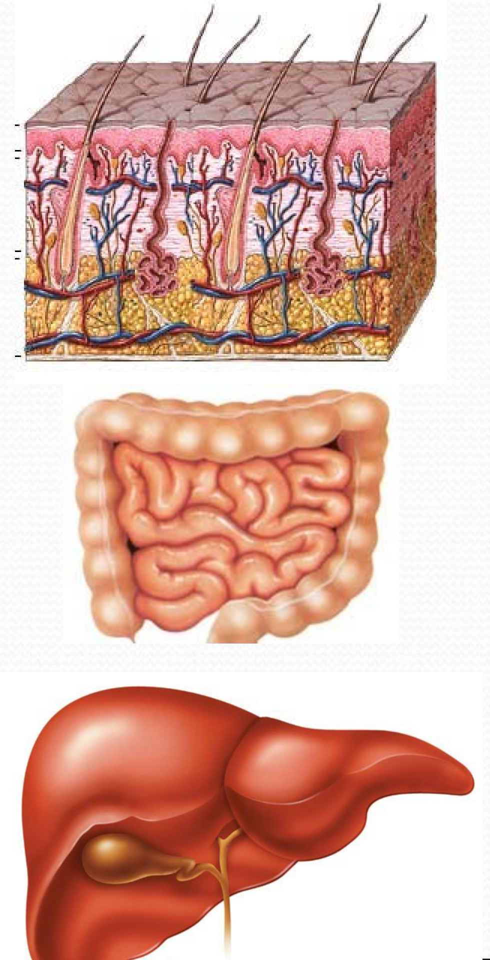
Regeneration in Nature

- Outstanding Examples
  - Planarian
  - Crayfish
  - Embryos
- Inverse Relationship
  - Increase complexity
  - Decrease regenerative ability

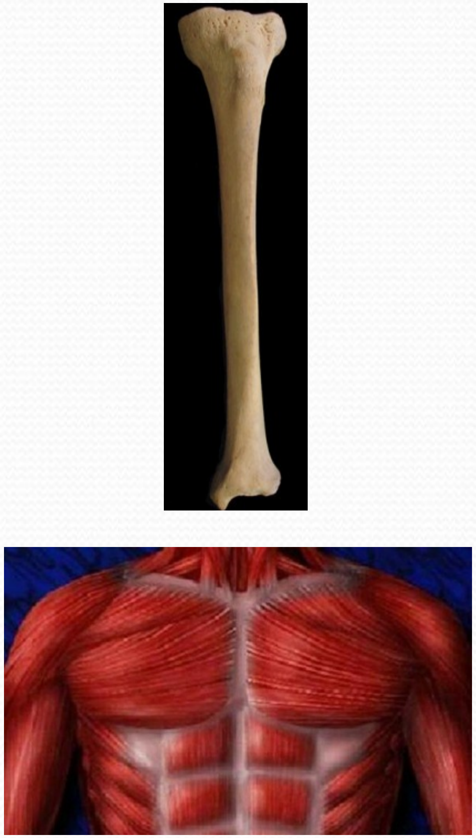


Regeneration in Humans

High



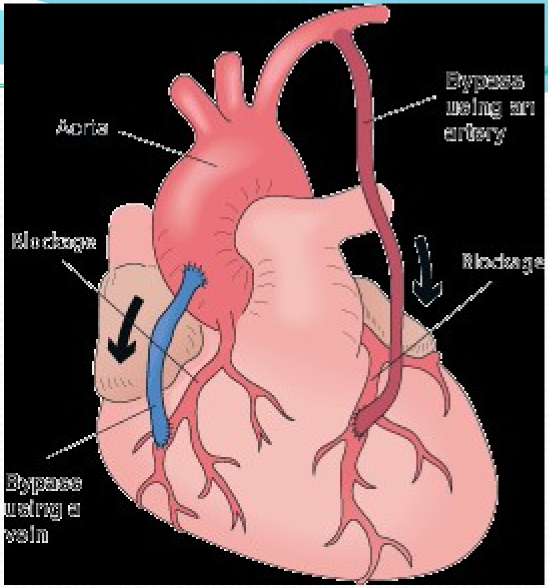
Moderate



Low

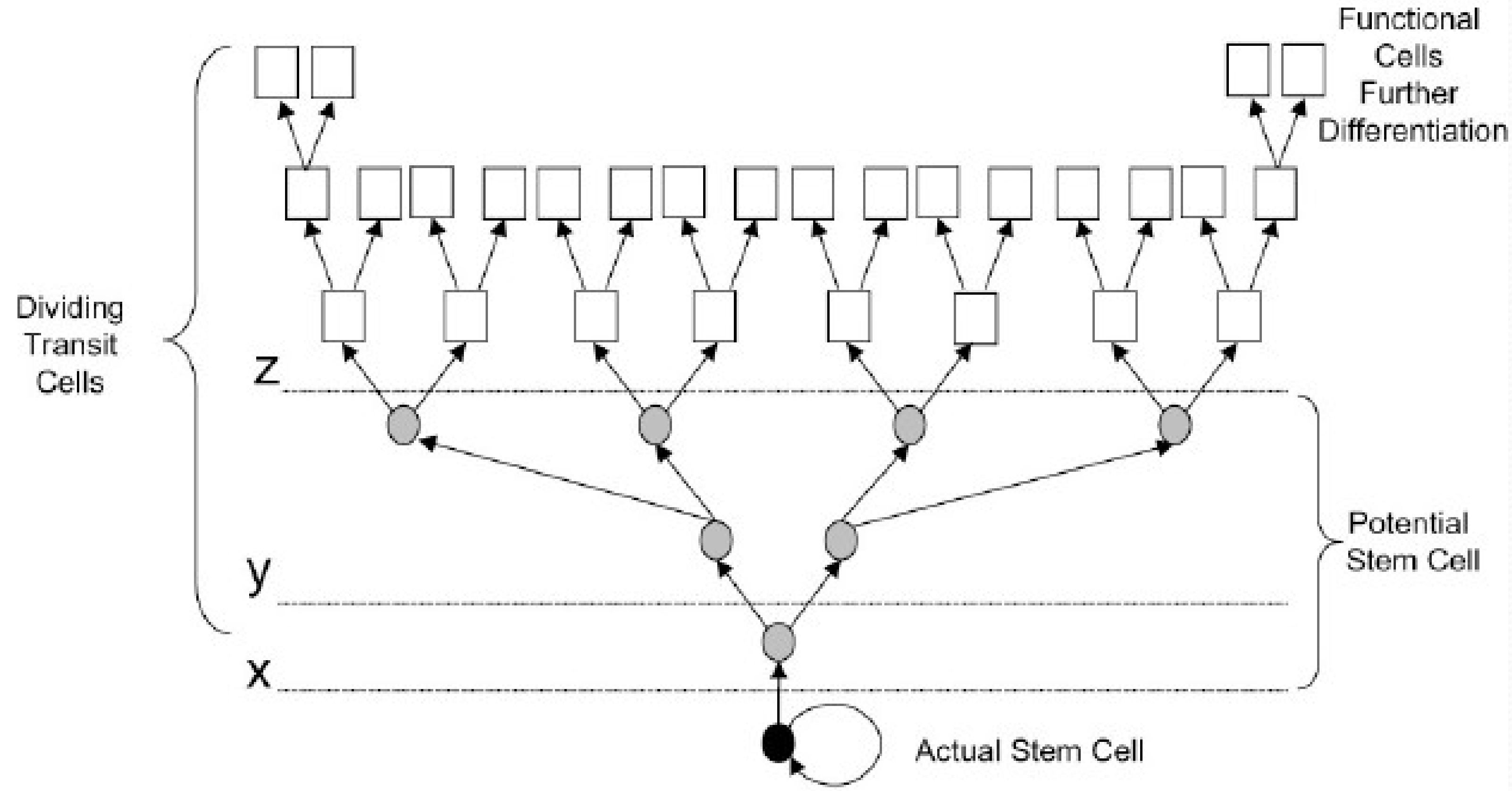
Clinical Needs

- Cardiovascular
  - Myocardial infarction
  - Stroke
- Bone
  - Non-union fractures
  - Tumor resections
- Nervous
  - Spinal Cord Injury
  - Degenerative diseases



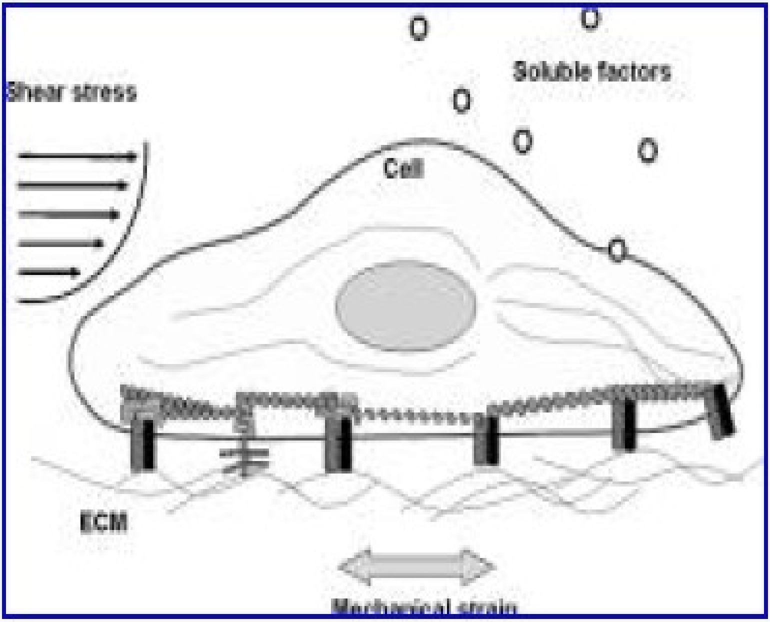
Stem Cells

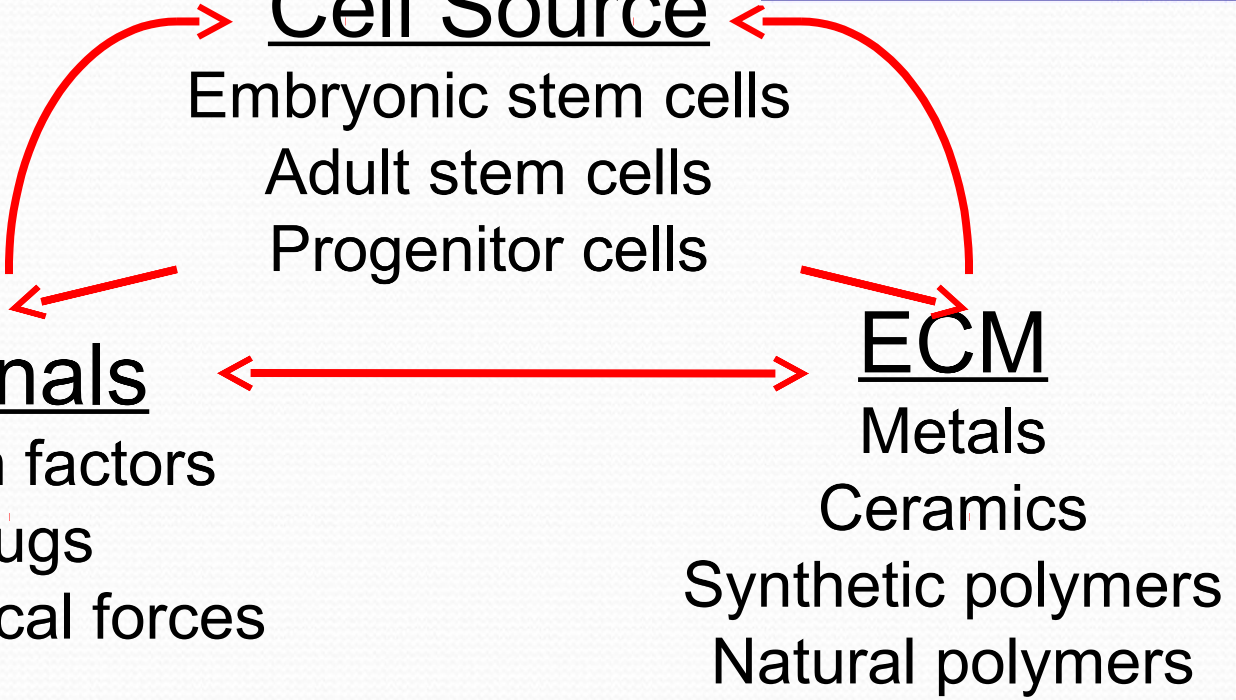
- Long-term self-renewal
- Clonogenic
- Environment-dependent differentiation



Tissue Engineering

- Repair/replace damaged tissues
  - Enhance natural regeneration





Cell Source

Embryonic stem cells
Adult stem cells
Progenitor cells

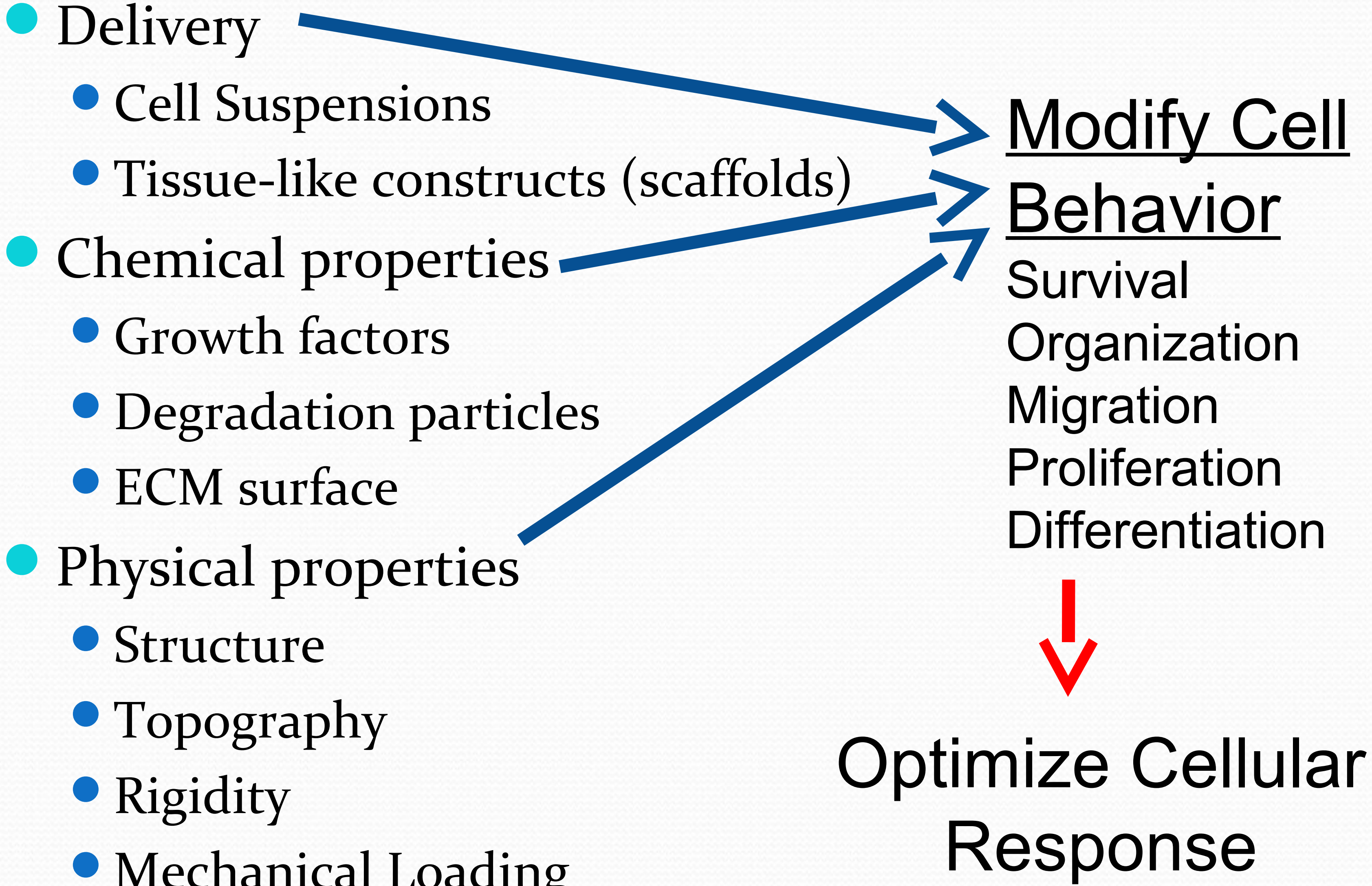
Signals

Growth factors
Drugs
Mechanical forces

ECM

Metals
Ceramics
Synthetic polymers
Natural polymers

Important Variables



- Delivery
  - Cell Suspensions
  - Tissue-like constructs (scaffolds)
- Chemical properties
  - Growth factors
  - Degradation particles
  - ECM surface
- Physical properties
  - Structure
  - Topography
  - Rigidity
  - Mechanical Loading

Modify Cell Behavior

Survival
Organization
Migration
Proliferation
Differentiation

Optimize Cellular Response

Stem and Progenitor Cells

- Isolation/Identification
  - Signature of cell surface markers
  - Surface adherence
  - Transcription factors
- Classifications
  - Embryonic Stem Cells
  - Adult Stem Cells
  - Induced Pluripotent Stem Cells

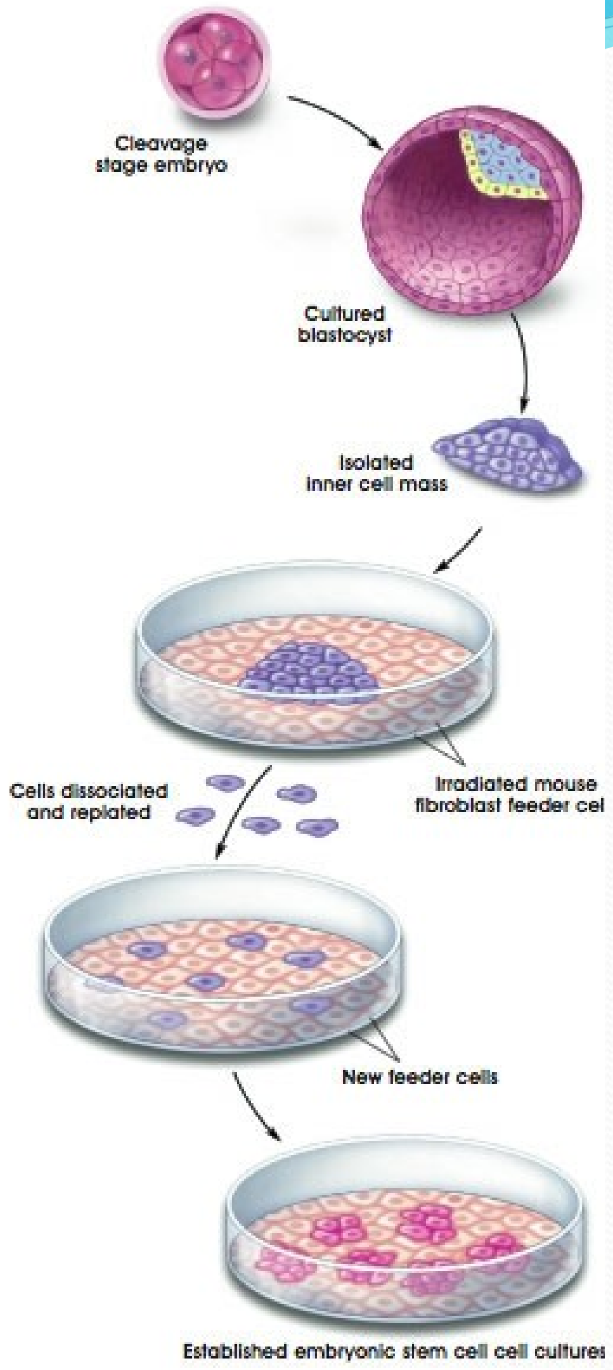
Embryonic Stem Cells

Strengths

- Highest level of pluripotency
  - All somatic cell types
- Unlimited self-renewal
  - Enhanced telomerase activity
- Markers
  - Oct-4, Nanog, SSEA-3/4

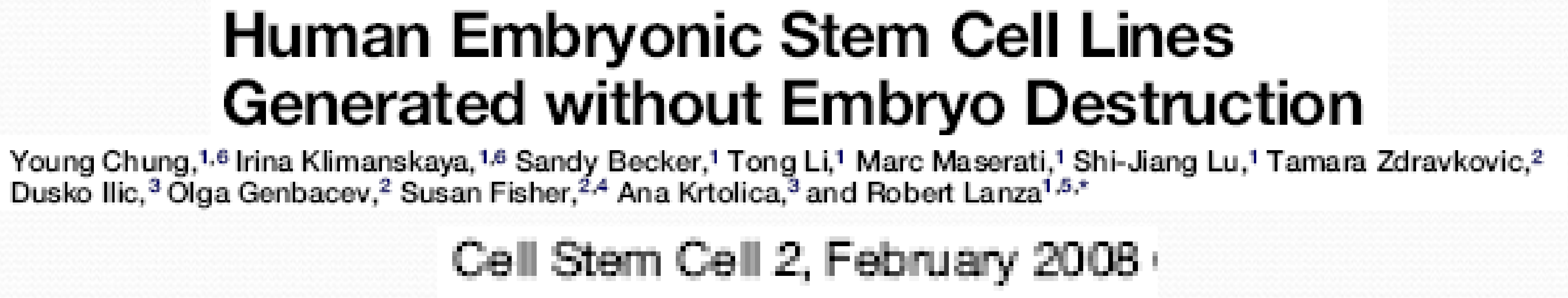
Limitations

- Teratoma Formation
- Animal pathogens
- Immune Response
- Ethics



Potential Solutions

- Teratoma Formation
  - Pre-differentiate cells in culture then insert
- Animal pathogens
  - Feeder-free culture conditions (Matrigel)
- Immune Response
  - Somatic cell nuclear transfer
  - Universalize DNA
- Ethics

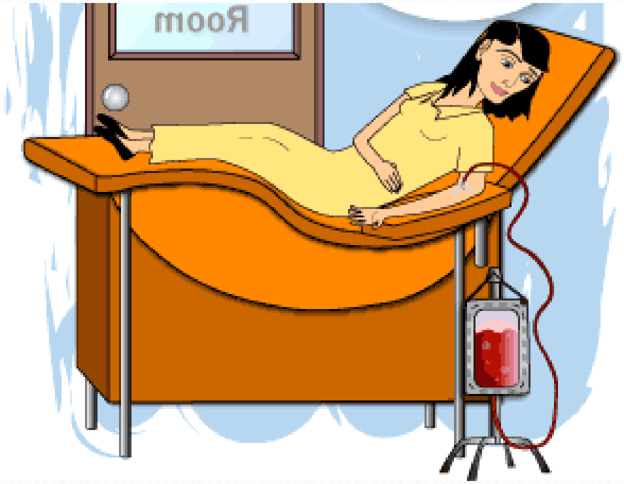


Human Embryonic Stem Cell Lines Generated without Embryo Destruction

Young Chung,[1,6] Irina Klimanskaya,[1,6] Sandy Becker,[1] Tong Li,[1] Marc Maserati,[1] Shi-Jiang Lu,[1] Tamara Zdravkovic,[2] Dusko Ilic,[3] Olga Genbacev,[2] Susan Fisher,[2,4] Ana Krtolica,[3] and Robert Lanza[1,5,*]

Cell Stem Cell 2, February 2008

Adult Stem Cells



Strengths

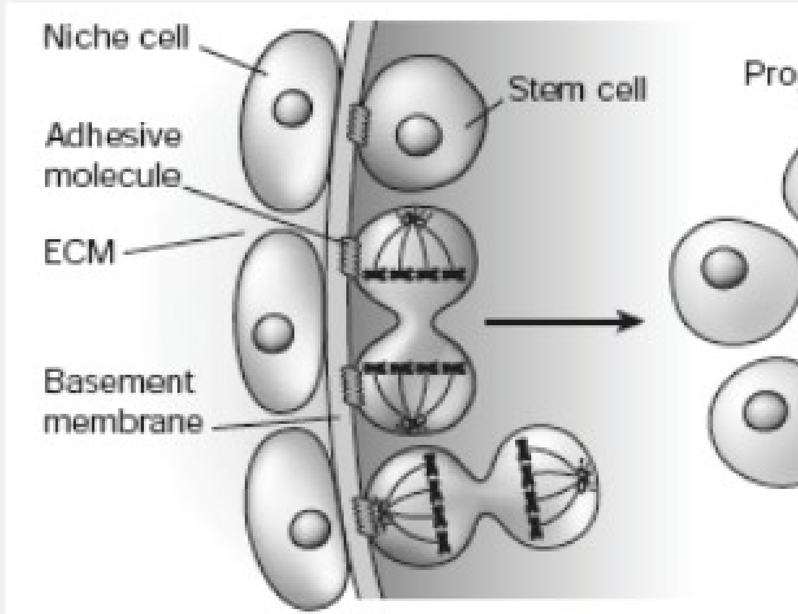
- Ethics, not controversial
- Immune-privileged
  - Allogenic, xenogenic transplantation
- Many sources
  - Most somatic tissues

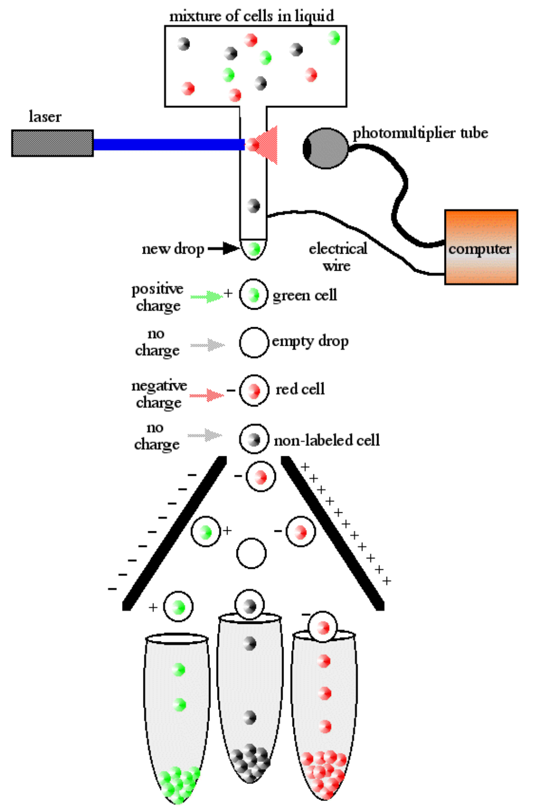
Limitations

- Differentiation Capacity?
- Self-renewal?
- Rarity among somatic cells

Potential Solutions

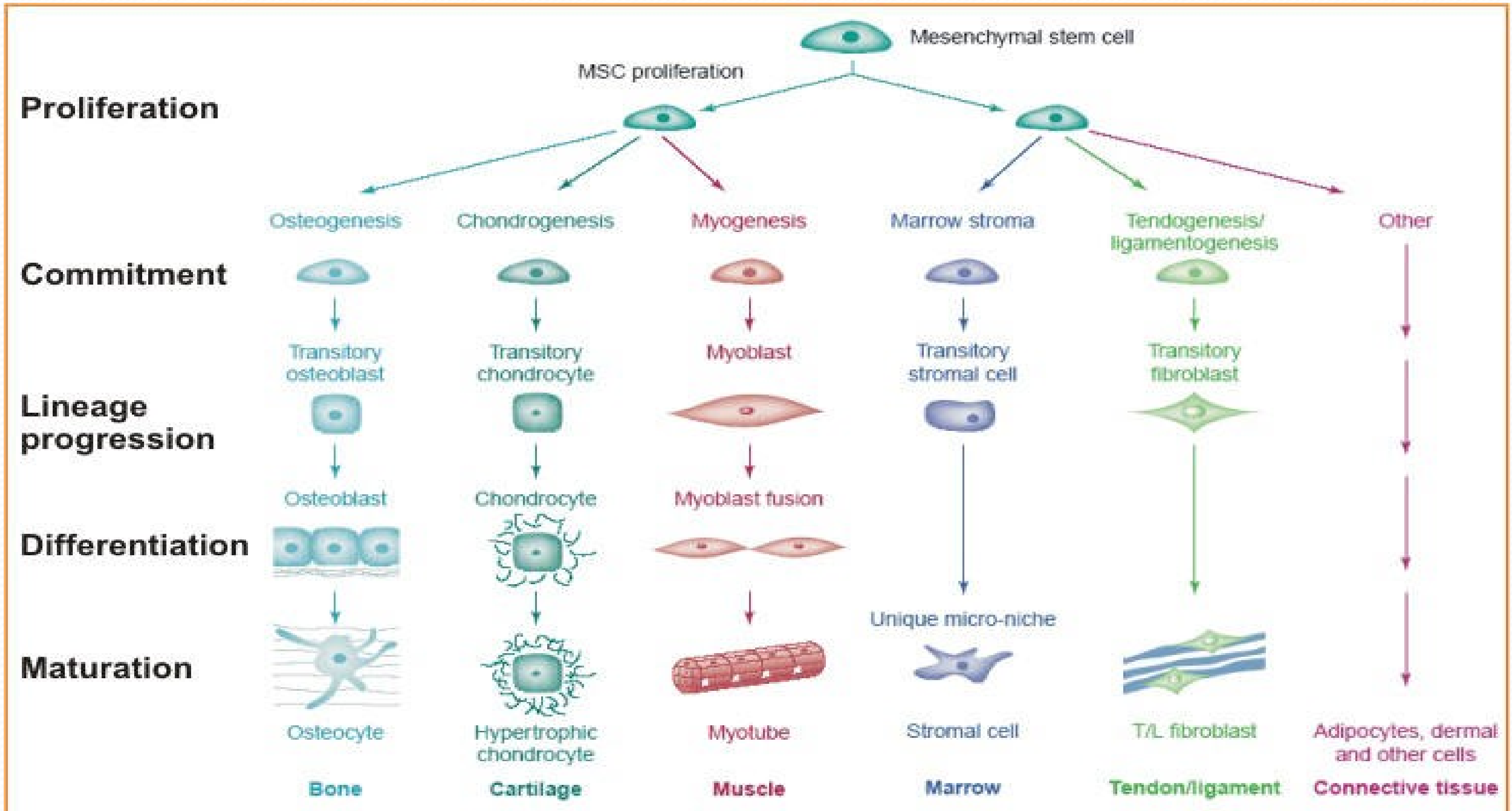
- Differentiation Capacity
  - Mimic stem cell niche
- Limited Self-renewal
  - Gene therapy
- Limited availability
  - Fluorescence-activated cell sorting
  - Adherence
    - Heterogenous population works better clinically





Mesenchymal Stem Cells

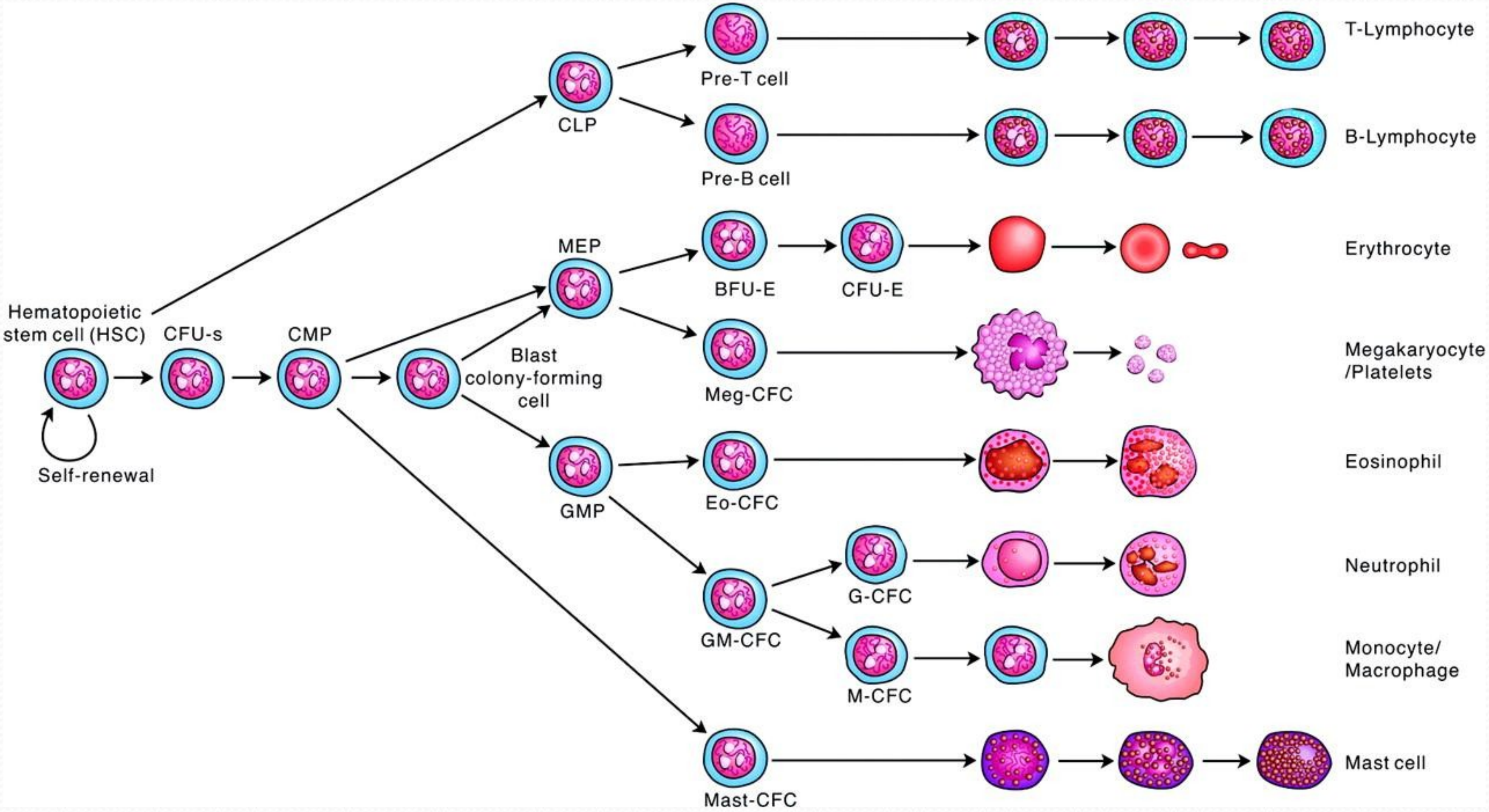
- Easy isolation, high expansion, reproducible



From Caplan & Bruder, TIMM 7:259

Hematopoietic Stem Cells

- Best-studied, used clinically for 30+ years



Induced Pluripotent Stem Cells

Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors

Kazutoshi Takahashi,[1] Koji Tanabe,[1] Mari Ohnuki,[1] Megumi Narita,[1,2] Tomoko Ichisaka,[1,2] Kiichiro Tomoda,[3] and Shinya Yamanaka[1,2,3,4,*]

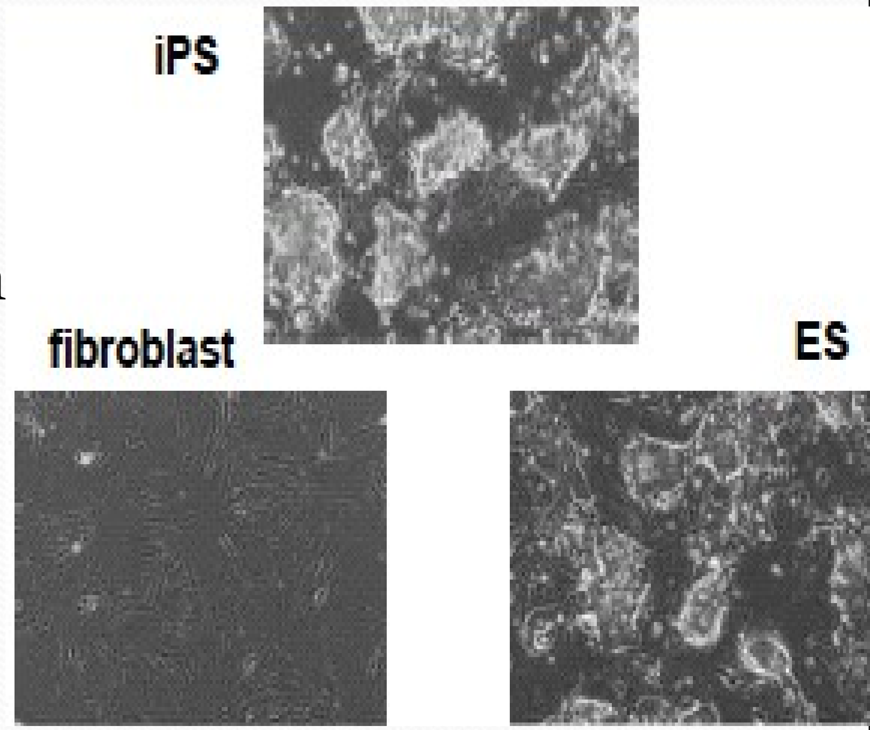
Cell 131, 1–12, November 30, 2007

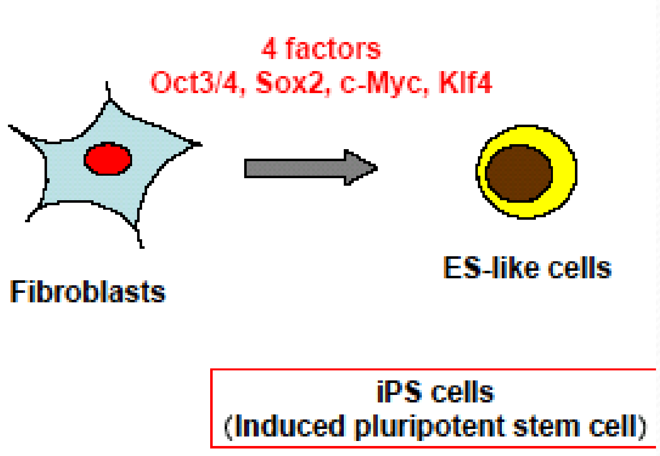
Strengths

- Patient DNA match
- Similar to embryonic stem cells?

Limitations

- Same genetic pre-dispositions
- Viral gene delivery mechanism





Potential Solutions

- Same genetic pre-dispositions
  - Gene therapy in culture
- Viral gene delivery mechanism
  - Polymer, liposome, controlled-release
- Use of known onco-genes
  - Try other combinations

Neurons derived from reprogrammed fibroblasts functionally integrate into the fetal brain and improve symptoms of rats with Parkinson's disease

Marius Wernig[*], Jian-Ping Zhao[†], Jan Pruszak[‡], Eva Hedlund[‡], Dongdong Fu[*], Frank Soldner[*], Vania Broccoli[§], Martha Constantine-Paton[†], Ole Isacson[‡], and Rudolf Jaenisch[*¶‖]

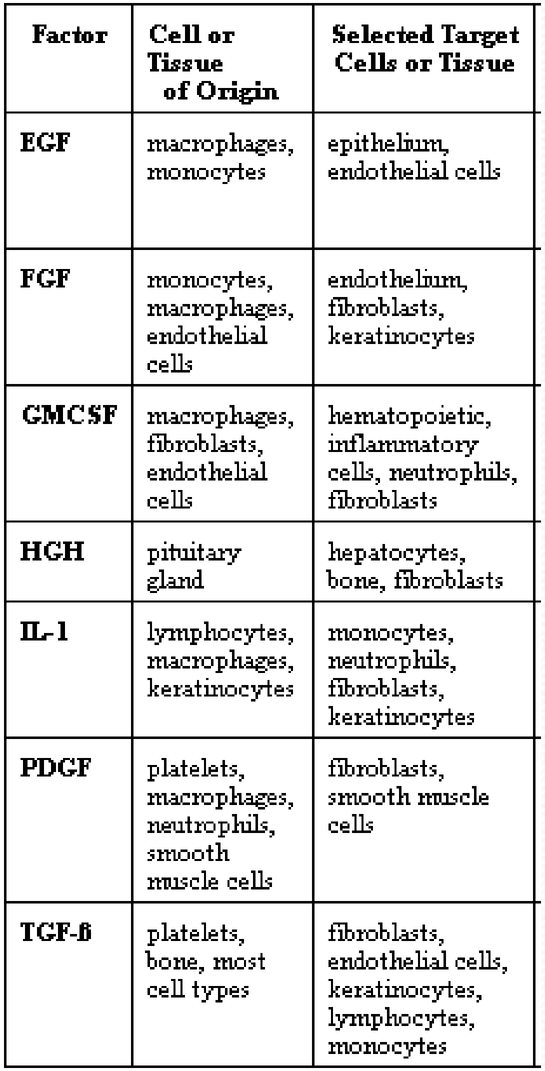
PNAS | April 15, 2008 | vol. 105 | no. 15

Soluble Chemical Factors

- Transduce signals
  - Cell type-dependent
  - Differentiation stage-dependent
    - Timing is critical
  - Dose-dependence

↓

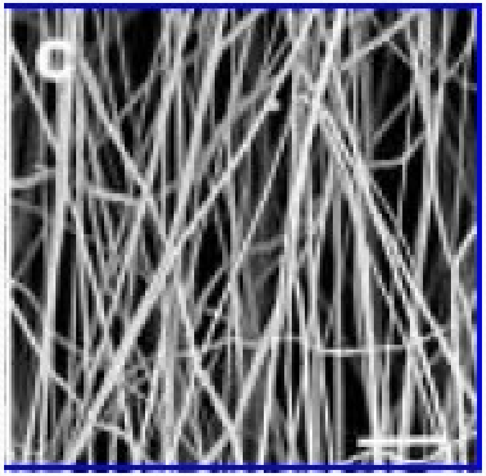
- Growth
- Survival
- Motility
- Differentiation



Factor	Cell or Tissue of Origin	Selected Target Cells or Tissue
EGF	macrophages, monocytes	epithelium, endothelial cells
FGF	monocytes, macrophages, endothelial cells	endothelium, fibroblasts, keratinocytes
GMCSF	macrophages, fibroblasts, endothelial cells	hematopoietic, inflammatory cells, neutrophils, fibroblasts
HGH	pituitary gland	hepatocytes, bone, fibroblasts
IL-1	lymphocytes, macrophages, keratinocytes	monocytes, neutrophils, fibroblasts, keratinocytes
PDGF	platelets, macrophages, neutrophils, smooth muscle cells	fibroblasts, smooth muscle cells
TGF-β	platelets, bone, most cell types	fibroblasts, endothelial cells, keratinocytes, lymphocytes, monocytes

Scaffold purpose

- Temporary structural support → Structural
  - Maintain shape
- Cellular microenvironment → Surface coating
  - High surface area/volume
  - ECM secretion
  - Integrin expression
  - Facilitate cell migration



Ideal Extracellular Matrix

- 3-dimensional
- Cross-linked
- Porous
- Biodegradable
- Proper surface chemistry
- Matching mechanical strength
- Biocompatible
- Promotes natural healing
- Accessibility
- Commercial Feasibility

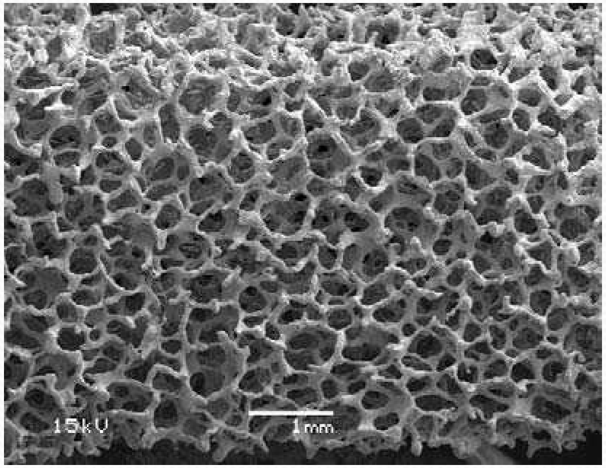
Modulate Properties
Physical, Chemical
Customize scaffold

Appropriate Trade-offs
Tissue
Disease condition

“Natural” Materials

- Polymers
  - Collagen
  - Laminin
  - Fibrin
  - Matrigel
  - Decellularized matrix
- Ceramics
  - Hydroxyapatite
  - Calcium phosphate
  - Bioglass

Perfusion-decellularized matrix: using nature's platform to engineer a bioartificial heart.
Ott, et al.
Nat Med. 2008 Feb;14(2):213



Important scaffold variables

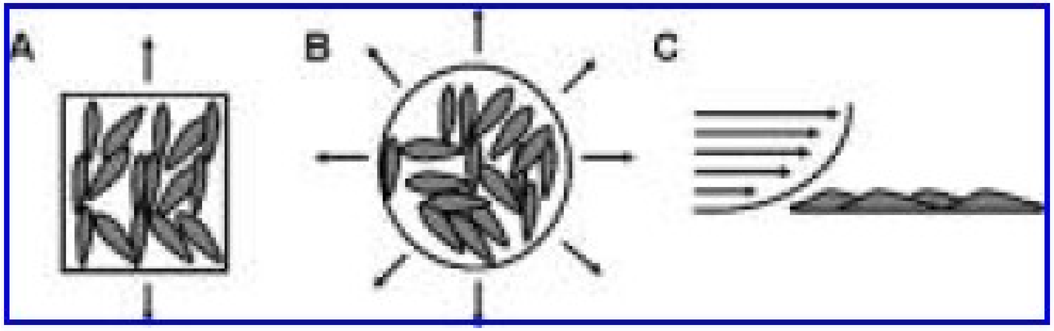
- Surface chemistry
- Matrix topography
  - Cell organization, alignment
  - Fiber alignment -> tissue development
- Rigidity
  - 5-23 kPa
- Porosity
  - Large interconnected
  - small disconnected

Mechanical Forces

- Flow-induced shear stress
  - Laminar blood flow
  - Rhythmic pulses
- Uniaxial, Equiaxial stretch
  - Magnitude
  - Frequency

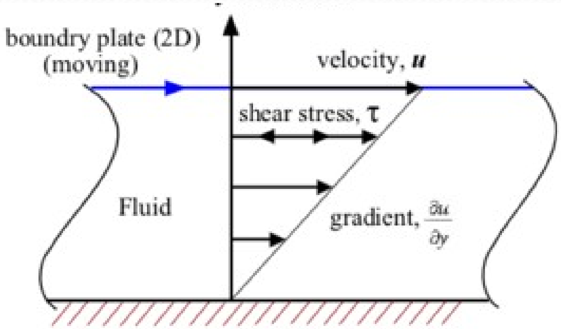
Mechanotransduction

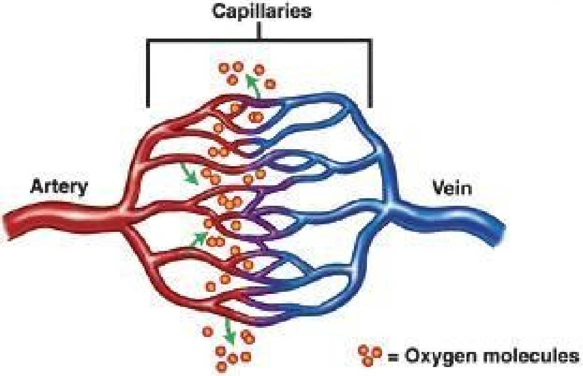
Conversion of a mechanical stimulus into a biochemical response

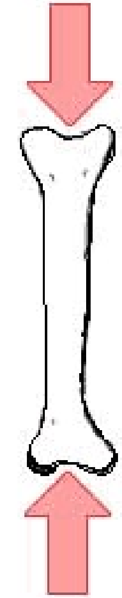


Flow-induced shear stress

- 2D parallel plate flow chamber
  - Hemodynamic force
  - Laminar flow
  - Pulsatile component
- 3D matrix
  - Interstitial flow
  - Bone: oscillating
- Cell-type specific







Models for Tissue Engineering

- *In vitro* differentiation
  - Construct tissues outside body before transplantation
  - Ultimate goal
    - Most economical
    - Least waiting time
- *In situ* methodology
  - Host remodeling of environment
- *Ex vivo* approach
  - Excision and remodeling in culture

Combine physical and chemical factors → Optimize stem cell differentiation and organization

Delivery Methods

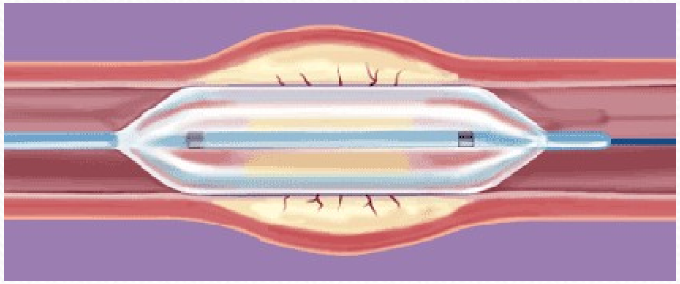
- Injectable stem cells
  - Cells or cell-polymer mix
  - Less invasive
  - Adopt shape of environment
  - Controlled growth factor release
- Solid scaffold manufacturing
  - Computer-aided design
  - Match defect shape

Cardiovascular Tissue Engineering

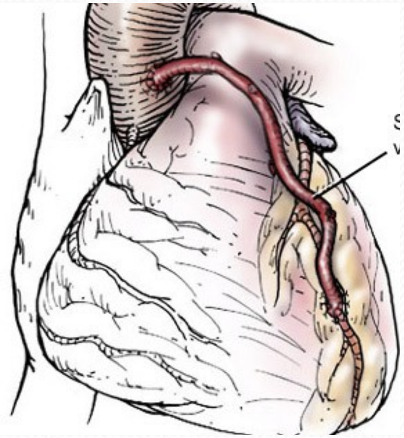
- Heals poorly after damage (non-functional scar tissue)
  - Myocardial infarction
    - 60% survival rate after 2 years
  - >40% tissue death requires transplantation
    - More patients than organ donors
- Heart attack and strokes
  - First and third leading causes of death
  - Patient often otherwise healthy

Current interventions

- Balloon angioplasty
  - Expanded at plaque site, contents collected
- Vascular stent
  - Deploy to maintain opening

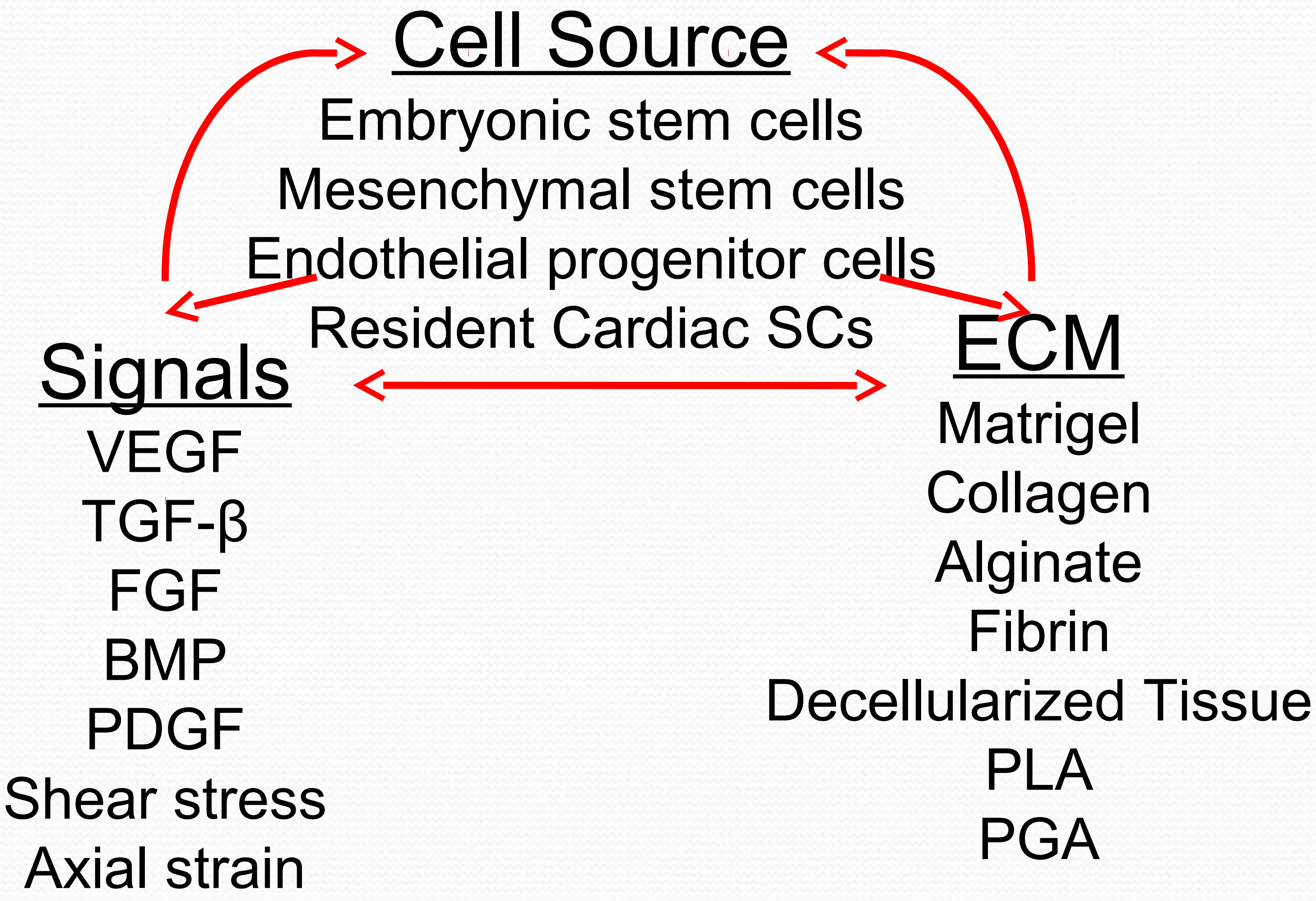


- Saphenous vein graft
  - Gold Standard
  - Form new conduit, bypass blockage



- All interventions ultimately fail
  - 10 years maximum lifetime

Cardiovascular Tissue Engineering



Cell Source

Embryonic stem cells
Mesenchymal stem cells
Endothelial progenitor cells
Resident Cardiac SCs

Signals

VEGF
TGF-β
FGF
BMP
PDGF
Shear stress
Axial strain

ECM

Matrigel
Collagen
Alginate
Fibrin
Decellularized Tissue
PLA
PGA

Clinical Questions

- What cell source do you use?
- How should cells be delivered?
- What cells within that pool are beneficial?
- How many cells do you need?
- When should you deliver the cells?
- What type of scaffold should be used?

These answers all depend on each other

Very sensitive to methodology!

- 2 nearly identical clinical trials, opposite results
  - Autologous Stem cell Transplantation in Acute Myocardial Infarction (ASTAMI)
  - Reinfusion of Enriched Progenitor cells And Infarct Remodeling in Acute Myocardial Infarction (REPAIR-AMI)
- Same inclusion criteria
- Same cell source (Bone marrow aspirates)
- Same delivery mechanism (intracoronary infusion)
- Same timing of delivery
- SIMILAR cell preparation methods

Seeger et al. European Heart Journal 28:766-772 (2007)

Cell preparation comparison

- Bone marrow aspirates diluted with 0.9% NaCl (1:5)
- Mononuclear cells isolated on Lymphoprep™ gradient 800rcf 20 min
- Washed 3 x 45 mL saline + 1% autologous plasma (250rcf)
- Stored overnight 4°C saline + 20 autologous plasma

- Bone marrow aspirates diluted with 0.9% NaCl (1:5)
- Mononuclear cells isolated on Ficoll™ gradient 800rcf 20 min
- Washed 3 x 45mL PBS (800rcf)
- Stored overnight room temperature in 10 + 20% autologous serum

Courtesy of Dr. Tor Jensen

Future Directions

- Standardization
  - Central cell processing facilities
  - Protocols
- Improved antimicrobial methods
  - Allergies
- Synthetic biology
  - Natural materials made synthetically, economically

Long-term: “clinical-grade” cell lines

- Animal-substance free conditions
  - Human feeder cells, chemically-defined media
  - Feeder-free culture
- No immune rejection, no immunosuppressive drugs
  - Somatic cell nuclear transfer
  - Genetic engineering, reprogramming
- Goals: understand normal/disease development, then repair/replace diseased organs and vice versa
  - Tissue engineering approach
    - ex vivo, in situ for now
    - In vitro for the future?

Summary

- Right combination of cell, scaffold, and factors depends on clinical problem
  - Extensive physician/scientist/engineering collaboration is vital to success
- Tissue engineering is leveraging our knowledge of cell biology and materials science to promote tissue regeneration where the natural process is not enough
  - Stem cells are an excellent tool for this task