Characteristics of living systems

- Reproduction
- Metabolism
- Movement
- Response to external stimuli
WE NEED A FEW FREE MINDS

MATRIX RELOADED
Signaling from the ECM

Chemical-growth factors, cytokines, hormones, etc
receptors for cell-ECM, cell-cell

Mechanical-muscle and fibroblast contraction,
fluid dynamics

Electrophysiological-expression of connexins and
ion channels by myocytes
and fibroblasts
Who makes the extracellular matrix in the heart?

Myocytes - basement membrane
Fibroblast family - collagen, glycoproteins, proteoglycans, growth factors
Transient cells - cytokines
Functions of the Extracellular Matrix

Form a substrate for cellular differentiation
Provide for tissue organization, cell size and shape
Communication between cell via specific receptors
Metabolism-enzymatic action
Anoikiosis-cell death pathway
Basic Principles of the ECM

Organization—qualitatively similar, quantitatively different

Cellularity—permanent (myocytes) vs transient cells (fibroblasts, others)

Density—quantitative amount of components

Fluid movement—vasculature, interstitium, lymph
CHEMICAL SIGNALLING

Large molecular weight
(proteoglycans, glycoproteins, collagens)

Latent form

Activation by proteases
MMP’s, Serine TPA, UPA

Receptor mediate response
Figure 9 The origin and lineage relationships of cardiac cell types. Each cardiac cell type is established by lineage diversification of embryonic cells which arise from one of three distinct origins: cardiogenic mesoderm, neural crest, or proepicardium. These data define the chronology and distribution for the development of all cell lineages in the avian heart.
Extracellular matrix in adult heart

Normal growth Connective tissue network
Hypertrophy-physiological vs pathological
Angiogenesis
Scar formation
Failure
Potential therapies
Serial reconstruction by confocal microscopy

Fig. 5. Comparison of subepicardial (A), midwall (B), and subendocardial (C) blocks extracted from the Wistar image volume showing myocytes and collagen (top) and collagen only (bottom). Block sizes were 300 μm on each side and presented with the common imaging plane on the front face.
ORGANIZATION OF MYOCYTES INTO LAMINAE  

Scanning electron microscopy
Scanning electron microscopy
Mechanical properties of the heart due to Connective tissue

(You know I did not do the math!)
Serial reconstruction by confocal microscopy

Fig. 5. Comparison of subepicardial (A), midwall (B), and subendocardial (C) blocks extracted from the Wistar image volume showing myocytes and collagen (top) and collagen only (bottom). Block sizes were 300 μm on each side and presented with the common imaging plane on the front face.
Dynamic Interactions In Cardiac Development, Disease and Homeostasis

Chemical Factors
- Growth Factors
- Cytokines

Mechanical Factors
- Stretch/Pressure

Myocytes
- Hyperplasia
- Hypertrophy

Fibroblasts
- Hyperplasia
- Synthesis of ECM

Electrical Conduction
- Ion Channels
(A) Infarct healing time course

(B) Matrix content time course
CARDIOMYOCYTE INTEGRIN EXPRESSION

F - FETAL
N - NEONATAL
A - NORMAL ADULT
P - PRESSURE-OVERLOAD HYPERTROPHY ADULT

Muscle Fibroblasts
Capillary Endothelial cell Pericytes
Basement membrane Lumen
Artery

Smooth muscle cell Adventia Media
Artery

Endothelial cell Lumen Pericytes

Myocytes

Fibroblasts

Collagen Receptor Integrins, DDR2
Cell Surface Receptor cadherins, connexins
Cell Surface Receptor new
Transmission electron microscopy
Transmission electron microscopy
Multilayer myocyte cultures

Layers can be made up to 5 myocytes thick. Layers can be subjected to cyclic or static stretch.
Static stretch

Cyclic stretch

(This is the version that actually worked!)
Multilayers of myocytes and collagen

2 layers
Mechanically stretching cells
(bioengineering again!)

Protein Metabolism in Aligned Myocytes With Myofibrils Arrayed in Parallel With the Axis of Stretch

- Myofibrillar Fraction
- Cytosolic Fraction
- Biosynthetically Labeled Myosin Heavy Chain
- Total Cellular Myosin Heavy Chain
- Biosynthetically Labeled Actin
- Total Cellular Actin

Percent Non-stretched Controls

Protein Metabolism in Aligned Myocytes With Myofibrils Arrayed Perpendicular to the Axis of Stretch

- Myofibrillar Fraction
- Cytosolic Fraction
- Biosynthetically Labeled Myosin Heavy Chain
- Total Cellular Myosin Heavy Chain
- Biosynthetically Labeled Actin
- Total Cellular Actin

Percent Non-stretched Controls
Stem cells and Induced Pluripotential Cells

Varies with the particular organ (good news/bad news)
Younger is better!
Wikipedia lists 18 potential diseases/therapy
Be careful what you wish for!
Regenerative potential
Embryonic stem cells-good but potential ethical problems
Amionic stem cells- good, abundant, some immunological considerations
Mesenchymal stem cells-variety of cell types, immunological considerations
Adipose stem cells-abundant, problematic, variety of cell types
Cardiac Regeneration and Stem Cells

Approaches

Injection of stem cells
Injection of IPS cells

Why do myocytes stop dividing?
Modified early cardiac genes?
Don’t hold your breath!
Role of the ECM in Regeneration

Form substrate for cellular integration similar to development (?)
Essential to multicellular signaling
Poorly understood, but high potential to promote cellular integration
Conclusions (So What!)

Cellular therapies are still very experimental but there is progress (improvement of function)

However, the old standards of exercise, diet and elimination of smoke reduce heart disease dramatically!
COLLABORATORS

International
Russia, Sweden, Norway, Finland, England, France
Germany, Canada, Japan, China, South Korea, New Zealand, Chile, Brazil

United States
UCLA, Univ. Arizona, UCSD, SDSU, Univ. Illinois,
Loyola Medical School, Northwestern, NYU, Harvard,
Univ. Chicago, LSU, MCG, Vermont, UNC, UVa,
Pennsylvania, Univ. Cincinnati, Michigan
Cell Populations Change During Development in the Murine Heart

- Fibroblast
- Myocytes
- Non-Myocyte/Non-Fibroblast

E18.5, Day 1, Day 5, Day 15, Adult, 12 wks

p<0.05
p<0.001
p<0.001
p<0.001
p<0.001
p<0.001

New students are welcome
Factors Affecting Heart Function
The Dynamic Interaction Model

**Chemical**
- Growth Factors
- Ang II, PDGF, EGF, FGF, IGF, VEGF, TGF
- Hormones
  - Insulin
  - Growth Hormone
  - Ang II

**Mechanical**
- Contraction
- Stretch
- Pressure

**Electrical**

**Growth Factor And Hormone Receptors**

**Myocytes**
- Hyperplasia
- Hypertrophy
- Myofibril formation
- Differentiation

**Fibroblasts**
- Hyperplasia
- Hypertrophy
- Differentiation
- Synthesis of ECM
epicardium

BMP2,4/TGFbeta's

subepicardium

VEGF/FGF

Angioblastic Pathway

Hemangioblastic Pathway

Smooth Muscle Cell Pathway

Fibroblastic Pathway

PDGF/TGF

BMP2

myocardium

endocardium

Stolen from Andy Wessels, MUSC
IL-6-Deficient Mice Display Gross Cardiac Abnormalities
Discoidin Domain Receptors

- Transmembrane proteins which bind collagen
- Receptor tyrosine kinase protein family
- Homologous to Discoidin 1 - lectin binding protein
- Two types: 1 and 2
Antibody 1611 DAPI Microspheres in capillaries merged
Role of ECM and Stem/IPS Cells
Provide a good home for subsequent differentiation
Poorly understood
Careful you may get a tumor!