Tissue Engineering in Microgravity

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Why Tissue Engineering?

- Millions suffer tissue or organ loss from diseases and accidents every year
  - Yearly cost of treatment exceeds $400 billion
- Major medical treatment is transplantation
  - Shortages of replacement tissue and organs
- Development of Alternative Sources for Transplantations by Engineering Tissue
- In vitro tissue models may allow better understanding of disease pathology to avoid organ failure

Biomedical Applications of Tissue Engineering

- **In vitro Growth of Tissues for Implantation**
  - **Replacement of Diseased or Damaged Tissues**
    - » Skin replacement for treatment of serious burns
- **Extracorporeal Support**
  - **External Devices Containing Tissue that Replace the Function of Internal Organ**
    - » Artificial liver
- **Human Disease Models**
  - **Differentiated Tissues for Pathogen Propagation**
    - » Models for HIV, Cyclospora
  - **Three-Dimensional Cancer Models**
    - » Prostate, Colon

Biomedical Applications of Tissue Engineering

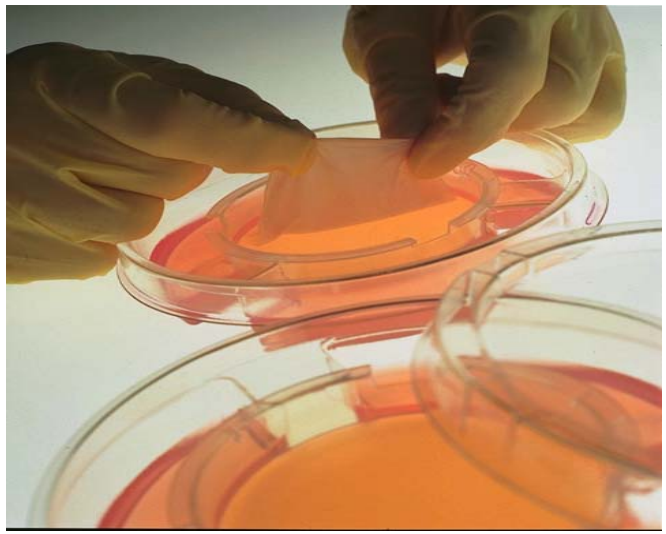
- **Drug Testing and Development**
  - **New Tissue models for drug development**
    - » Renal Toxicity, Heart
- **Biomaterial-guided Tissue Regeneration**
  - **Implantation of Biomaterials to Induce Tissue Regeneration**
    - » Absorbable collagen matrix for guiding tissue regeneration in periodontal surgery.

Immune System Problems

- Immunosuppressive Drugs
  - Serious Complications
- Autologous
  - Use the person's cells
  - Best approach if possible
- Encapsulation: Immunoisolation
  - Biopolymer coating to keep immune system out
  - Pancreatic Islets
    - » 1-2% of Pancreatic Volume
- Future: Genetically Modified Cells
  - Major Histocompatibility Complex Genes
  - Mesenchymal Stem Cells

Current Commercial Products

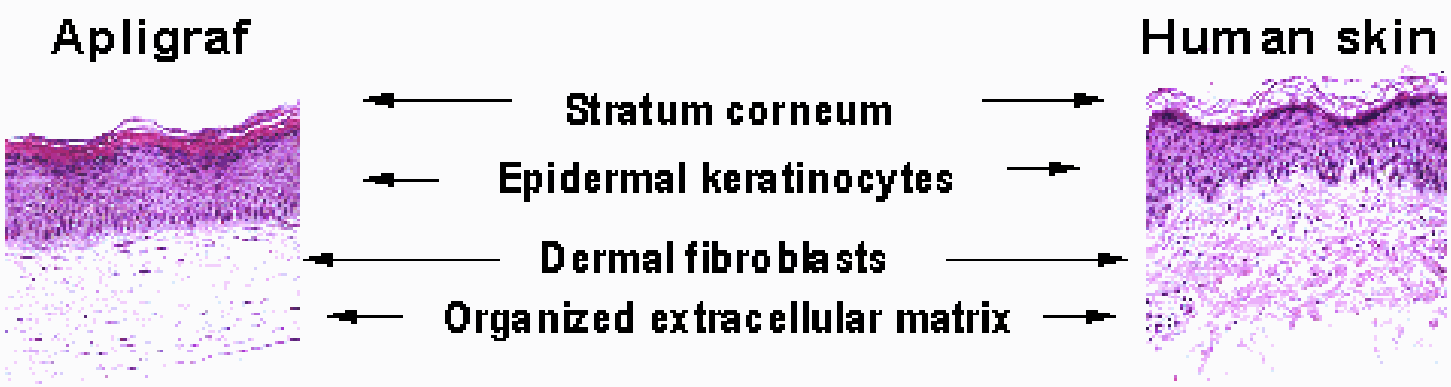
- **Human Skin Equivalent with Cells**
  - **Autologous**
    - » Genzyme (Epicel): Epidermal Grafts
    - » 16 Days
    - » Close a serious burn wound: If you live
    - » Currently one layer (two layer: strength)
  - **Neonatal foreskin**
    - » Used for skin ulcers
    - » Stimulates the host tissue to regenerate: Not there at end
    - » Advanced Tissue Science: Dermagraft (frozen)
    - » Organogenesis: Apligraf - Two layers



- **Cell-based Procedure to Repair Knee Injuries**
  - **Autologous**
    - » Genzyme (Carticel)
    - » Inject chondrocytes under periosteal flap: Jury is out

Human Skin Equivalent

- **Organogenesis Inc.**
  - Apligraf: skin construct with upper epidermal and lower dermal layer comprised of viable human skin cells
  - No blood vessels, hair follicles, sweat glands, melanocytes
  - 20 Days to produce product



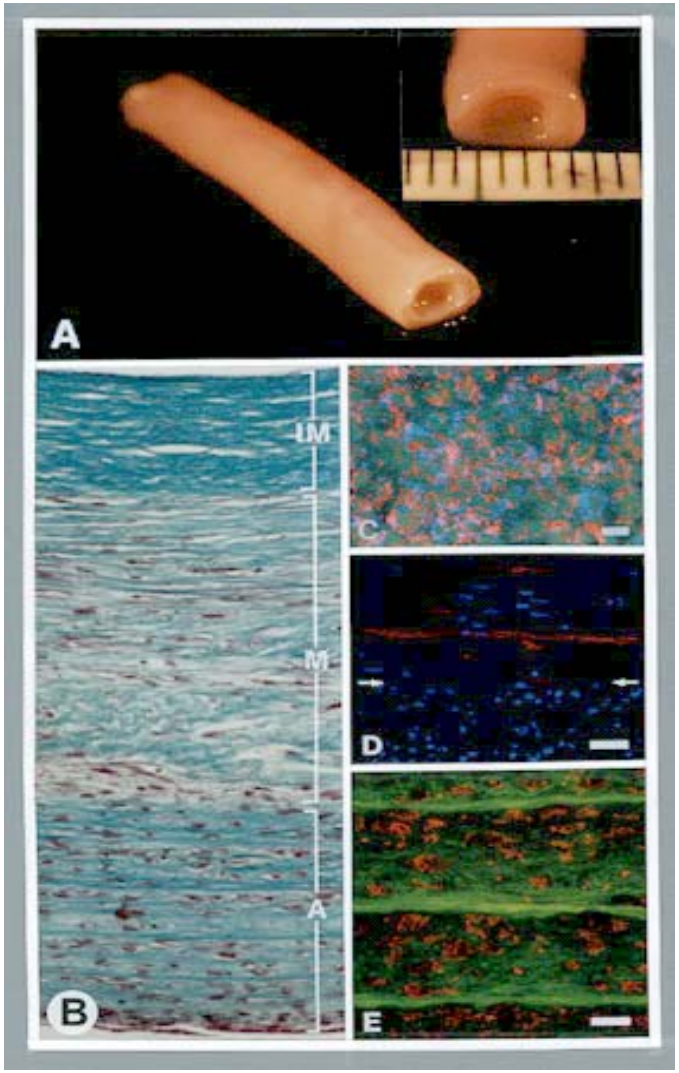
Apligraf compared to human skin under a microscope.

Growth Approach is Important

- Monolayer Growth
  - Can be applied to cell proliferation
  - Monolayers can be used to buildup tissue
- Perfused Systems
  - Force Fluid through the tissue
  - Support larger tissue constructs
  - Provide asymmetrical growth conditions
  - Mechanical loads
  - Pulsed-flow
- Coupling of monolayer proliferation and perfused systems
  - Blood vessels

Blood Vessel Formation

- Monolayer Technique
  - Build Tissue Layer by Layer
- Grow Tissues Independently
  - Vascular smooth muscle cells
  - Fibroblasts
  - Endothelial: seeded on lumen
- Three-layer Structure
  - ECM with elastin
- Differentiation Markers
  - Desmin
- Burst strength comparable to native blood vessels

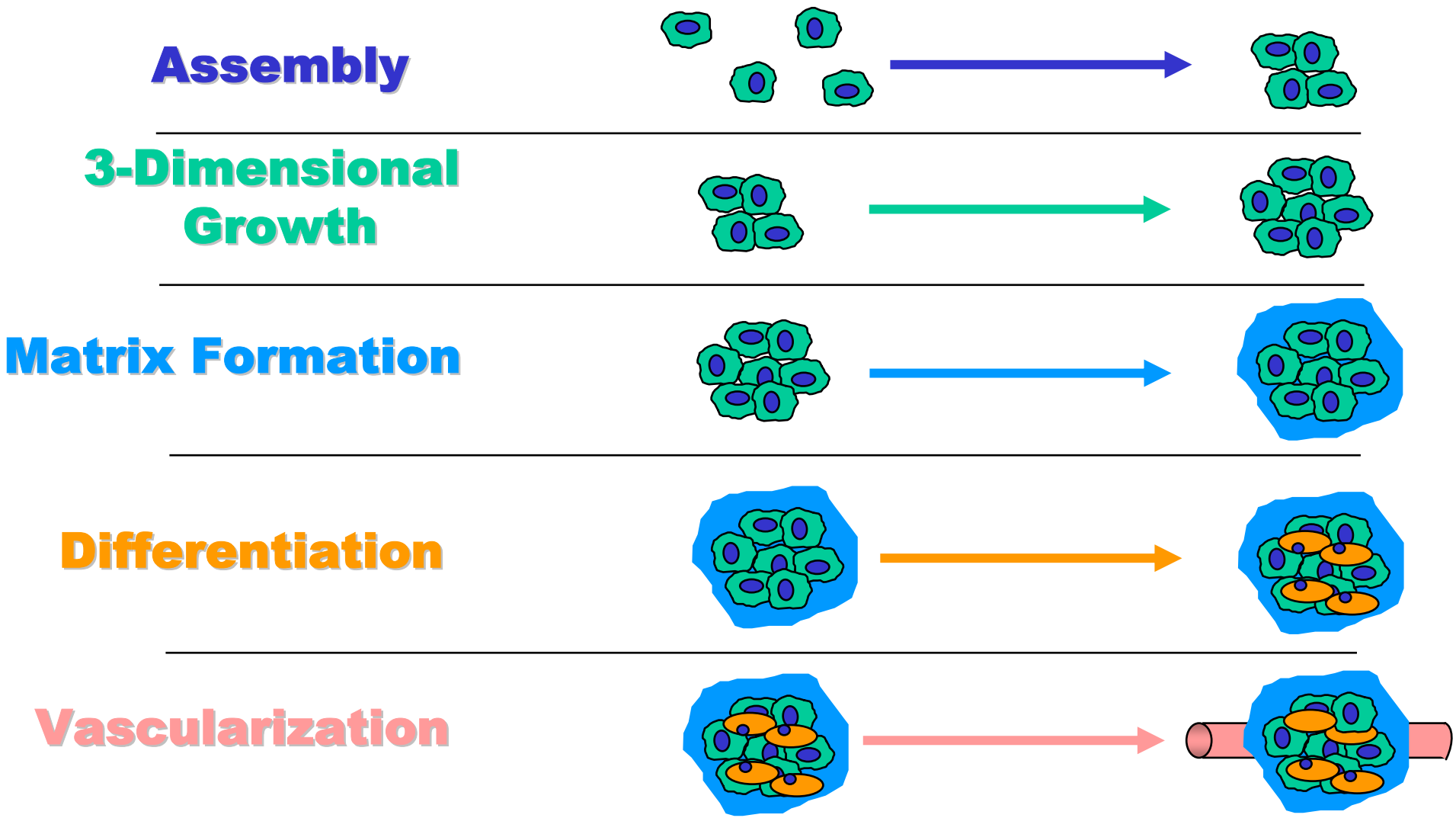


FASEB Vol. 12, 1998, p 47

Cellular Requirements for Engineering Tissue

- Proliferation of Cells Required
  - Start with limited number of cells
  - Expand large number of times
- Cellular Assembly into 3-D Constructs
  - Cell-matrix adhesion: intergrins
  - Cell-cell adhesion: cadherins
  - Intercellular Junction Formation
- ECM formation Required
- Differentiation Required
- Angiogenesis
  - Co-culture with endothelial cells
- Innervation

Tissue Engineering in 5 Steps

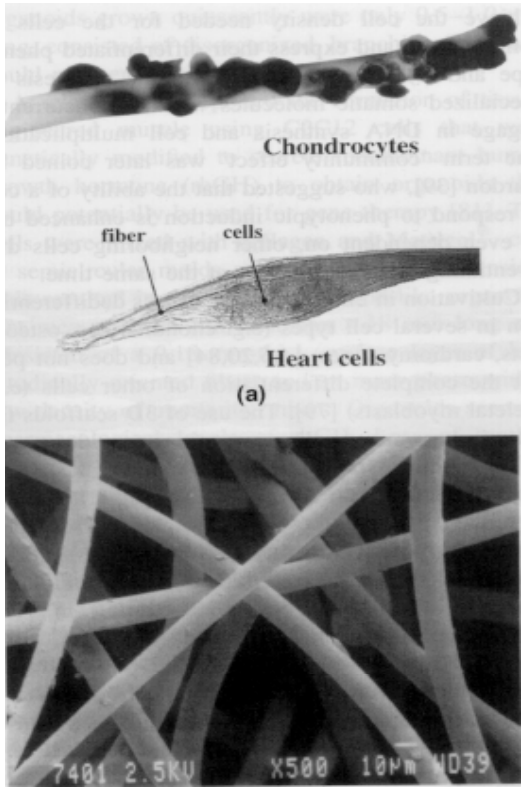


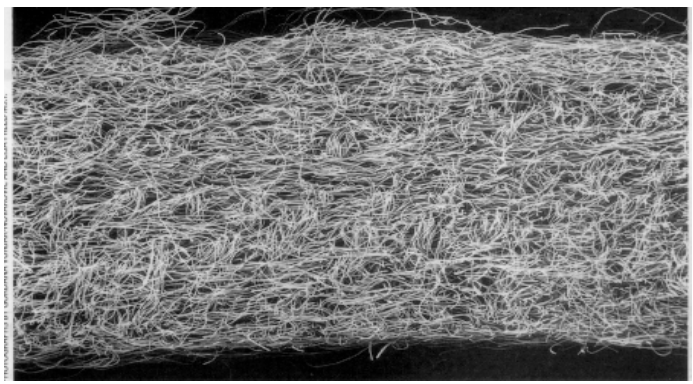
Four Primary Requirements for Engineering Tissue

- Cell Source
  - Proliferation and Differentiation Required
  - Pluri-potent Stem Cells
- Biomaterial Scaffold: Biopolymers
  - Provides Appropriate Substratum to Support Cell-cell, Cell-matrix Interactions
- Bioreactors
  - Maintains Physiological Conditions
  - Uniform Concentrations of Gases and Nutrients
- Specific Factors
  - Growth factors, hormones, metabolites
  - Depends on tissue type and developmental stage

Scaffolds for Tissue Engineering

- **Desirable Properties**
  - **Biodegradable & Biocompatible**
  - **Highly porous**
    - » High permeability (PGA: 97% Porous)
  - **Cell adhesion**
    - » ECM establishes adhesion (fibronection)
    - » Strengthened by CAM's (cadherins)
  - **Tailor and control**
    - » Shape, strength, speed of degradation, and microstructure
  - **Mimic natural materials**
    - » Fibronectin RGD sequence in polymers improves cell adhesion
  - **Materials**
    - » Suture material: polyglycolic acid
    - » Collagen, Alginate, Hyaluronic Acid





Limitations on Engineered Tissue Size

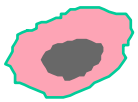
- **Tissue Size is Limited By**
  - **Transport of nutrients and gases**
  - **Metabolic rates of component cells and permeability of the construct**
- **Angiogenesis**
  - **Most cells are no more than 100 um from the nearest capillary**
  - **Capillaries: effective mass transfer**
    - » small diameter (6-8 um)
    - » Residence time of blood is greater than radial diffusion time
- **Mixed and Perfused Systems**
  - **Force flow of fluid through tissue**
  - **Too much fluid shear damages cells and tissues**

Engineered Tissue Thickness

- Total Cardiac Output Recieved
  - Skeletal & cardiac muscle ~ 25% (~75% strenuous exercise)
  - Cartilage ~ 2%
  - Bone ~ 10%
- Tissue that are normally vascularized
  - Bone, muscle
    - » Mass transfer limited
- Avascular cartilage
- Current thickness of engineered tissues
  - Cartilage ~ 5 mm
    - » Thickness is appropriate for human articular cartilage repair
  - Bone-like ~ .5 mm
  - Cardic-like ~ .18 mm

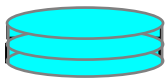
Cell-Polymer-Bioreactor System

Precursor Cells:



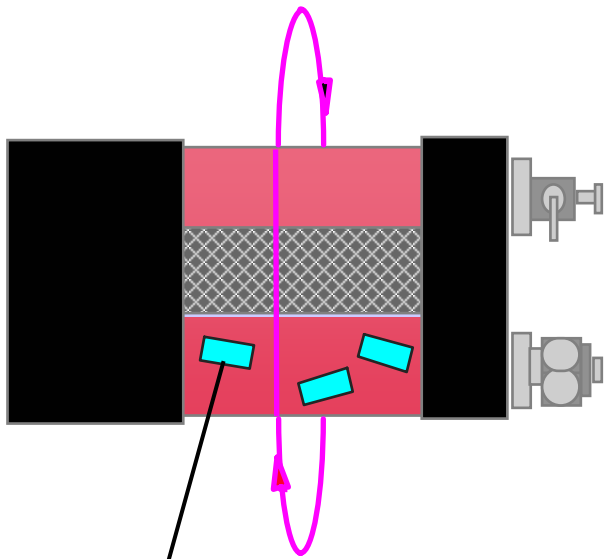
Polymer scaffold:

e.g. fibrous mesh
or porous sponge



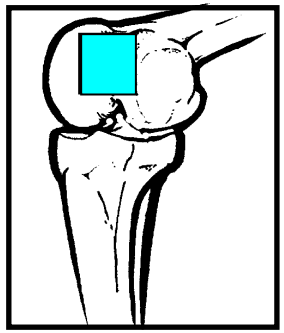
Bioreactor:

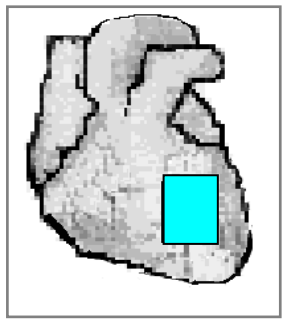
e.g. rotating vessel



Engineered Tissue Construct

Applications:





Cartilage Cell Sources

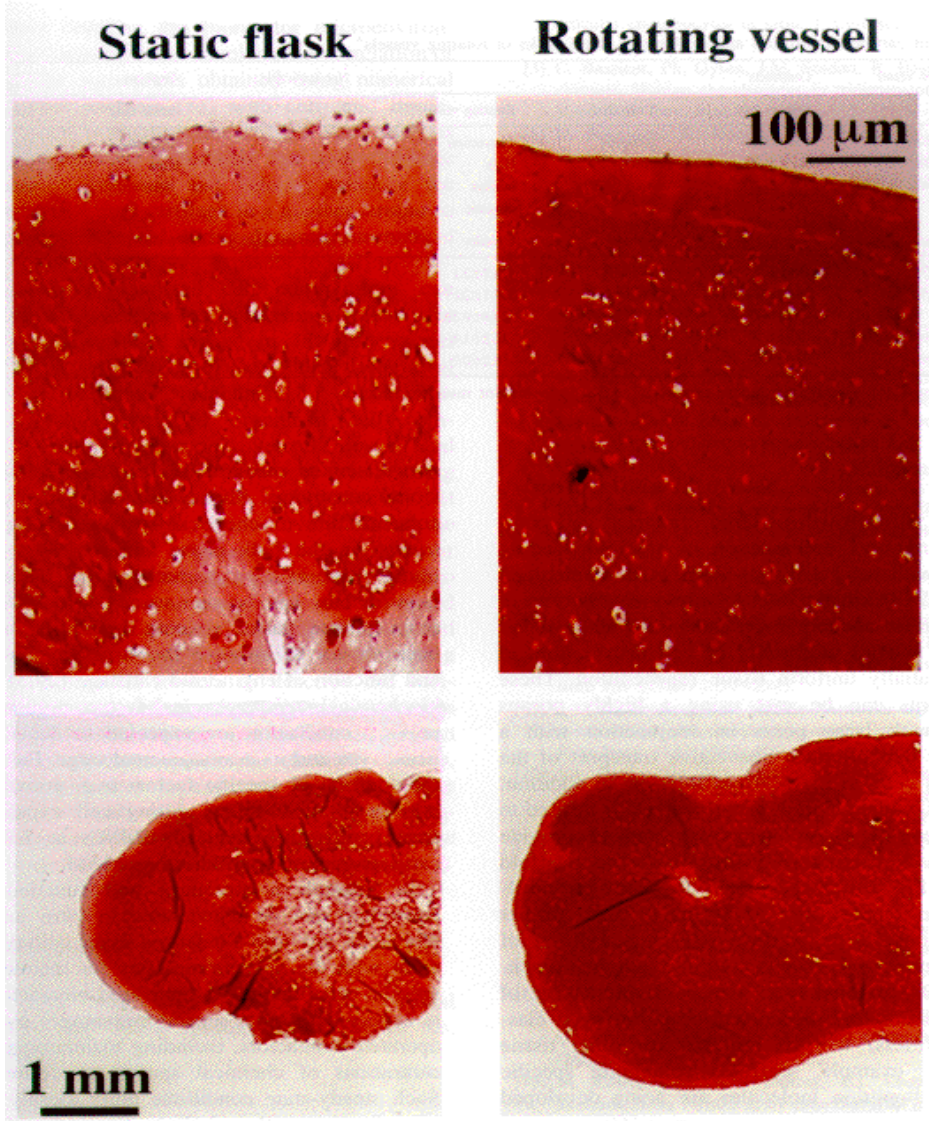
- Need sufficient number of chondrogenic cells
  - Cell density plays critical role in the initiation of chondrogenesis
- Chondrocytes
  - Bovine calves
  - Obtained from articular cartilage and expanded
- Bone Marrow Stromal Cells
  - Differentiate into several mesenchymal lineages
    - » Osteoblasts, chrondrocytes, adipocytes, myocytes
  - Involved in natural repair of tissue
  - Growth factors required (FGF-2, TGF-beta 1)
  - Embryonic chicks and bovine calves

Advantages of Bone Marrow Stromal Cells

- Low numbers of cells required
  - Rapidly expanded in monolayers and maintain differentiation potential
- Relative simplicity of the procedure to harvest bone marrow
- High biosynthetic activity in older individuals
- Possibility of engineering composites of bone and cartilage for osteochondral defects

Tissue Engineering of Cartilage

- **Cell Seeding Density and Assembly**
  - **Critical to promoting cell-cell contacts**
    - » 4 to 10 million per 10x5mm scaffold
    - » At Lower seeding levels, insufficient ECM produced and construct loses structural integrity
  - **Associated with high rates of ECM biosynthesis chondrocytes**
  - **Expression of a chondrogenic phenotype by progenitor cells in marrow**



Effects of Media on Engineered Cartilage

TABLE 1. Effects of Medium Composition on Construct Properties

Measured Parameter	Group 1	Group 2
Medium composition (over 5 weeks)		
Oxygen tension (mm Hg)	86.5 ± 7.3 (60)*	42.7 ± 4.5 (60)
pH	6.98 ± 0.07 (60)*	6.73 ± 0.09 (60)
Lactate to glucose ratio (mol/mol)	1.65	2.17
Construct properties (at 5 weeks)		
Wet weight (mg)	139 ± 12 (3)*	101 ± 8.0 (3)
Cells (millions per construct)	13.5 ± 1.29 (3)*	9.96 ± 1.52 (3)
Glycosaminoglycan (% of wet weight)	4.18 ± 0.22 (3)*	3.07 ± 0.28 (3)
Total collagen (% of wet weight)	2.76 ± 0.03*	0.77 ± 0.03 (3)
Macromolecular incorporation of $^{35}SO_4$ (ng/μgDNA day)	110 ± 17 (3)*	37 ± 1.0 (3)
Macromolecular incorporation of ^{3}H (ng/μgDNA day)	104 ± 21 (3)	117 ± 8.0 (3)
Fraction of incorporated ^{3}H in hydroxyproline (% of total)	21.1 ± 0.8 (3)*	4.7 ± 1.2 (3)

The number of samples is given in parentheses.

*Significant difference between groups.

Effects Bioreactor Vessel on Engineered Cartilage

TABLE 2. Effects of Bioreactor Vessel and Cultivation Time on Construct Properties

Construct Culture Vessel and Time (versus native articular cartilage)	Glycosaminoglycan (% of wet weight)	Total Collagen (% of wet weight)	Equilibrium Modulus (MPa)
Static flask, 6 weeks constructs	2.73 ± 0.20 (6)	1.41 ± 0.08 (6)	0.053 ± 0.011 (3)
Mixed flask, 6 weeks constructs	2.19 ± 0.17 (6)	2.74 ± 0.16 (6)	0.051 ± 0.004 (4)
Rotating bioreactor, 6 weeks constructs	4.71 ± 0.41 (6)†	3.79 ± 0.05 (6)†	0.172 ± 0.035 (4)†
Rotating bioreactor, 3 day constructs	0.71 ± 0.03 (3)	0.48 ± 0.08 (3)	~ 0
Rotating bioreactor, 6 week constructs	4.71 ± 0.41 (6)	3.79 ± 0.05 (6)	0.172 ± 0.035 (4)
Rotating bioreactor, 3 month constructs	6.03 ± 0.84 (3)	2.7 ± 0.75 (3)	0.108 ± 0.047 (2)
Rotating bioreactor, 7 month constructs	8.83 ± 0.93*,‡	3.68 ± 0.27 (3)‡	0.932 ± 0.049 (3)*
Bovine calf cartilage, freshly explanted	6.81 ± 1.12 (24)	9.69 ± 1.68 (24)	0.939 ± 0.026 (6)

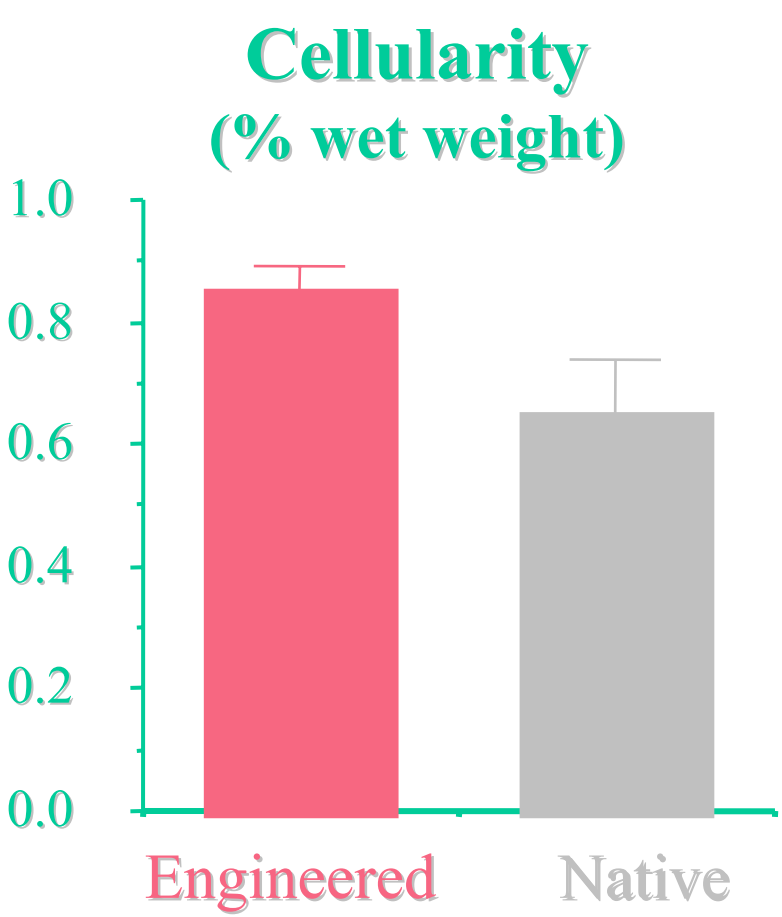
The number of samples is given in parentheses.

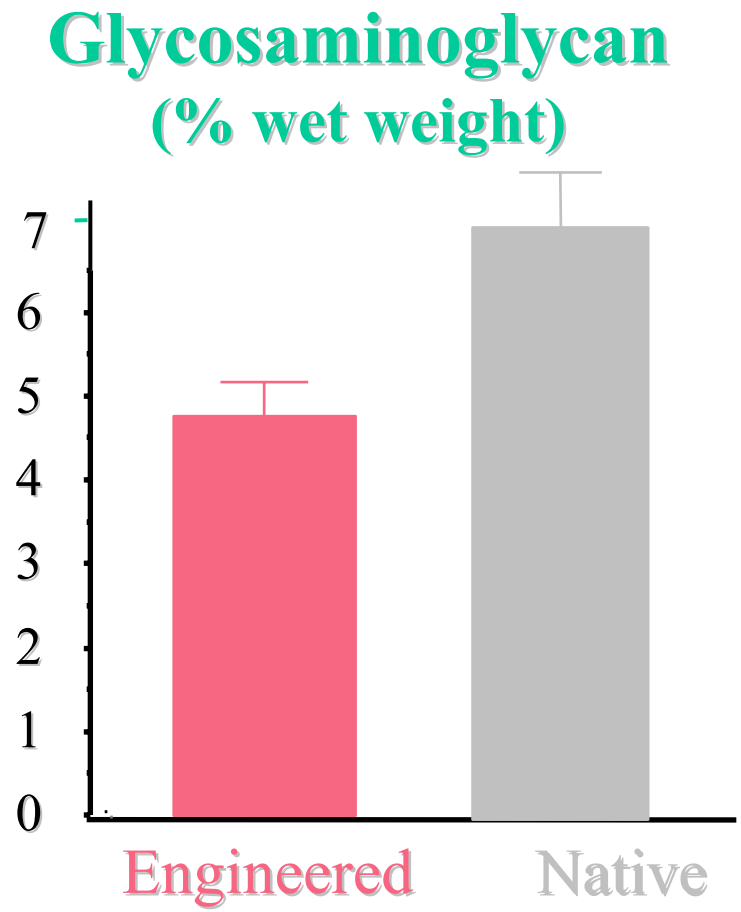
*Significant difference between constructs cultured for 7 months and those cultured in rotating bioreactors for shorter times.

†Significant difference between constructs cultured in rotating bioreactors and those cultured in either static flasks or mixed flasks.

‡Significant difference between 7-month constructs and native articular cartilage.

Engineered* vs. native cartilage
(*cultured 6 weeks in rotating bioreactors)

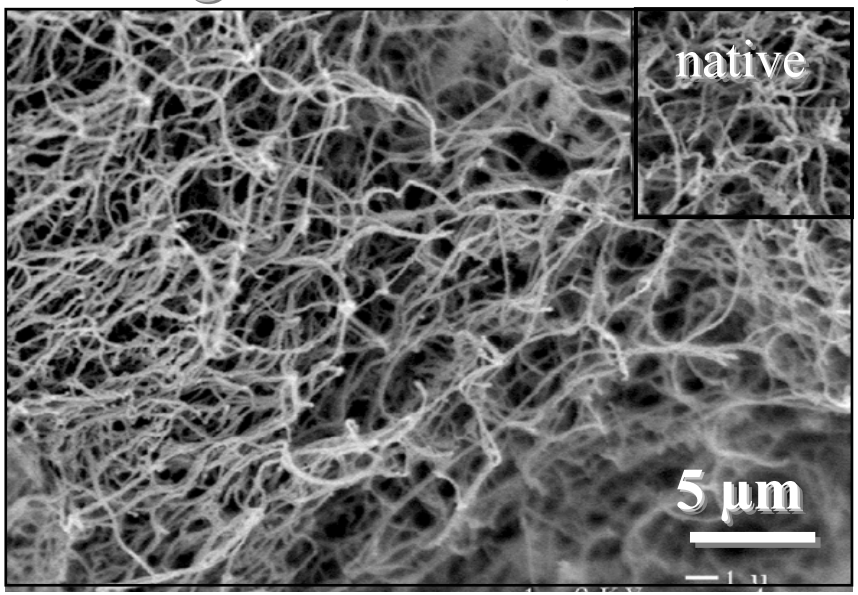




Freed et al., Exp Cell Res 240: 58, 1998

Structure and Protein Expression in Engineered Cartilage

Collagen network, SEM



Riesle et al., J Cell Biochem 71: 313, 1998

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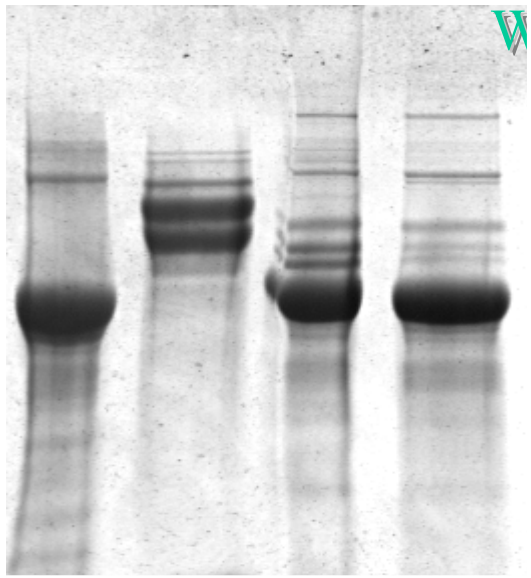
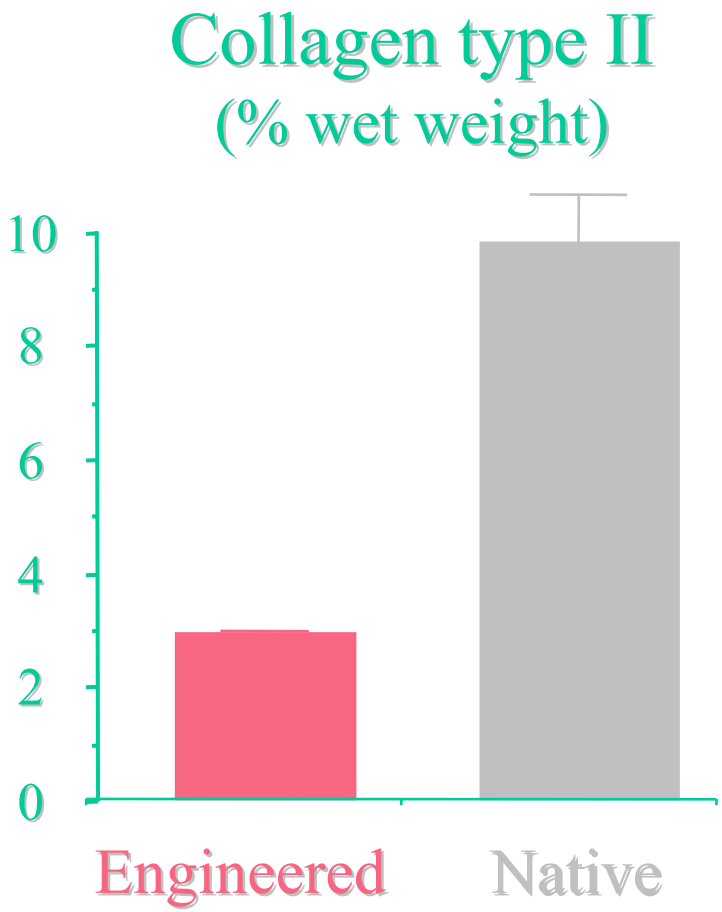
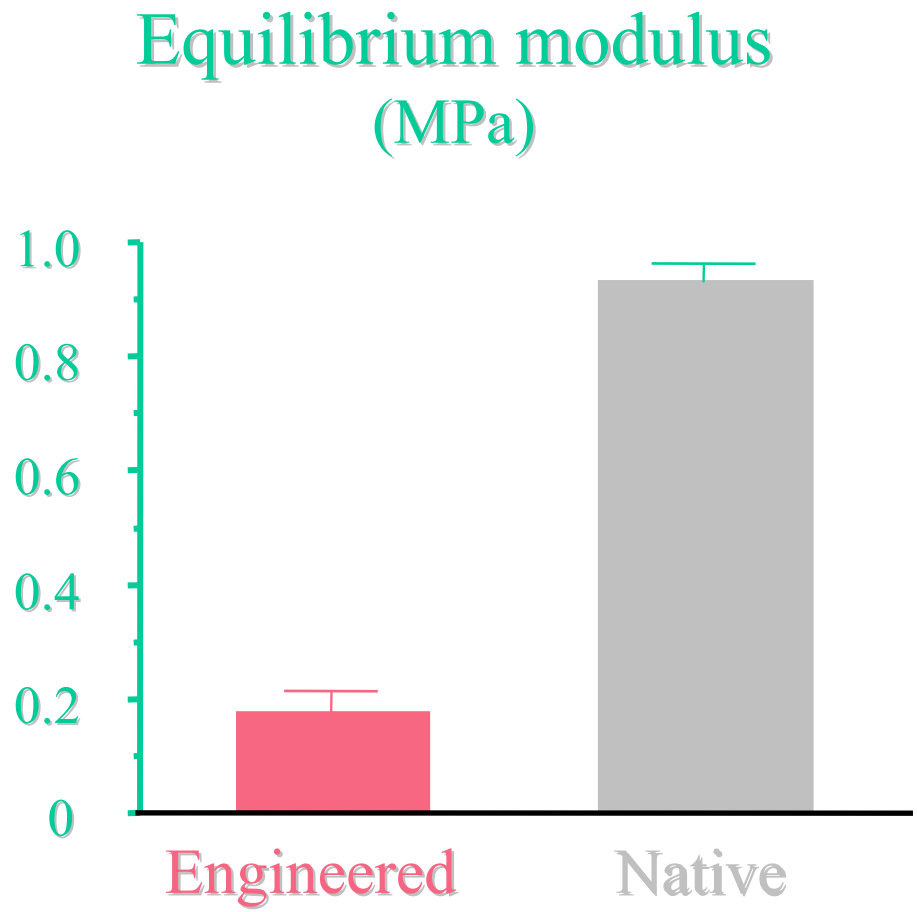


Collagen II Western blot

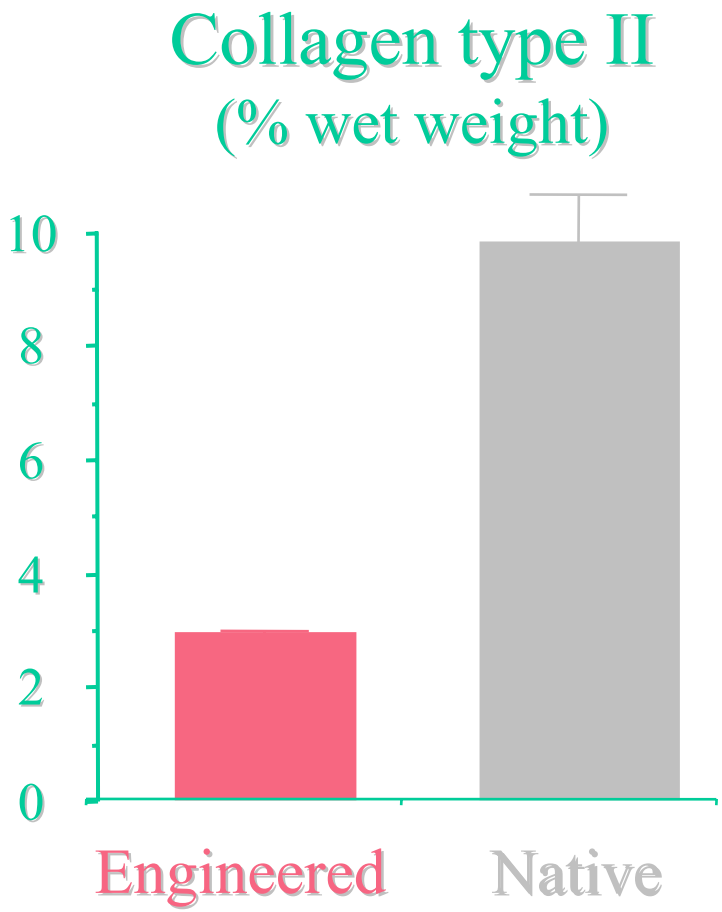
Engineered* vs. native cartilage
(*cultured 6 weeks in rotating bioreactors)

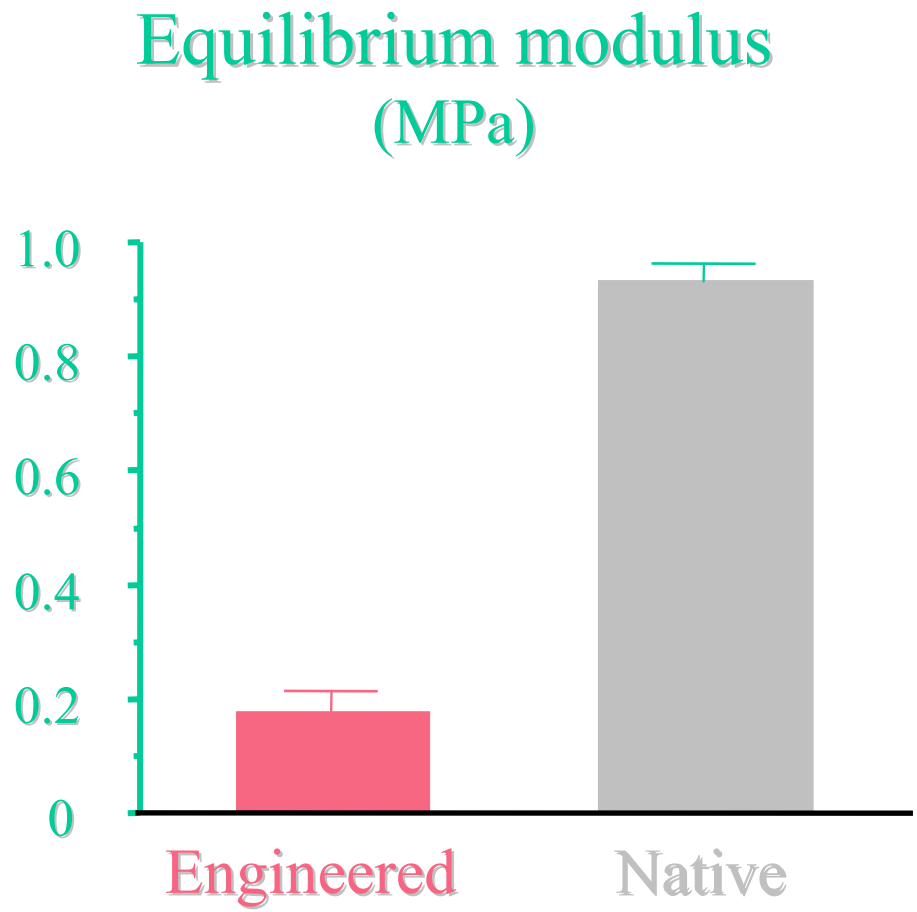




Vunjak-Novakovic et al., JOR 17: 130, 1999

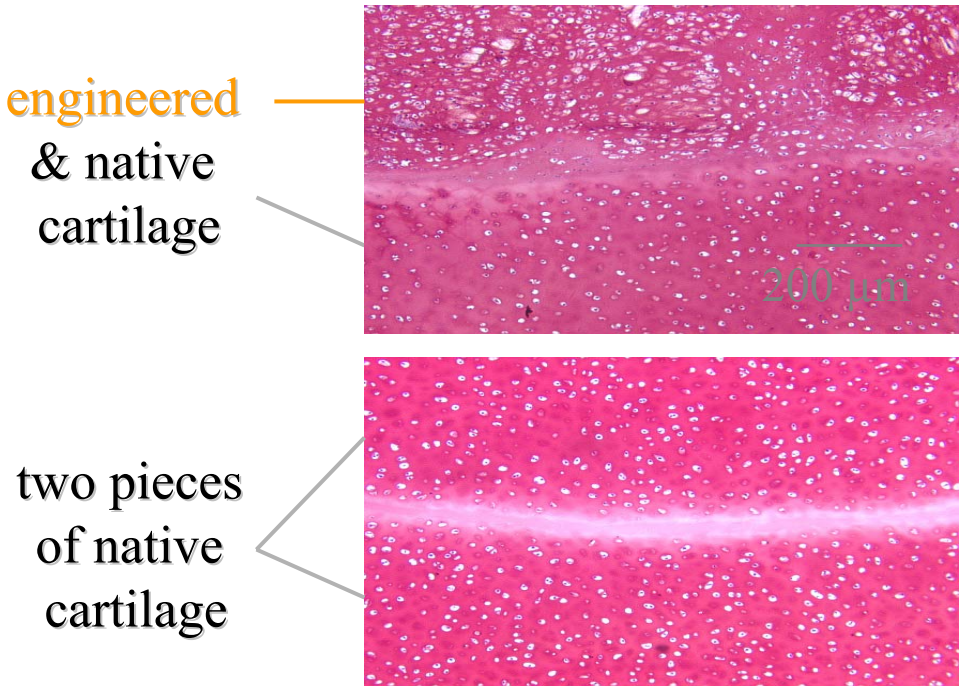
Engineered* vs. native cartilage
(*cultured 6 weeks in rotating bioreactors)

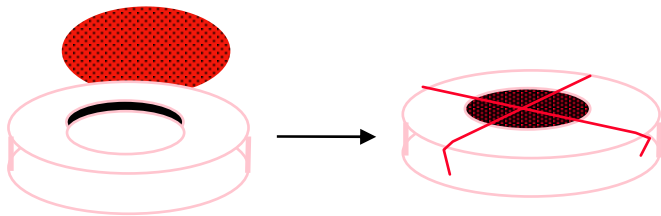




Vunjak-Novakovic et al., JOR 17: 130, 1999

Engineered cartilage integrated with native cartilage





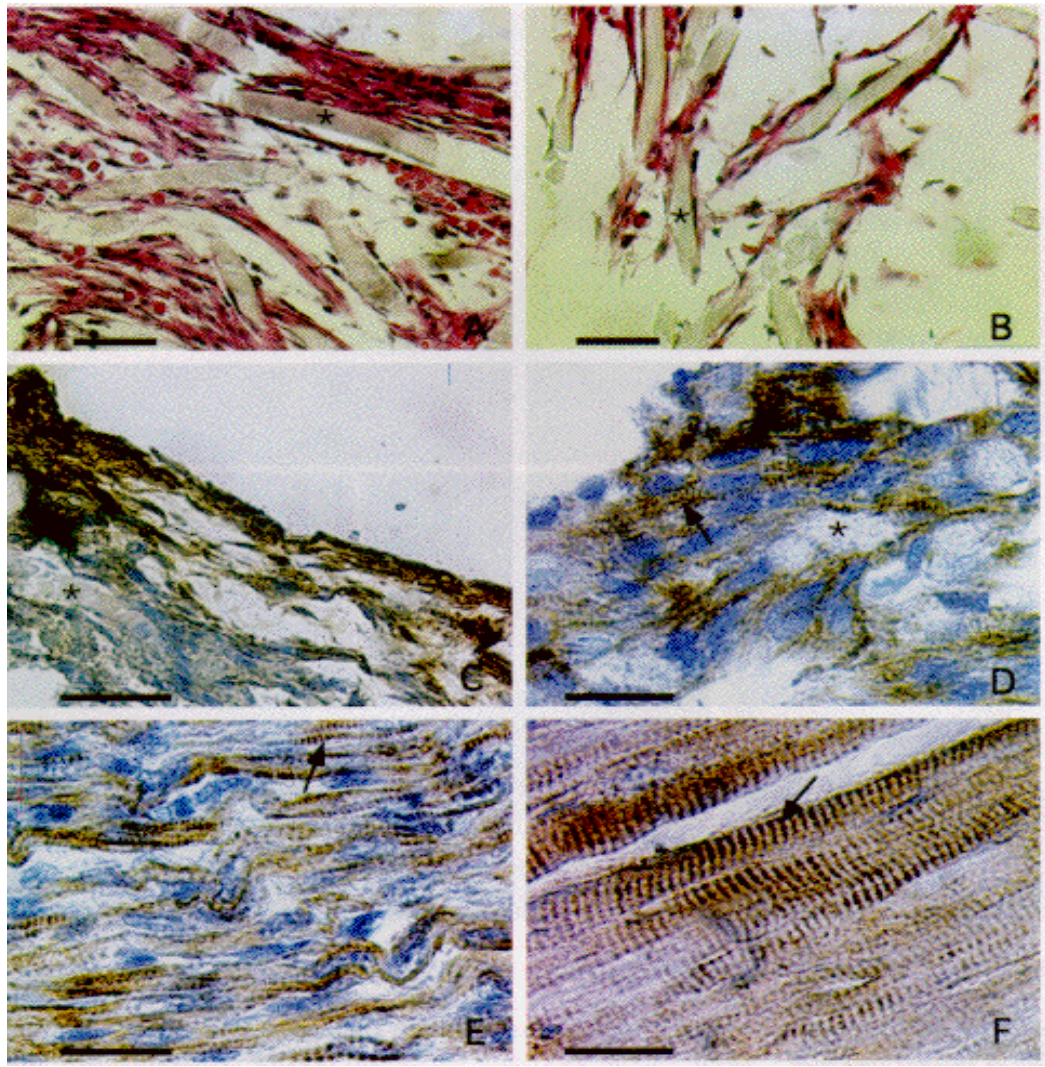
Obradovic et al.,
Trans ORS 25: 616, 2000

Engineered Heart Tissue

- Develop cardiac constructs for developmental, physiological, and pharmacological studies
- Compared with monolayer cultures, 3-D multilayer cultures more closely resemble intact cardiac tissue
  - Cellular differentiation
  - Electrical properties
- In vivo cardiac repair
  - If constructs can be grown sufficiently large and functional
- Check functionality with impulse propagation
- Cardiac Myocyte Cell Source
  - Neonatal Rat ventricles
    - » Enzymatic digestion of ventricles
    - » Monolayer expansion
    - » Cell seeding on scaffold (5x2 mm)

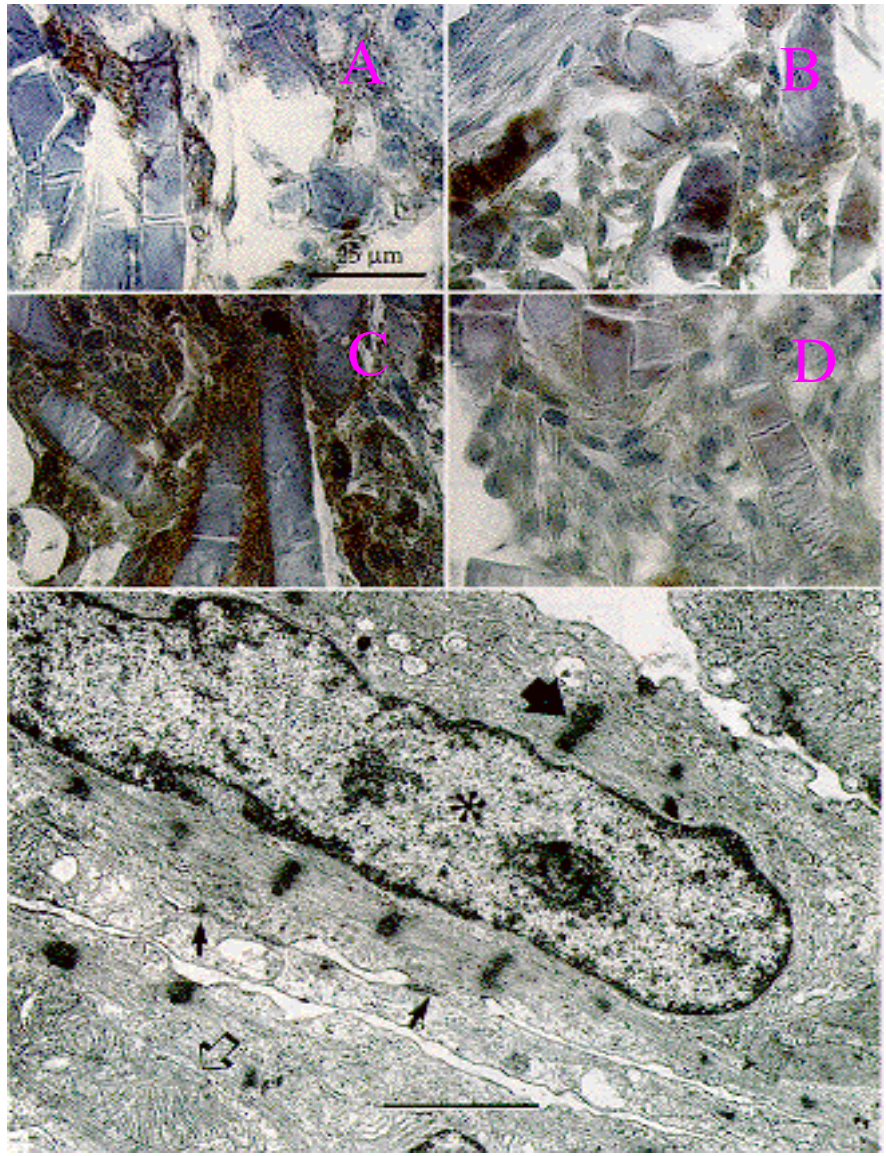
Engineered Heart Tissue

- **Histology**
  - **Cells in outermost part of construct formed 3-D tissuelike structures**
    - » Attached to other cells and spreading along PGA fibers
  - **100-200 um thick outer tissue**
  - **At construct center, cells were sparsely distributed**
- **Immunohistochemistry**
  - **Majority of cells expressed muscle-specific sarcomeric tropomyosin (brown color)**



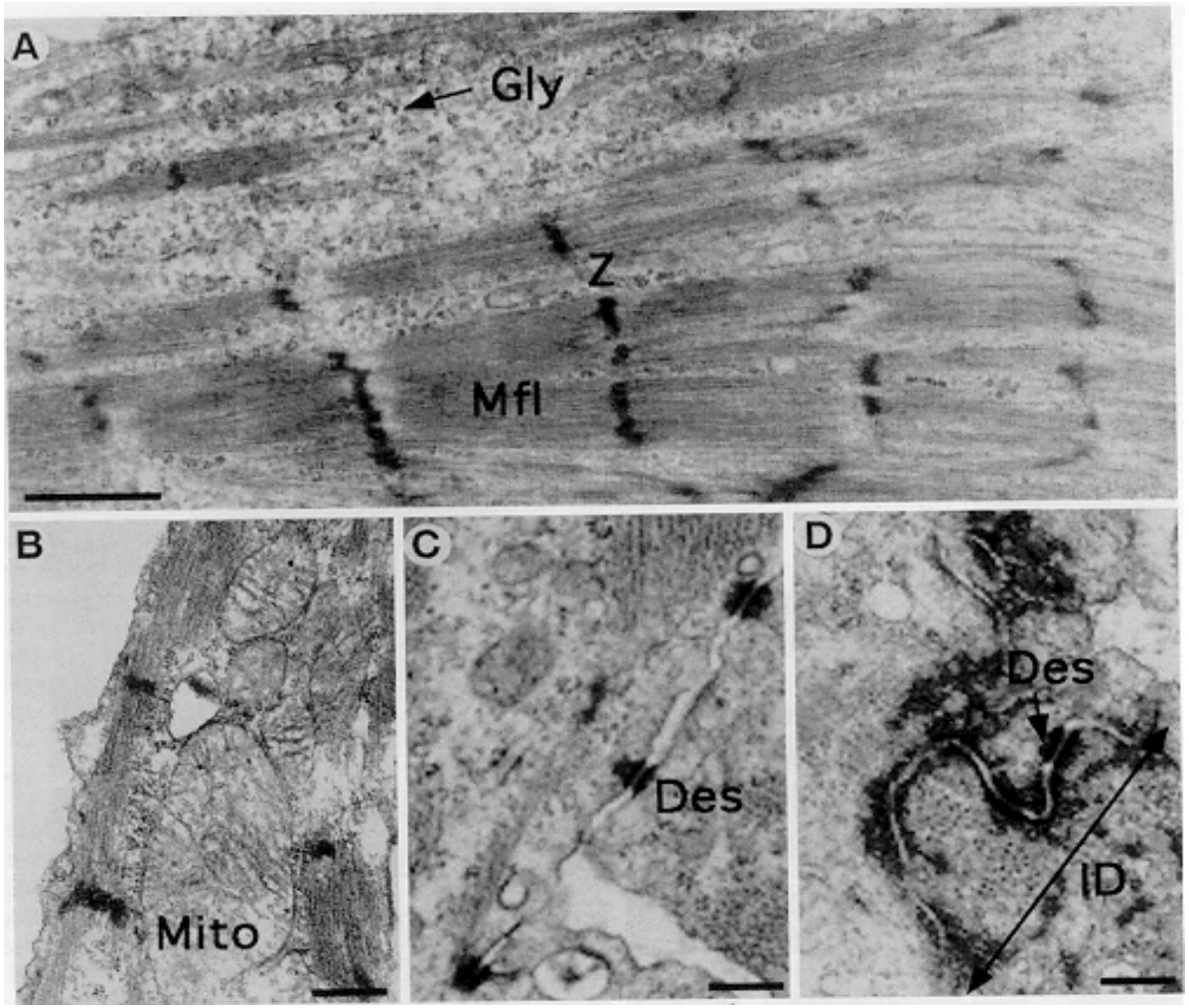
Engineered Heart Tissue

- **Cardiac Constructs**
  - **One week of culture**
  - **Cell Seeding Density**
    - » 4-8 million cells per scaffold
    - » Allowed synchronous contractions over macroscopic areas
- **Immunohistochemical Labeling: Muscle Specifc**
  - **A) Muscle desmin (IF)**
  - **B) Cardiac myosin**
  - **C) Cardiac troponin-T**
  - **D) Sarcomeric tropomyosin**
- **TEM**
  - **Desmosomes (little arrows)**
  - **Myofibrils: Z lines (broad arrow)**



Engineered Heart Tissue

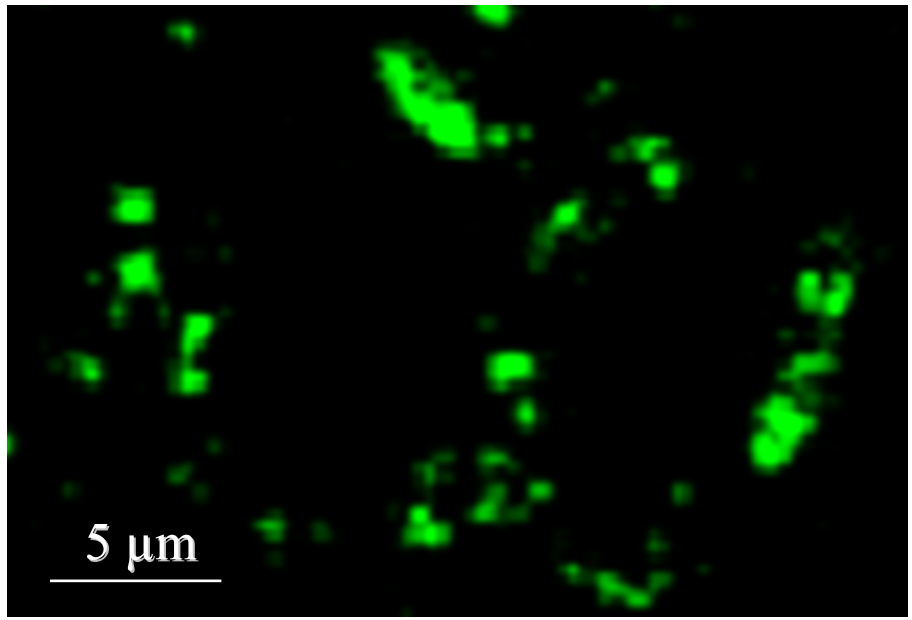
- TEM: Cardiac Myocytes
  - Myofilaments with well defined: sarcomeres, Z-lines, and glycogen granules
  - Mitochondria
  - Intercellular Junctions: Desmosomes
  - Intercellular Junctions: Desmosomes and Intercalated disc



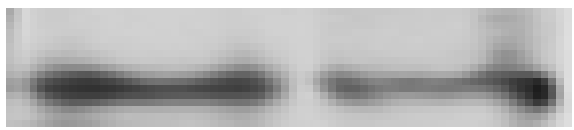
Engineered Heart Tissue

- Intercellular Coupling via Gap Junctions
  - Connexin- 43
  - 43 kD subunits found in Gap Junctions
  - Electrically couples cardiac cells
  - Ion currents flow to propagate action potentials

Connexin-43, immunolabeling

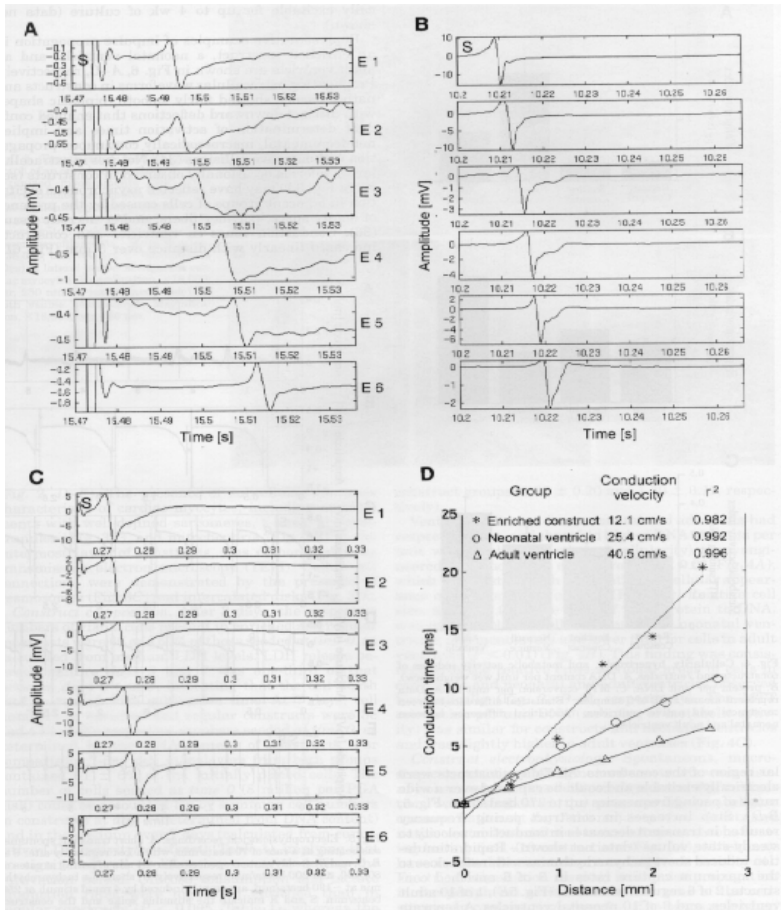


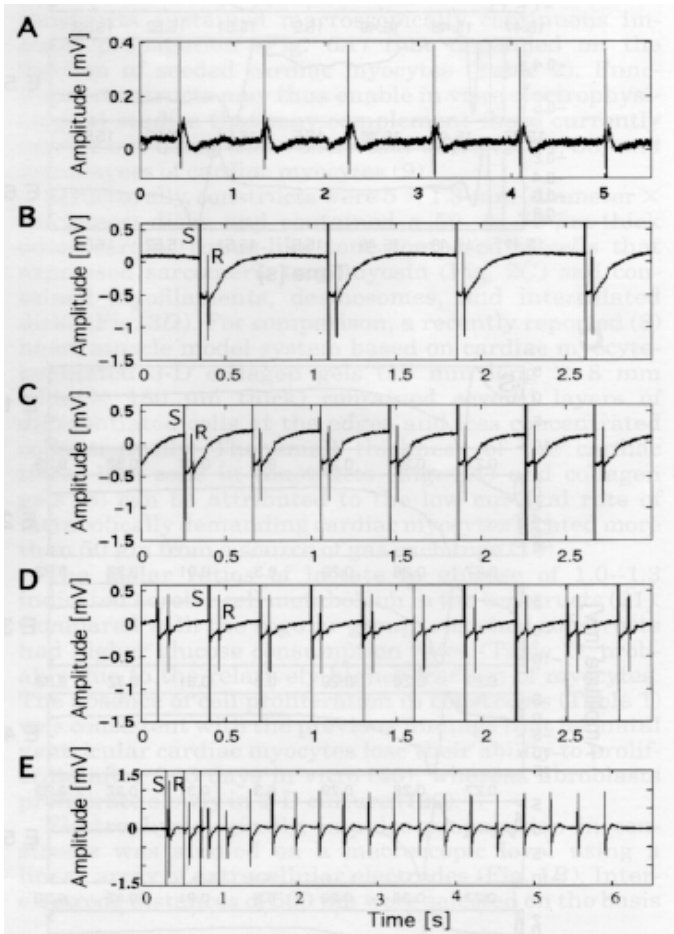
Connexin-43 Western blot



Engineered Heart Tissue

- **Impulse Propagation and Pacing Frequencies**
  - **Steady state response at 80, 150, and 200 beats/min**





Engineered Heart Tissue

- **Inferior electrophysiological properties compared with native ventricles**
  - **High excitation thresholds and low response amplitudes**
    - » Low construct cellularity
  - **Low conduction velocities**
    - » Decreased cell coupling (Gap Junctions)

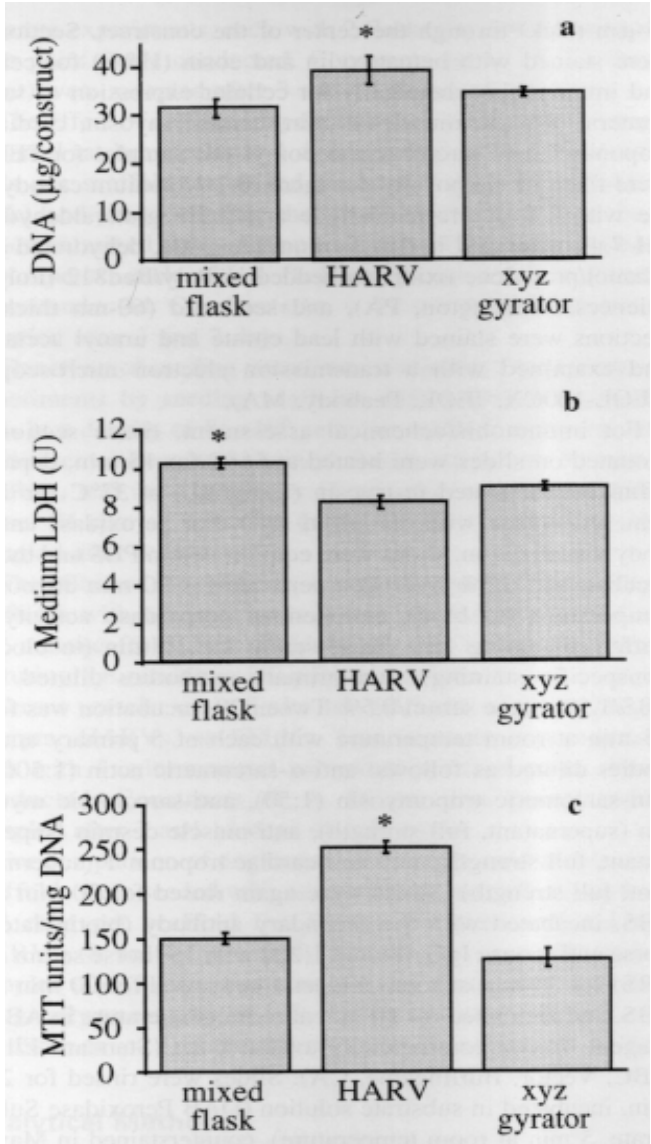
Table 2. *Electrophysiological parameters in 7-day constructs and native ventricles*

Group	*n*	Excitation Threshold, V	Conduction Velocity, cm/s	Maximum Amplitude, mV	Average Amplitude, mV	Maximum Capture Rate, beats/min
Constructs						
Regular*	6	2.70 ± 0.24	9.35 ± 0.27	0.52 ± 0.05	0.26 ± 0.09	111.7 ± 9.5
Enriched*	6	2.97 ± 0.30	11.89 ± 0.46†	0.90 ± 0.14†	0.43 ± 0.14	175.0 ± 21.3†
Ventricles						
Neonatal	10	0.74 ± 0.20	21.82 ± 1.48	31.91 ± 3.53	18.34 ± 4.31	475.0 ± 25.0
Adult	10	1.34 ± 0.17‡	31.69 ± 4.44‡	25.82 ± 2.81	14.62 ± 3.59	281.2 ± 21.0‡

Data represent means ± SE; *n* = no. of constructs or ventricles. *Significant difference between constructs and ventricles; †significant difference between enriched and regular constructs; ‡significant difference between neonatal and adult ventricles.

Engineered Heart Tissue

- Constructs seeded in low shear vessels
  - Highest cell densityand most uniformly distributed cells
  - Higher DNA contents
  - Lowest index of cell damage and cell death
  - Highest metabolic activity index
- Should result in improved electrical properties



Human Tissue Models that Enable Biomedical Research

- **Universal Pathogen Culture System**
  - **Liver, epithelial, lymphoid co-culture**
    - » Multiple tissue provide correct microenvironment for most common human pathogens
  - **EBV, Ebola, Monkeypox**