Cardiac Tissue Engineering

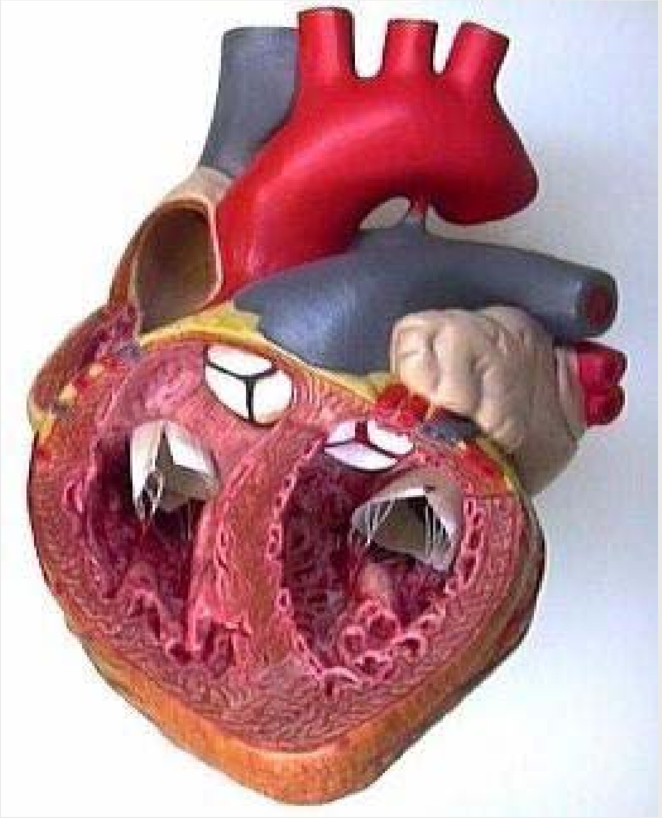


Fig. 1

Siama Durrani

Overview of Presentation

- Heart
- Role for Tissue Engineering
- Issues and Progress
- Overview of Results
- Suggestion
- References

Heart[1]

- Human heart is a hollow, muscular organ with 4 chambers
- Blood pumped in continuous cycle called circulation
  - Blood has to move throughout body at all times
  - Heart is a pump whose job it to keep blood moving
  - The heart supplies blood and oxygen to all parts of the body

Heart, Cont'd.[2]

- Arising from mesoderm, undergoes series of morphogenetic changes[2]
  - Begins beating 21 days after conception
- The heart is surrounded by a fluid filled sac called the pericardium.
- The Heart Wall is composed of
  - Epicardium
  - Myocardium
  - Endocardium

Myocardium

- Complex interactions of numerous cell types give heart ability to pump blood

Types of Cardiac Muscle

- Nodal: characteristic of both muscle and nervous tissue
  - Sinus and AV node
  - Bundle of His
- Contractile
  - Ventricular wall
- Conductile
  - Bulk of heart.

Types of Cardiac Cells

- Myocardial cells (working cell)
  - Contain contractile filaments, form muscle layers of myocardium
- Specialized electrical conduction cells
  - Nodal Cells, etc.

Characteristics of Cardiac Muscle Cells aka Cardiac Myocytes[3]

- 10-20μm diameter and 50-100μm length
- Located in heart only
- Striations present
- Uni/binucleate
- Electrically excitable
- Self-stimulating; isolate myocytes via trypsin treatment will beat independently
- Conduct action potential
- Contract and generate force like skeletal muscle, however, involuntary muscle
- Branching cells connect via intercalated discs bound by gap junctions mediated by connexin 43

Key Cardiac Cell Characteristics

- Excitability
- Automaticity
- Conductivity
- Contractility

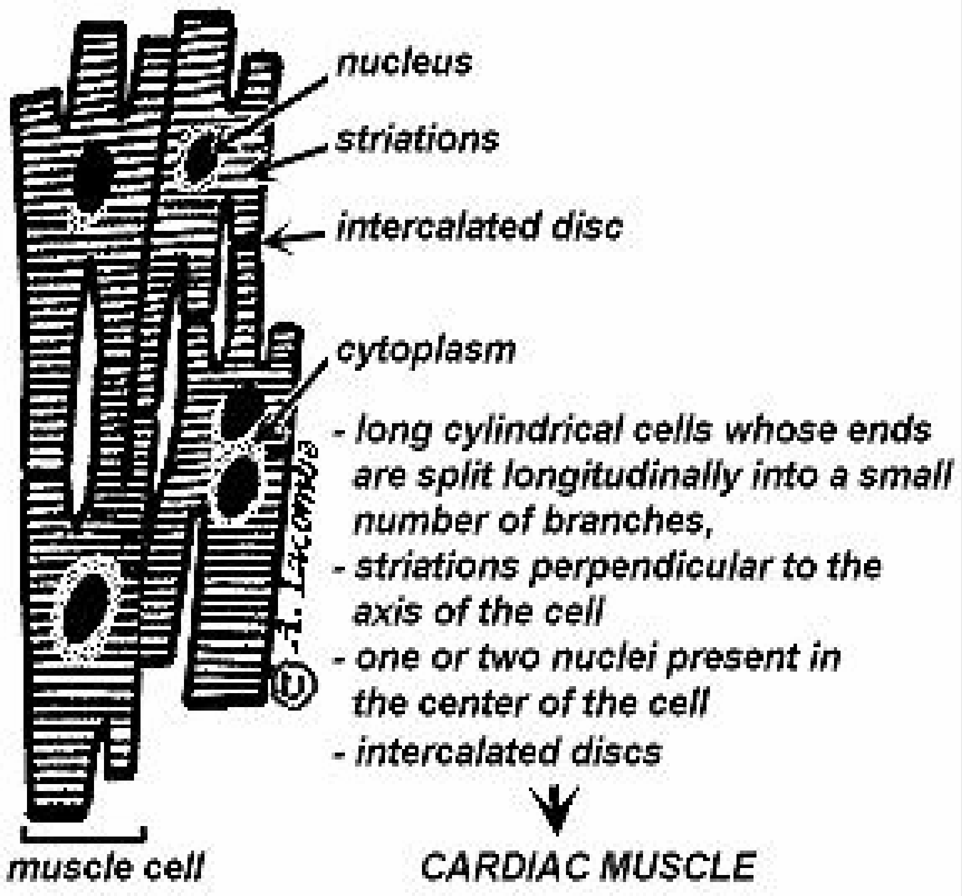


FIG. 2

Functional Characteristics

- Contract without neural stimulation
- Regulated by pacemakers
- Innervation from nervous system can alter pacemaker activity
- Cardiac muscle contraction lasts 10 times longer than skeletal muscle

Cardiac Cell Contraction

- Appearance of action potential, triggered by pacemaker
- Release of calcium from SR and contraction of sacromere
- Once AP in action travel cell to cell via gap junctions
- Without pacemaker, cells would not contract
- Heart rate(beats/min) depend on information from autonomic nervous system

Cardiac Anatomy

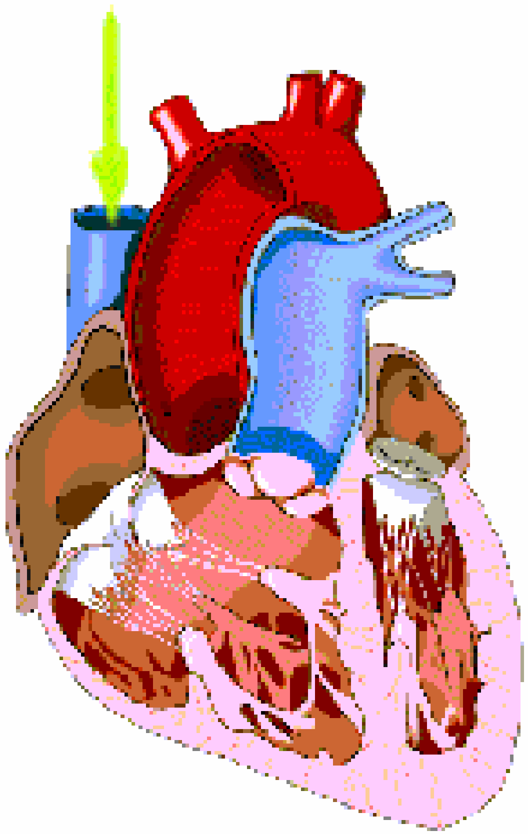


FIG. 3

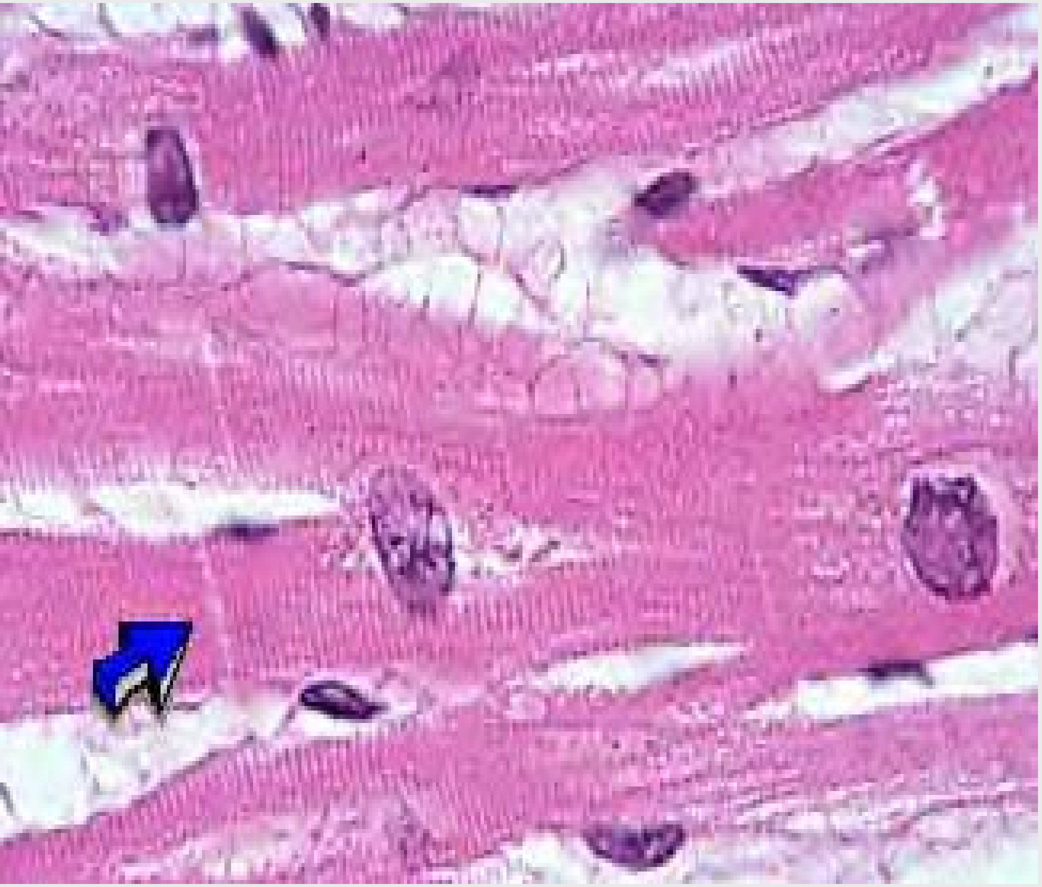
- Right Atrium
- Tricuspid Valve
- Right Ventricle
- Pulmonic Valve
- Pulmonary Arteries
- Pulmonic Veins
- Left Atrium
- Mitral Valve
- Left Ventricle
- Aortic Valve
- Aorta

Cardiac Cycle

- **The sequence of events that occur when the heart beats.**
  - Diastole - Ventricles are relaxed.
  - Systole - Ventricles contract.
    - R.A. receives "used blood"
    - Blood pushed through the tricuspid valve to the R.V.
    - R.V. pumps to lungs through pulmonic valve to Pulmonary Arteries, providing blood to both lungs
    - Blood circulated through the lungs, CO_2 removed and O_2 added
    - Returns through Pulmonary Veins, empties into L.A.
    - Blood empties into the L.V.
    - L.A. pushes the Mitral Valve open.
    - As L.V. pumps, pressure closes mitral valve and opens aortic valve
    - Blood passes through to the Aorta, where it will be delivered to the rest of the body.

Myocyte Representation

FIG. 4



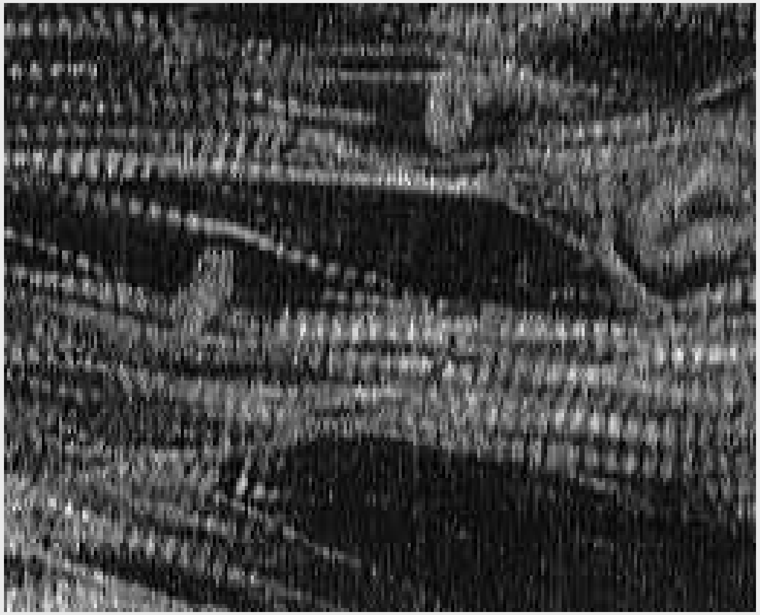


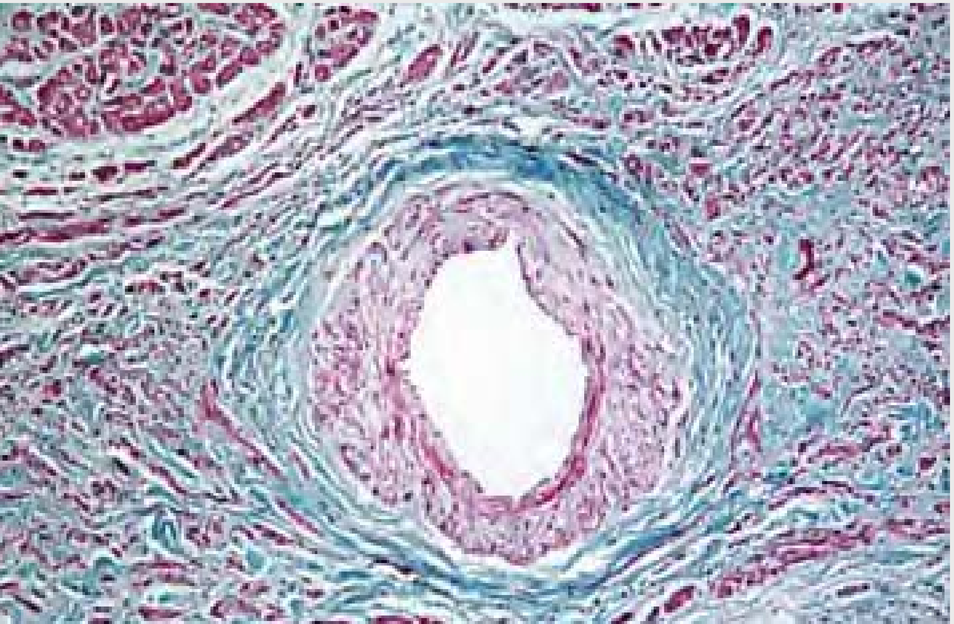
FIG. 5

Cardiac muscle in longitudinal section can be identified by centrally placed round to oblong nuclei, striations, branching, and intercalated discs

Cardiac Cell, Continued

- **S.A. NODE (PACEMAKER) CELLS**
  - Smaller than other cardiac cells
  - Weakly contractile
  - Unstable Resting Membrane Potential

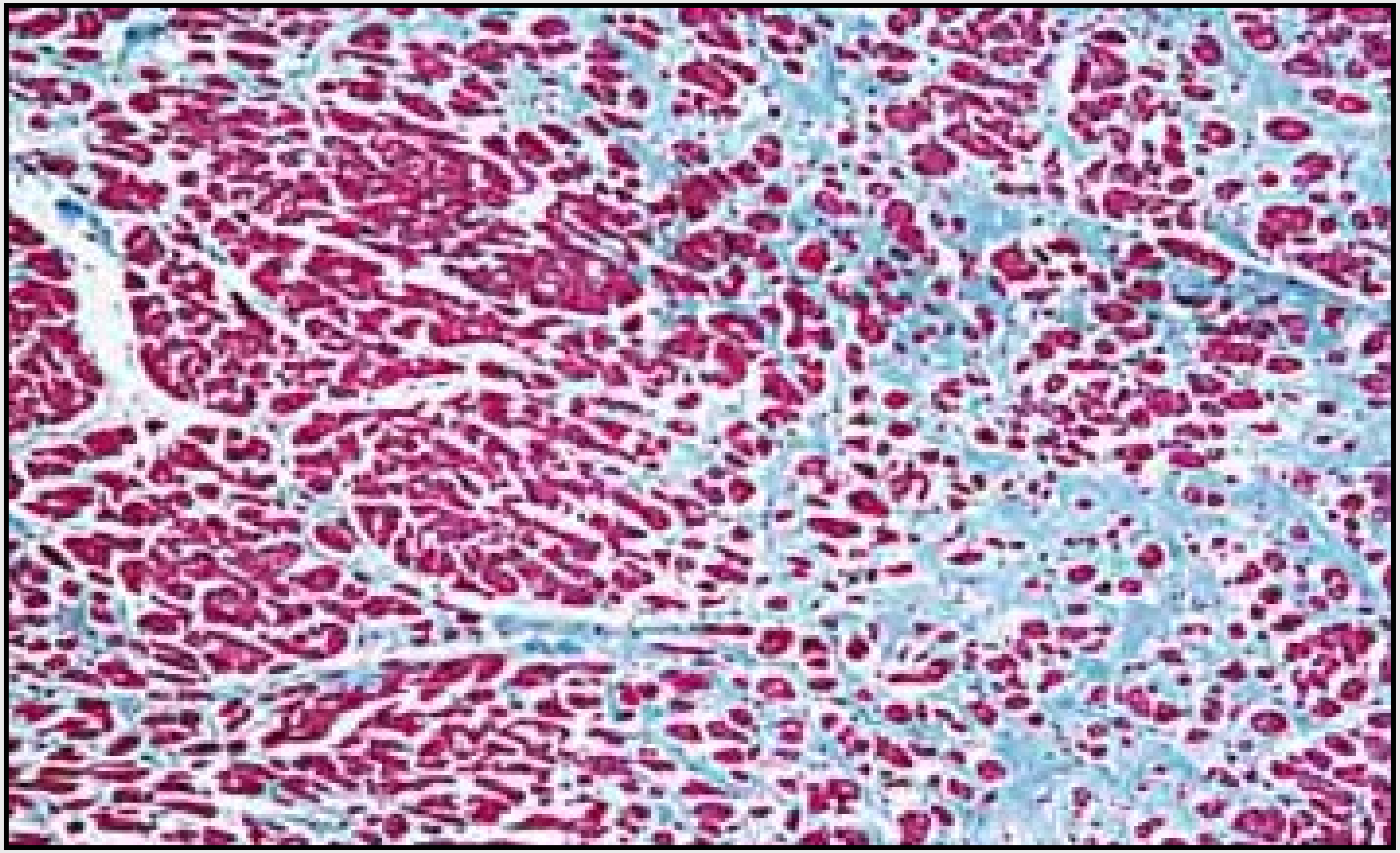
FIG. 6



The SA Node surrounds the nodal artery from which it receives a rich blood supply. These smaller, modified muscle cells generate the electrical signal that controls the heart.

Nodal vs. Myocytes

FIG. 7



This closer look more clearly shows the smaller nodal cells to the right and the larger cardiac cells to the left.

Intercalated Discs

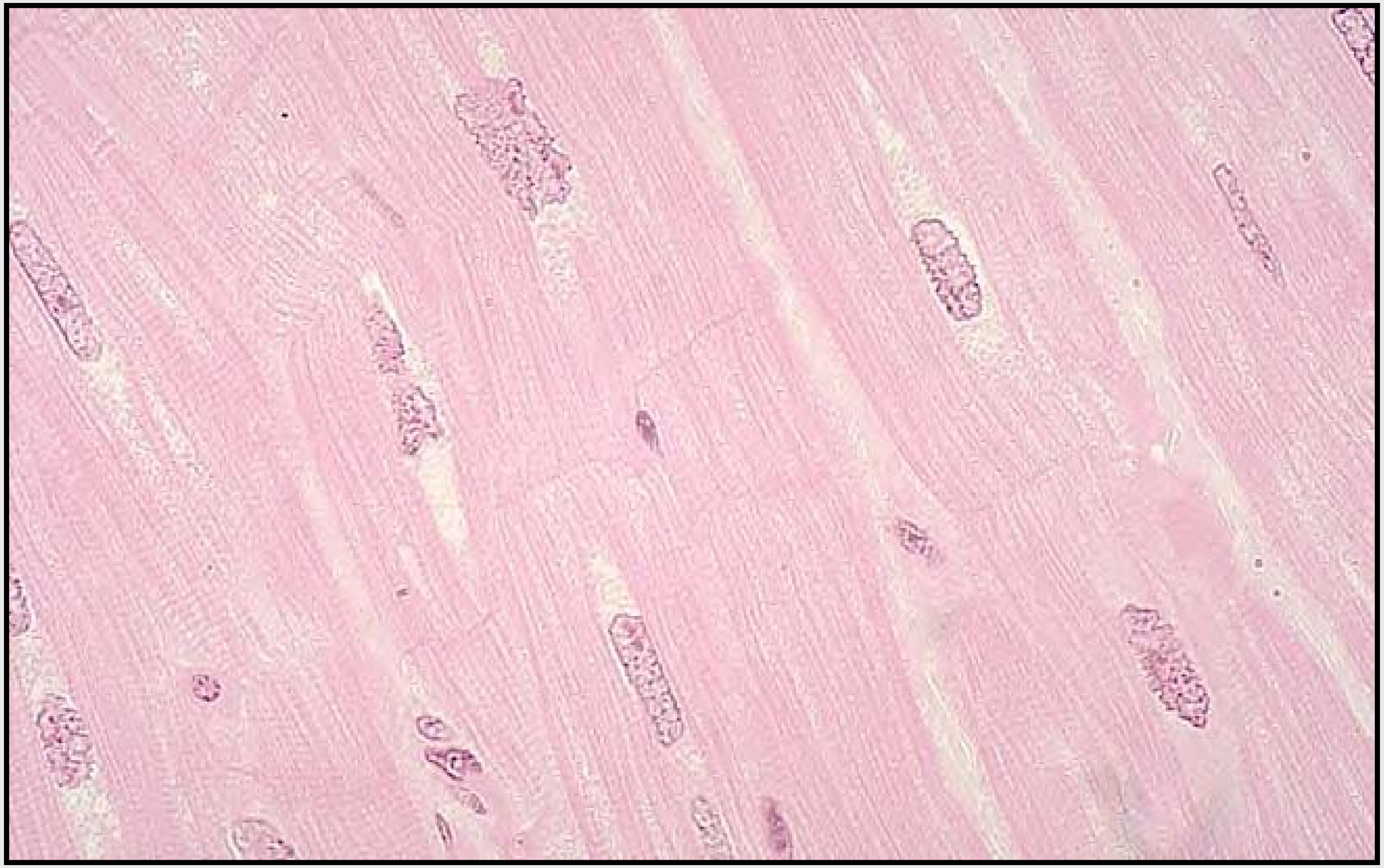


FIG. 8

Cells in Valves

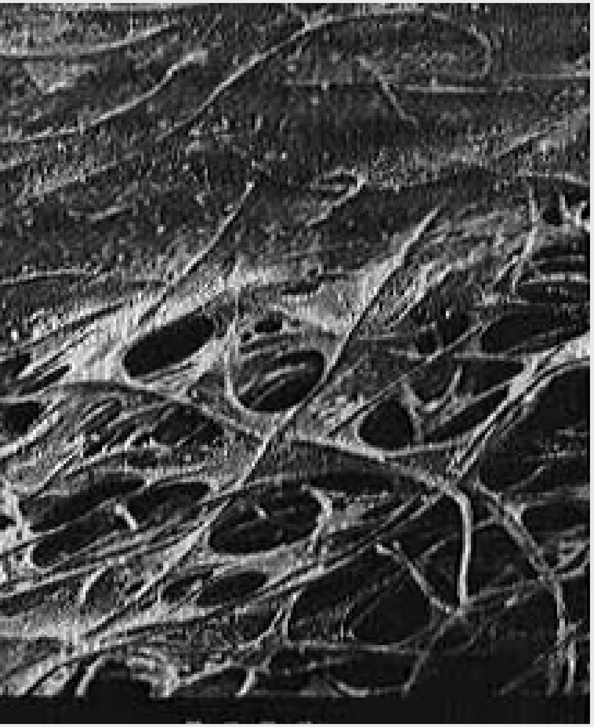


FIG. 9

- 3 distinct cell types found in both pulmonary and aortic valves:
  - Fibroblasts
  - Myofibroblasts
  - Fetal-type SM
    - Distribution related to valve layers

Image of fibroblast-polymer formation as seen via SEM; results of which made PGA-mesh feasible scaffold model for vessels or cardiac valves

Electrical Pathway

- **Heart contracts and relaxes automatically**
  - 72 times per minute
- **Signal for every contraction originate in Heart itself, in SA node**
  - Initiated by action potential
  - SA node depolarizes to threshold because node ‘leaky’ to sodium, + ions enter potential ↑
  - Threshold reached, AP fired
    - Signal travels from SA to atria
    - AV passes signal from atria to ventricles, via Bundle of His
    - Travels to both ventricles, via Purkinje fibers

Electrical Pathway

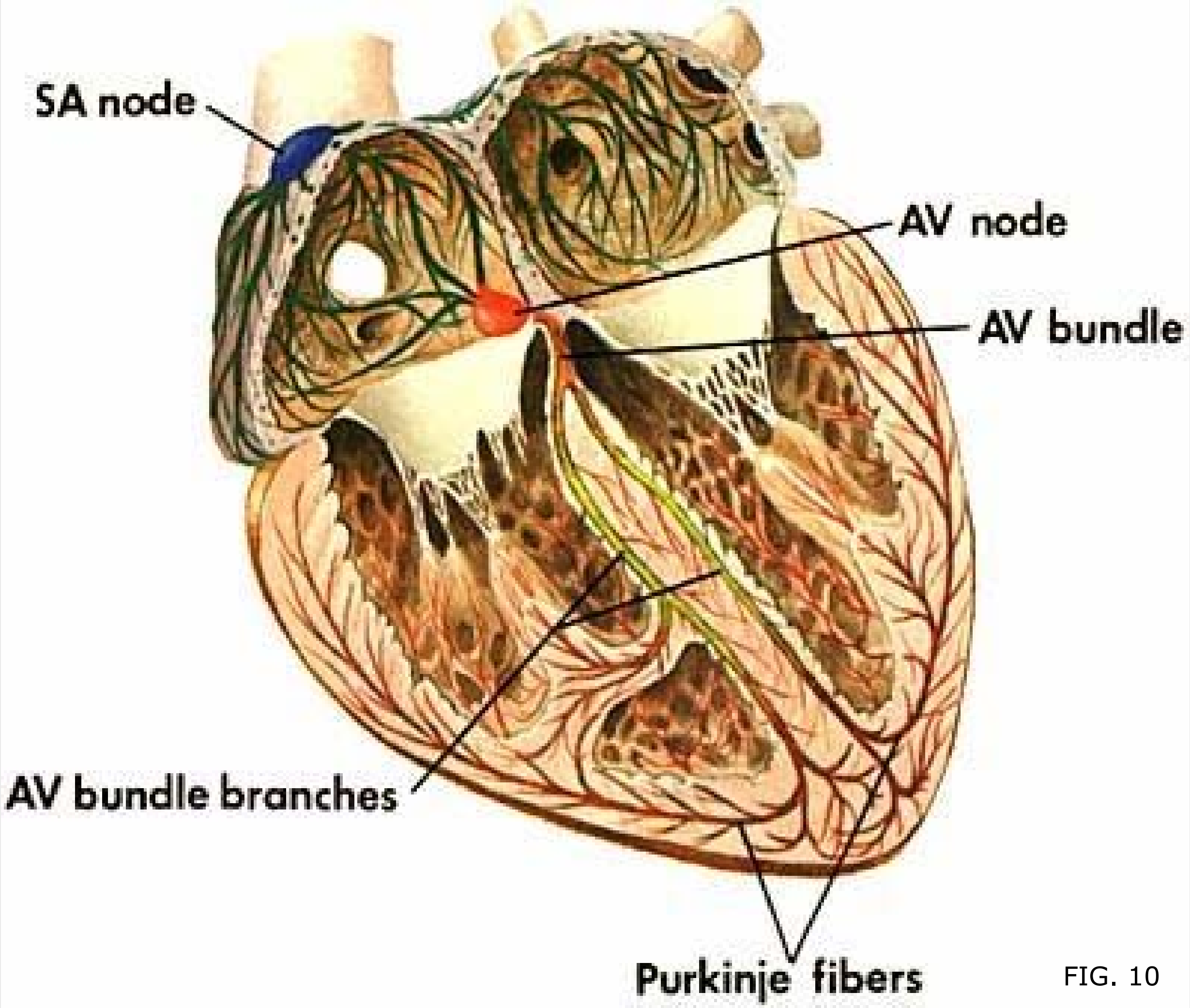


FIG. 10

Purkinje

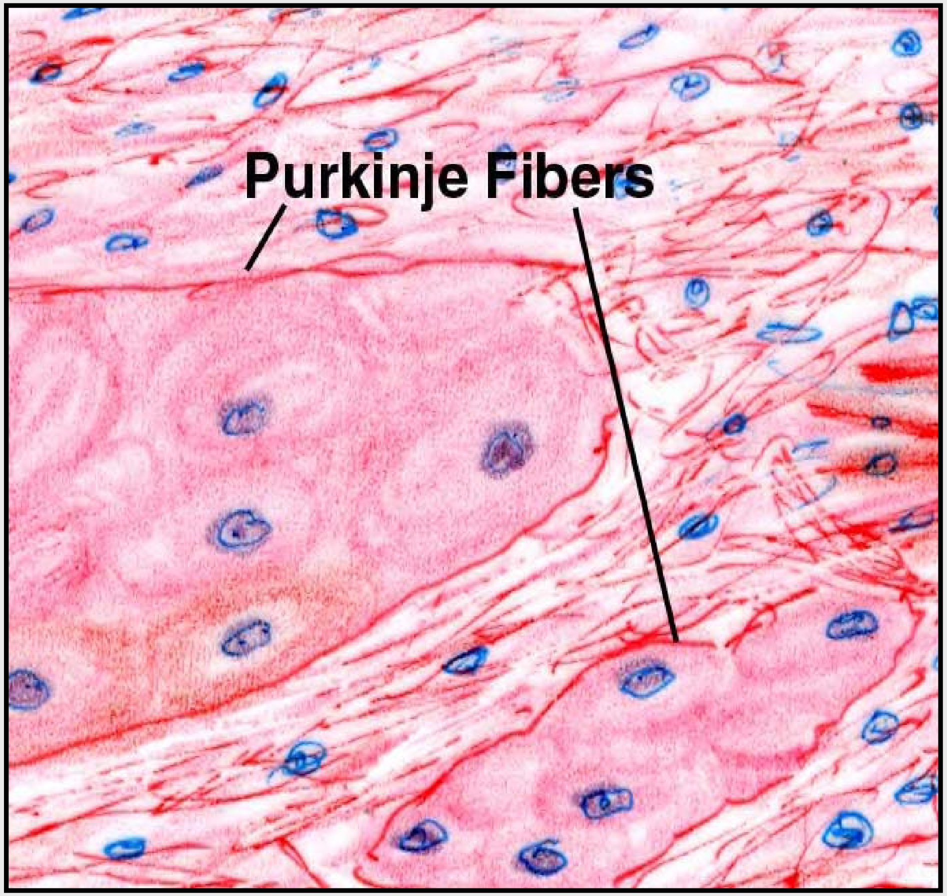


Fig. 11

Cardiac Cells and ECM

- ECM produced by fibroblastic cells surround cardiac myocytes.
- Fibroblasts
  - Synthesis/deposition of ECM components
  - Synthesis/release of enzymes for ECM turnover
  - Mechanical tension in collagen network
- ECM receptors
  - Integrins
  - Immunoglobulin superfamily
  - Cadherins
  - Selectins

Receptors on Cardiac Sarcolemma

- Alpha-receptors: causes vessel constriction
- Beta-receptors: vessel relaxing
- Subtype beta-1 found mostly in heart
- Bind catecholamines (nor/epi)

Receptors Expressed by CARDIAC MYOCYTES

Receptors		Cardiomyocyte	Mouse
Acetycholine	m1	X	
	m2	X	X
Adenosine	A1	X	
	A2a	X	
	A2b	X	X
α-Adrenergic	a_{1A}		X
	a_{2C}		X
β–Adrenergic	b_1	X	
	b_2	X	
Adernomedullin		X	
ANF		X	X
Angiotensis	AT1	X	
	AT2	X	

Receptors		Cardiomyocyte	Mouse
Ap4A		X	
ATP		X	
Bradykinin		X	
Calcitonin gene-related peptide		X	
cAMP		X	
Ciliary neurotrophic factor		X	
Corticotropin-releasing hormone		X	
Cytokines	cardiotrophin-1	X	
	Interferon g	X	
	IL-1b	X	
Dopamine	d1b	X	

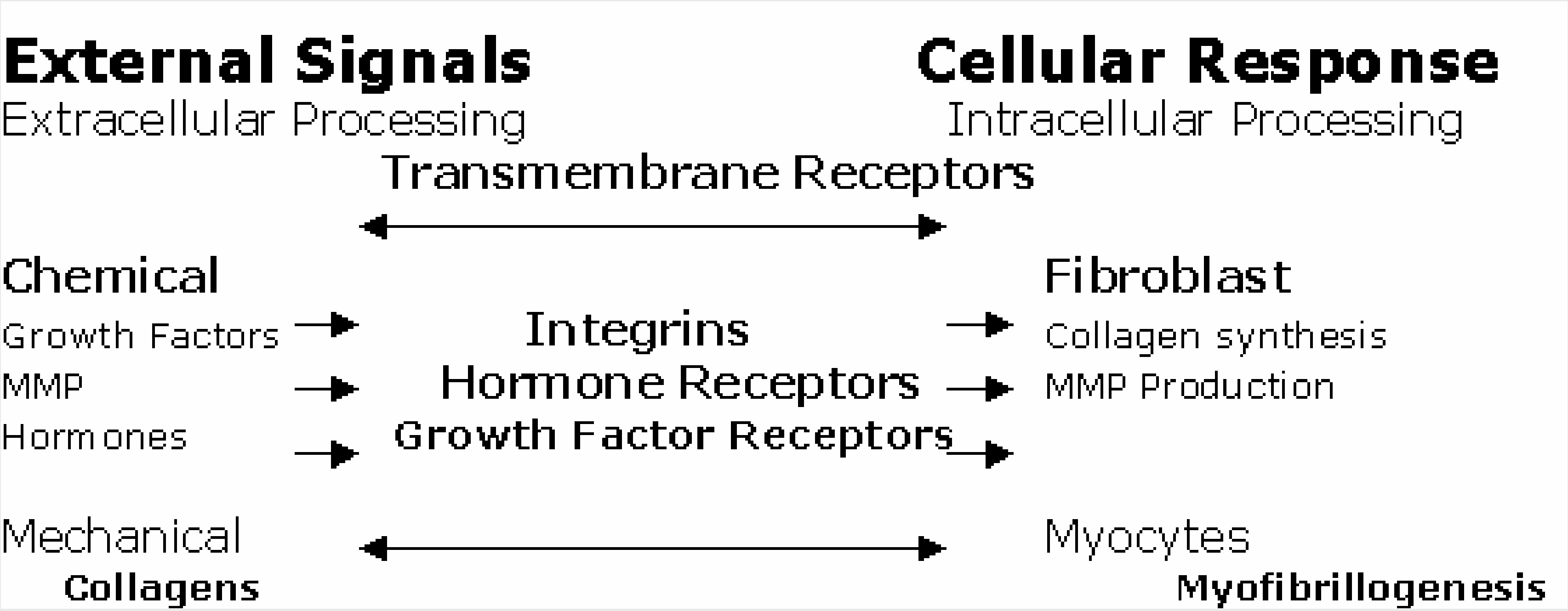
Cardiomyocyte Receptors, Cont'd.

Receptors		Cardiomyocyte	Mouse
Endothelin	ETA	X	
[Met5]Enkephalin		X	
Ephrin A5		X	X
Fatty Acids	bFGF-1		X
	bFGF-2	X	
Glucagon		X	
Histamine	H2		X
Insulin		X	
Insulin-like growth factor-1		X	
Insulin-like growth factor-2		X	
Neuregulin		X	
Neuropeptide	Y1	X	

Receptors		Cardiomyocyte	Mouse
Opioid	kappa	X	
	Delta	X	X
PAF		X	
PDGF		X	
PTHrp		X	
Prostaglandin	E1	X	
	F1a	X	
Serotonin(5-hydroxy-tryptamine)		X	X
Taurine		X	
TGFb1		X	
Thrombin		X	

Receptors		Cardiomyocyte	Mouse
Transferrin		X	
Urotensin II	PAR-1	X	
	PAR-2	X	
Vasopressin			X
VIP		X	

Dynamic Interaction[4]



Cardiac Integrins[5]

- Cardiac cell shape regulated by interactions of ECM and members of integrin family of matrix receptors.
- Phenotypic information stored in tertiary structure and chemical identity of ECM.
- Information actively communicated by $\alpha 1 \beta 1$ integrin molecule into intracellular signal that regulates cardiac cell shape and myofibrillar organization.
- Angiotensin II plays role in controlling cardiac looping and myofibril proliferation

Mechanical/Chemical Signaling

- Growth factors: PDGF, TGFβ, FGF, IGF role in cardiac development and maintenance adult phenotype
- Ang-II integration of mech/chem signaling: AT receptor activate G protein
- Stretch induced growth of cardiac myocyte/fibroblast mediated by Ang-II
- Antibodies block specific integrins and can block collagen gel formation
- Dynamic interactions

Nerve Supply of Heart

- Innervated by both sympathetic and parasmpathetic supply.
- Perhaps microchip can alleviate concerns in future.

Effects of Catecholamines and other compounds

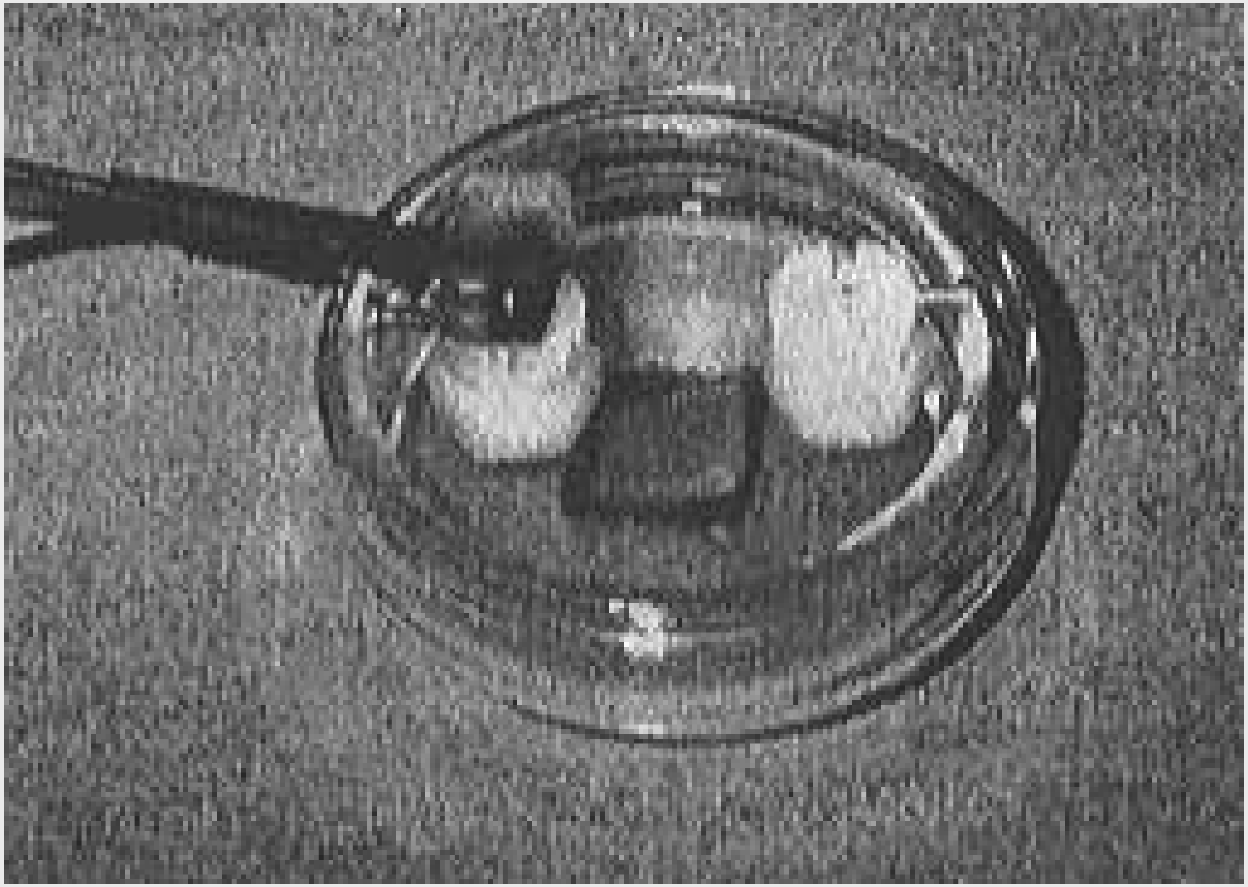
- Catecholamines:
  - Increased rate of myocardium contraction.
  - Increased force of contraction of the myocardium.
  - Increased speed of conduction of the wave of excitation over the heart.
- Other compounds with hormone-like effects on the heart:
  - Serotonin Acetycholine
  - Atropine Dopamine
  - Histamine Glucagon
  - Glutamate ANG-II
  - Bradykinin Vasopressin

Monitoring Cardiac Activity

- **Flow of Electricity → AV node initiation → AV delay → Conduction via Purkinje → Repolarization of Ventricle**

- **ELECTROCARDIOGRAM (E.C.G.)**
  - Measures electrical activity of the heart, but tells little about mechanical activity.

Monitoring Cellular Activity



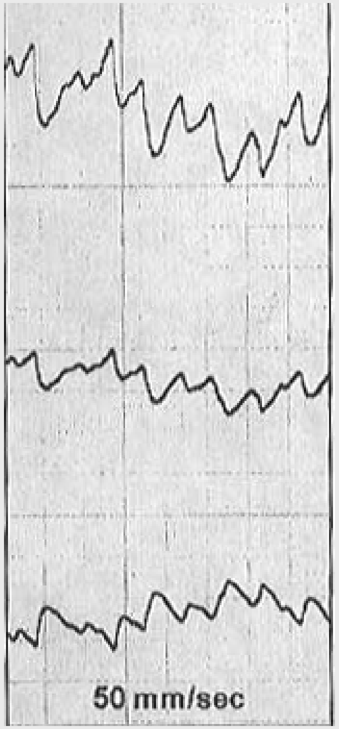


Fig. 12

Roles for Tissue Engineering in Cardiovascular System[6]

- Rapidly expanding field to address organ shortage problem
  - Since 1900, CVD #1 killer every year except 1918
  - Cost of transplants
  - Technical difficulty
  - Drug regimes to prevent organ rejection
- Goal: to "restore function through the delivery of living elements which become integrated into the patient"

Roles for T.E., cont'd.

- Creation of new tissues in cardiovascular surgery
  - Heart valves
  - Cardiac muscles
  - Myocardium
  - Pericardium
  - Blood Vessels
  - Cardiac Pacemaker

Key Issues and Concerns

- Scaffolding
- Cell Sources and Expansion
- Endothelial Cell Growth and Differentiation
- Myocyte Growth and Differentiation
- Heart Valves
- Angiogenesis
- Mechanical Analysis of Heart
- Electrical Modeling and Analysis
- Biocompatibility

Scaffolds in Cardiac T.E.[7]

- Scaffold plays central role in creation of 3D structure – why SF shape ?
  - Physical support and cell attachment template
- Properties
  - Tissue-like mechanical properties
  - Complete immunologic integrity
  - Nontoxic
  - Mechanical/chemical properties (e.g. GF) should be modifiable
  - Control of degradation
  - Low diffusion barrier

Examples of Scaffolds

- Synthetic polymers : PUR, PGA, PLA, PHB, blocks copolymers,polyanhydrides, polyortho esters
- Natural polymers: chitosan, glycosaminoglycans,collagen, fibrin, collagen, hyaluronan
- Biological: acellularized porcine aortic conduits
- Inorganic: TCP, calcium carbonate, nonsintered hydroxapatite
- Composites

Fibrin Gel[8]

- Idea: Produce complete structure in one part from biodegradable polymer in injection molding technique
  - Seeded w/ myofibroblasts
- Can be produced from patients' own blood as scaffold, and can be seeded with fibroblasts
- = No immune reaction since autologous scaffold
- Characterization of Fibrin Gel
  - Degradation by plasminogen in culture can be successfully controlled via aprotinin
  - Moldable

PHA Scaffold [9]

- Bacterially derived polyester used as scaffold in conjunction w/ bioreactor
- Harvested cells from ovine carotid artery
- Seeded scaffold w/ 8million vascular cells
- After 5 days exposed to pulsatile flow, placed in bioreactor

Other Approaches

- Innovation to overcome Scaffold limitations
  - Problem not growth, but after certain size begin to kill cells on interior due to lack of nutrients
  - Cardiac tissue reconstruction based on cell sheet engineering (Japan)
  - Polymer Scaffold and Bioreactor Vessels (MIT Scientists and NASA)

Japan and Germany [10]

Germany

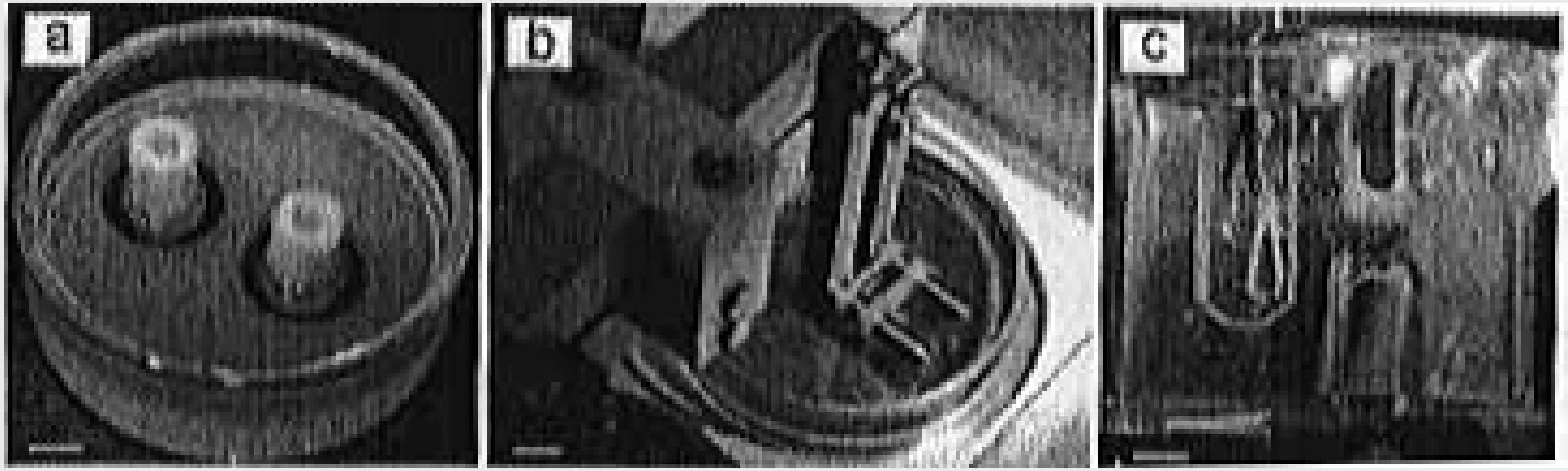


Fig. 13

Japan

No scaffold – cell sheets made on culture dish – harvesting of cell sheets makes 3D structures

MIT and NASA[11]

- Approach: Seed cardiac cells onto 3D polymer scaffold that slowly degrades as cells develop into tissue
- 1994, cells began beating as one, Lisa Freed, team leader @ Harvard-MIT
- Since then active investigations to characterize tissues' structural and electrical properties and define parameters for growth
- First in-space t.e. experiment
  - Cardiac cells cultivated on 5mm diameter scaffold – cell/scaffold placed in bioreactor
  - Coined phrase "patches for broken heart"
    - 5million heart cells

Microgravity T.E.

Fig. 14

- NASA developed rotating bioreactor
- Bioreactors of this configuration adapted for t.e. by adjusting speed of rotation to maintain large tissues (disks 5 to 10 mm diameter and 1 to 5 mm thick) freely suspended during culturing.
- Engineered cartilage capable of withstanding mechanical loading and cardiac tissue that contracted in response to electrical stimuli were grown in these reactors.
- **Valve materials grown in reactor have twice the mechanical strength and secrete more collagen, elastin than those grown in petri dish**

Results from Microgravity

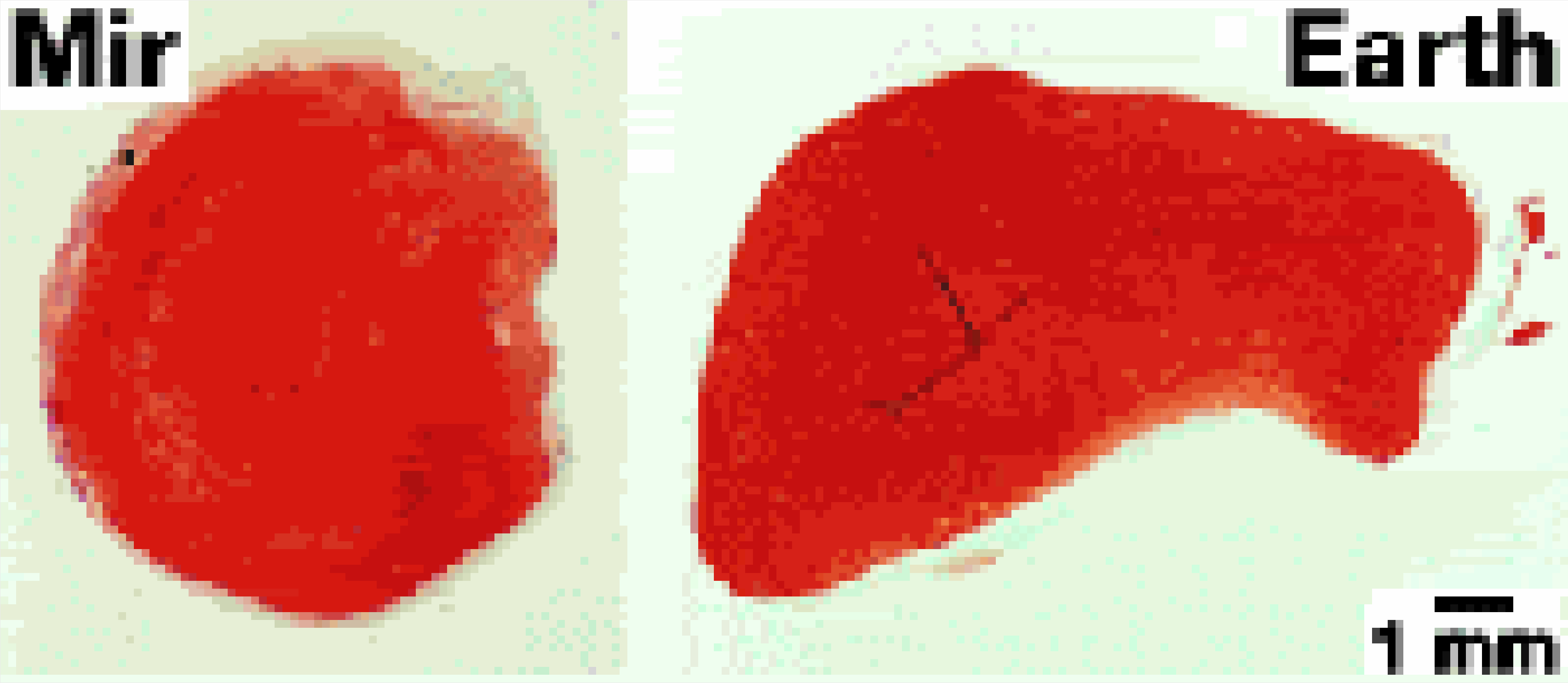


Fig. 15

Cardiac Patches a Reality

- Attach cells to fibrous polymer mesh
- Allow to attach on all surfaces and pores
- Scaffold containing cells placed in NASA bioreactor
- In 1 week, the 5million cells begin forming connections
- Patch of tissue 5mm diameter, 1/10 mm thick – beat spontaneously – very sensitive to stimuli –
- Want to make thicker by building conduits

Cardiac Patch

Fig. 16

Stem Cell Potential

- Lack of proliferative activity of adult cardiomyocytes
  - Look to Stem cells
    - Researchers regenerate heart tissue in mice with human adult stem cells
    - Potential for ES to divide and differentiate into cardiomyocytes in myocardial environment.
    - Calcium plays important role for ES differentiation.
    - Trying to isolate molecular markers
    - Calreticulin sequesters CA in embryonic stores
    - Increase number of cardiomyocytes by increasing markers = accelerated differentiation.
    - Overexpression of MLC promoters (accelerate differentiation
    - Increase cell number (EGFP expression

Current Research[12]

- Novel bioartificial myocardial tissue
  - Obstacle:lack of proliferative adult myocyte activity
  - Generally cell-polymer scaffolds have weak integrity, short contractility, inhomogeneous seeding, etc.
  - Used collagen-matrix, microscopic assessment of cell distribution, tested activity via electrodes
  - New doors for myocardial tissue replacement
- Cardiac Pacemaker
  - Wanted to determine if transplanted cells contribute to contraction/electrical excitation of host myocardium.
    - Fetal canine atrial myocytes delivered into L.V.
    - After 4weeks, e.p mapping
    - Expression of Connexin-43 between donor and recipient suggest formation of gap junctions.

Cardiac Tissue Engineering Summary

- Cardiomyocytes → Scaffolds → Biografts → Transplantation/Patient
- Materials Used:
  - Polystyrenes, PMMA, PDMS, Collagen Matrices
- Clinical Benefits:
  - Possibly better incorporation ?
  - Increased longevity ?
- Problems to Overcome:
  - Large scale production
  - Antigenicity issues

Suggestion

- Fibrin gel or collagen gel scaffold
- Densely populated with umbilical artery smooth muscle cells, lined with human endothelial cells on one side with fibroblasts on the other
- Bioreactor
- In vivo testing

Further Areas of Investigation

- Optimize scaffold porosity and design growth factor release method
- Mass production of vascular cells from stem cells
- Integrate angiogenic factors into scaffold
- Future
  - Biomedical engineers grow new hearts using cells taken from the patients themselves as seeds. Such hearts would be young, alive, and welcomed by the immune systems of their recipients.

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