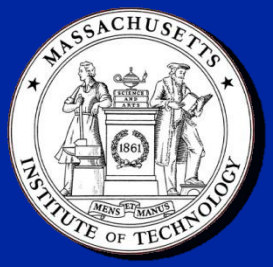

Massachusetts Institute of Technology
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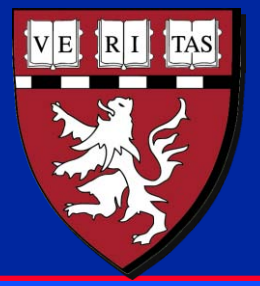
VA Boston Healthcare System



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Cardiac Muscle Tissue Engineering

M. Spector, Ph.D.

TISSUE ENGINEERING VS. REGENERATIVE MEDICINE*

TISSUE ENGINEERING

Regeneration *In Vitro*

Produce the fully formed tissue *in vitro* by seeding cells into a biomaterial matrix, and then implant the regenerated tissue into the body.

REGENERATIVE MED.

Regeneration *In Vivo*

Implant the biomaterial matrix with, or without seeded cells, into the body to facilitate regeneration of the tissue *in vivo*.

Inject cells (*e.g.*, MSCs).

TISSUE ENGINEERING VS. REGENERATIVE MEDICINE

TISSUE ENGINEERING

Regeneration *In Vitro*

Advantages

- Evaluation of tissue prior to implantation

Disadvantages

- For incorporation, must be remodeling
- Stress-induced architecture cannot yet be produced *in vitro*

REGENERATIVE MED.

Regeneration *In Vivo*

Advantages

- Incorporation and formation under the influence of endogenous regulators (including mechanical strains)

Disadvantages

- Dislodgment and degrad. by mech. stresses *in vivo*

CARDIAC MUSCLE TISSUE ENGR./REGENERATIVE MED.

- SCAFFOLD (MATRIX)
  - Collagen
  - Matrigel
- CELLS
  - Neonatal cardiomyocytes
  - Mesenchymal stem cells
  - Embryonic stem cells
- REGULATORS
  - Cytokines (growth factors)
  - Mechanical loading
  - Electric stimulation

Which Tissues Can Regenerate Spontaneously?

	Yes	No
Connective Tissues		
• Bone	√	
• Articular Cartilage, Ligament, Intervertebral Disc, Others		√
Epithelia (e.g., epidermis)	√	
Muscle		
• Cardiac, Skeletal		√
• Smooth	√	
Nerve		√

TISSUE ENGINEERING ENDPOINTS

- **Morphological/Histological/Biochemical**
- **Functional**
  - **Synchronous contraction with the recipient heart**
- **Clinical**
  - **Improved cardiac function**

Cardiac Anatomy

Image removed due to copyright restrictions.
Medical illustrations of human heart, cross-section view.

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Cardiac Infarct Resulting from Coronary Artery Occlusion

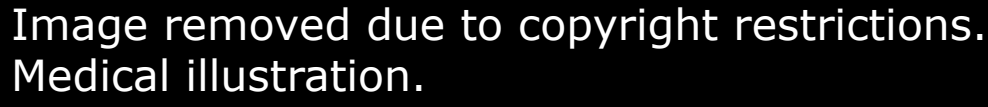


Image removed due to copyright restrictions.
Medical illustration.

Cardiomyocytes

Images removed due to copyright restrictions.

Neonatal rat cardiomyocytes by ICC/IF:
Red: actin
Green: heavy chain cardiac myosin primary antibody
Blue: DAPI-labelled DNA

K. Shu

Cardiac Contraction

- Contractile proteins:
  - α-cardiac actin
  - Myosin heavy chain (MHC)
  - Tropomyosin
    - Troponin-T
    - Troponin-I
    - Troponin-C
  - Connexin-43
  - Titin (connectin)

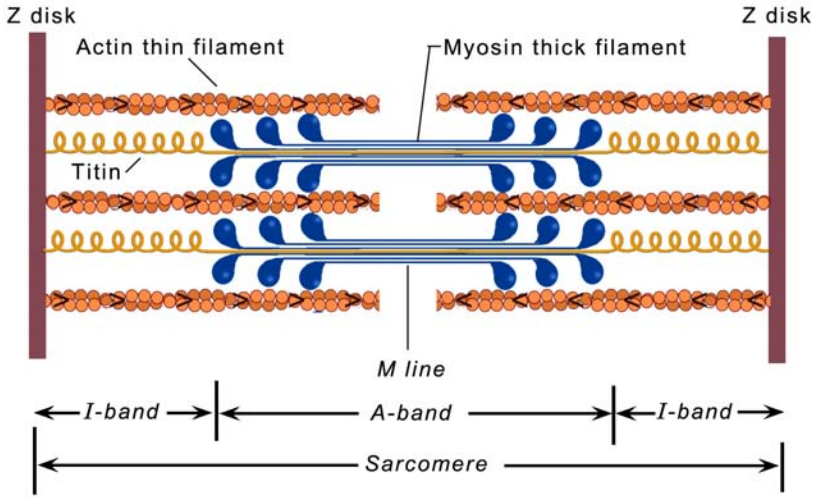


Figure by MIT OpenCourseWare.

An Elastic, Biodegradable Cardiac Patch Induces Contractile Smooth Muscle and Improves Cardiac Remodeling and Function in Subacute Myocardial Infarction

(J Am Coll Cardiol 2007;49:2292–300) © 2007

Kazuro L. Fujimoto, MD,*† Kimimasa Tobita, MD,†‡§ W. David Merryman, PHD,§ Jianjun Guan, PHD,*† Nobuo Momoi, MD, PHD,‡ Donna B. Stolz, PHD,‖ Michael S. Sacks, PHD,†§ Bradley B. Keller, MD,†‡‖ William R. Wagner, PHD*†§

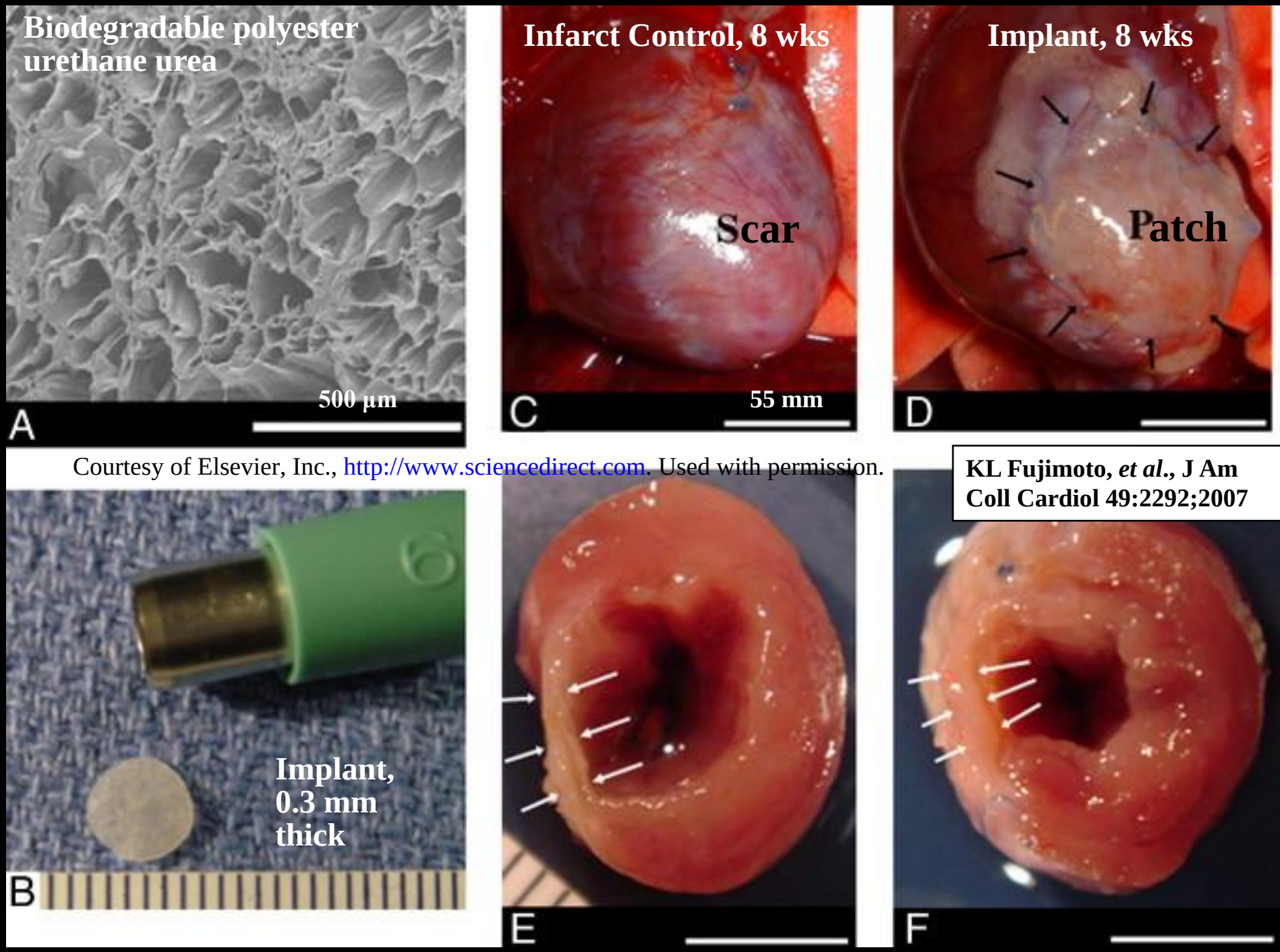
Pittsburgh, Pennsylvania

Our objective in this study was to apply an elastic, biodegradable polyester urethane urea (PEUU) cardiac patch onto subacute infarcts and to examine the resulting cardiac ventricular remodeling and performance.

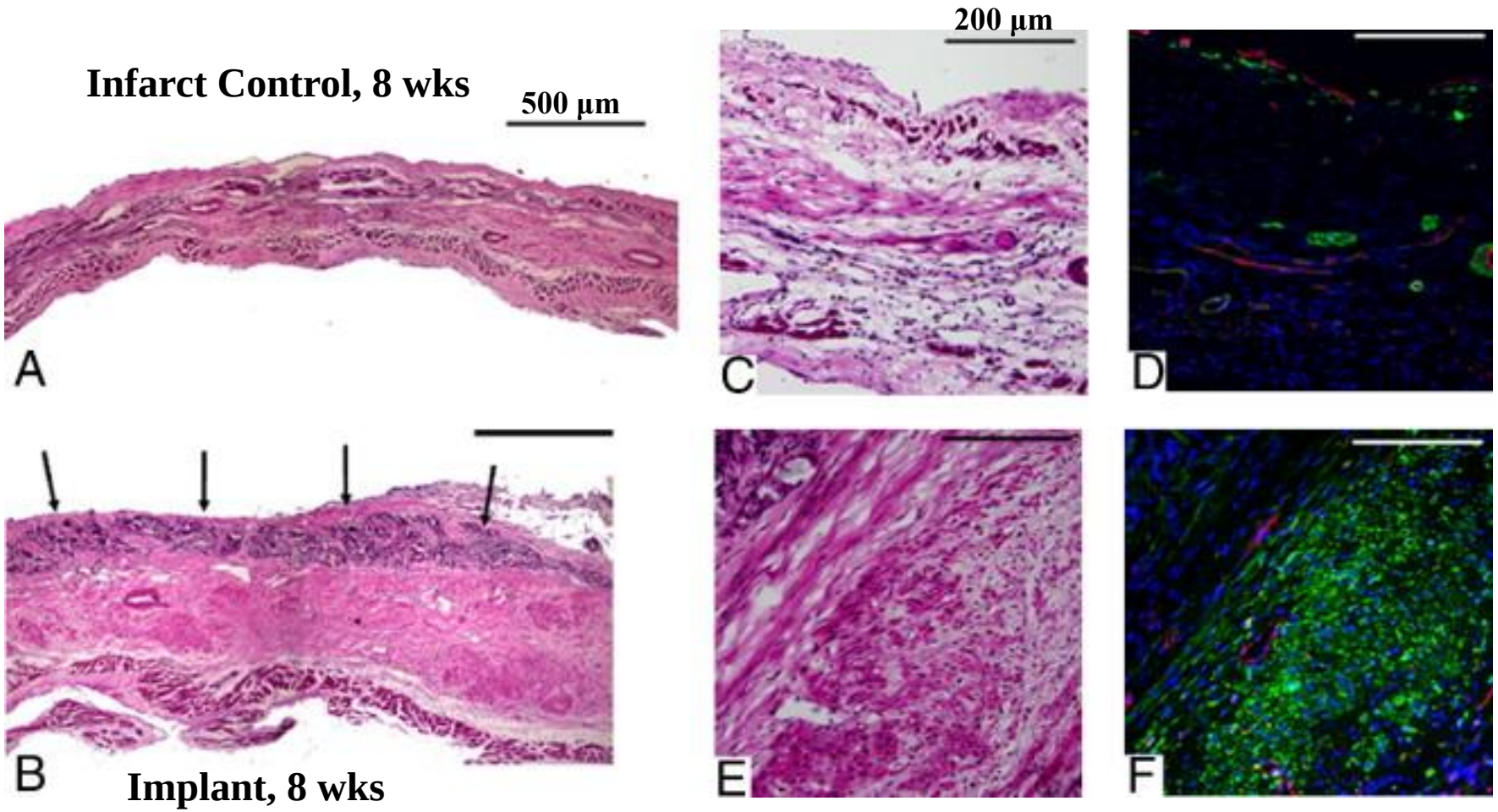
Lewis rats underwent proximal left coronary ligation. Two weeks after coronary ligation, a 6-mm diameter microporous PEUU patch was implanted directly on the infarcted LV wall surface (PEUU patch group, n = 14). Sham surgery was performed as an infarction control (n = 12). The LV contractile function, regional myocardial wall compliance, and tissue histology were assessed 8 weeks after patch implantation.

The end-diastolic LV cavity area (EDA) did not change, and the fractional area change (FAC) increased in the PEUU patch group ($p < 0.05$ vs. week 0), while EDA increased and FAC decreased in the infarction control group ($p < 0.05$). The PEUU patch was largely resorbed 8 weeks after implantation and the LV wall was thicker than infarction control ($p < 0.05$ vs. control group). Abundant smooth muscle bundles with mature contractile phenotype were found in the infarcted myocardium of the PEUU group. The myocardial compliance of the PEUU group was distributed between normal myocardium and infarction control ($p < 0.001$).

Implantation of a novel biodegradable PEUU patch onto a subacute myocardial infarction promoted contractile phenotype smooth muscle tissue formation and improved cardiac remodeling and contractile function at the chronic stage. Our findings suggest a new therapeutic option against post-infarct cardiac failure.



KL Fujimoto, *et al.*, J Am Coll Cardiol 49:2292;2007

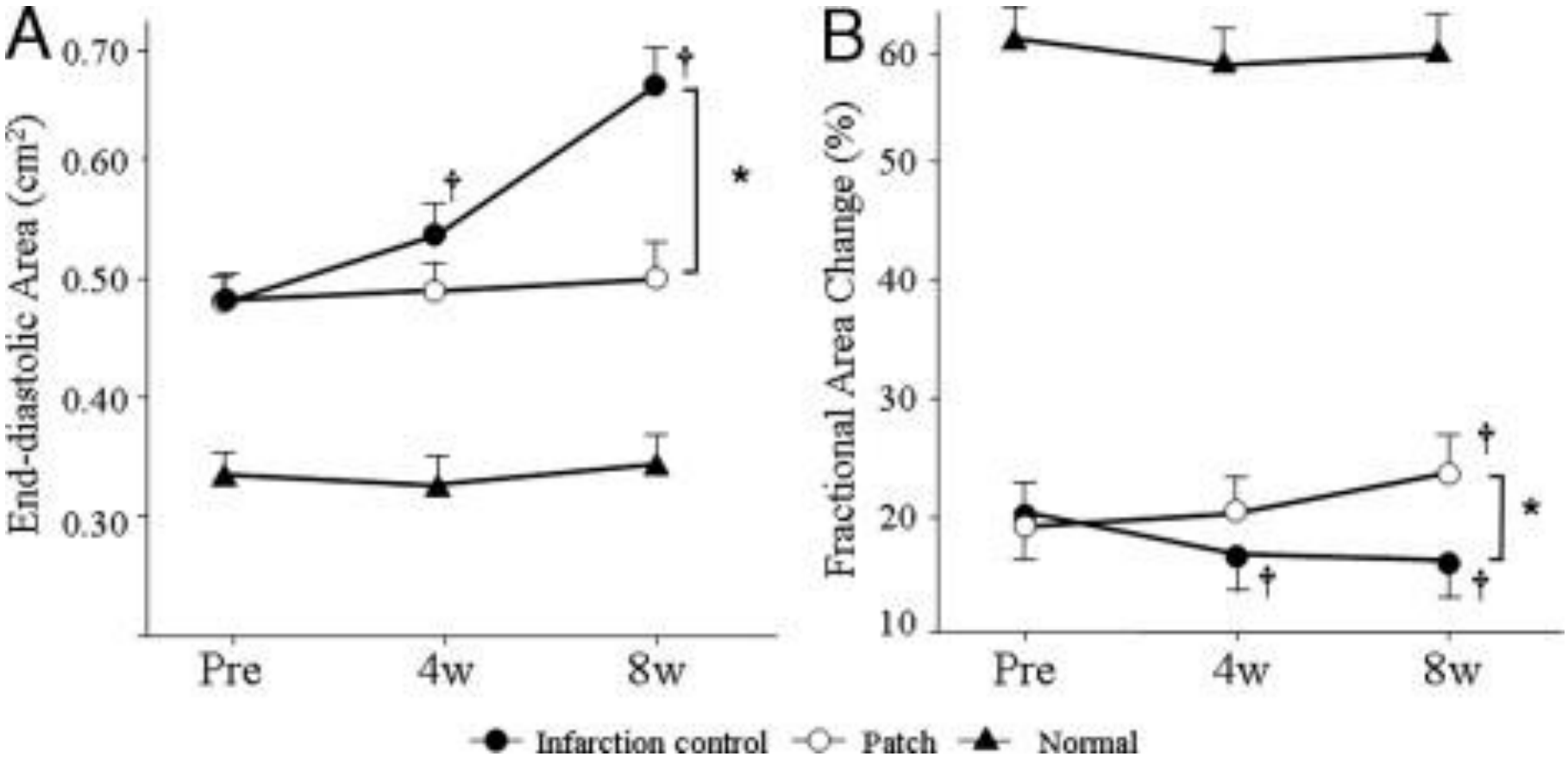


Courtesy of Elsevier, Inc., http://www.sciencedirect.com. Used with permission.

- **Black arrows indicate the top of the PEUU implanted area.**
- **α-SMA staining appears green**
- **CD31 staining appears red**
- **Nuclear staining appears blue**
- **Increased smooth muscle actin is apparent in the PEUU patched group**

KL Fujimoto, *et al.*, J Am Coll Cardiol 49:2292;2007

Echocardiography



KL Fujimoto, *et al.*, J Am Coll Cardiol 49:2292;2007

Functional assembly of engineered myocardium by electrical stimulation of cardiac myocytes cultured on scaffolds

Milica Radisic*†, Hyoungshin Park*†, Helen Shing‡, Thomas Consi*, Frederick J. Schoen‡, Robert Langer*, Lisa E. Freed*, and Gordana Vunjak-Novakovic*§

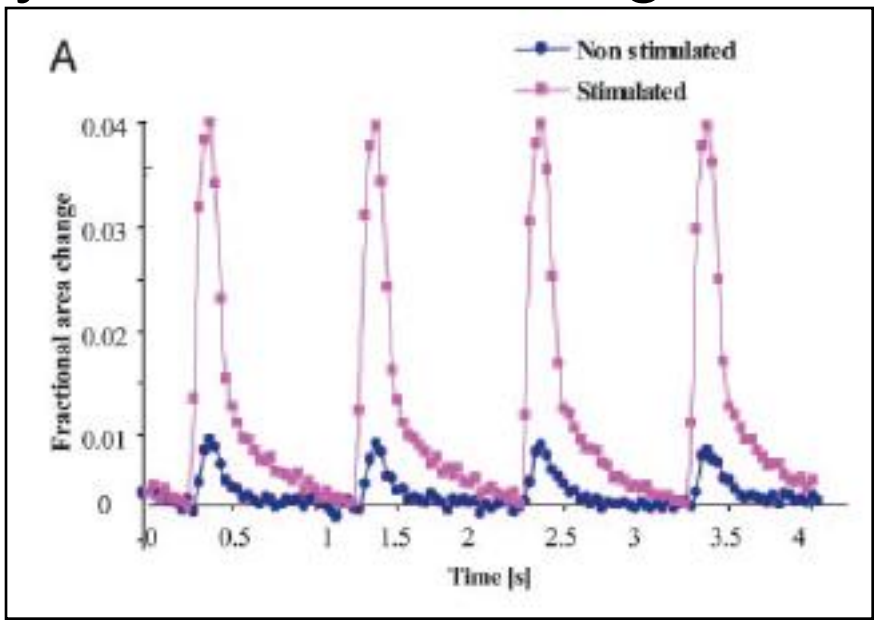
*Harvard–MIT Division of Health Sciences and Technology, Massachusetts Institute of Technology, 77 Massachusetts Avenue, E25-342, Cambridge, MA 02139; and ‡Department of Pathology, Harvard Medical School, 75 Francis Street, Boston, MA 02115

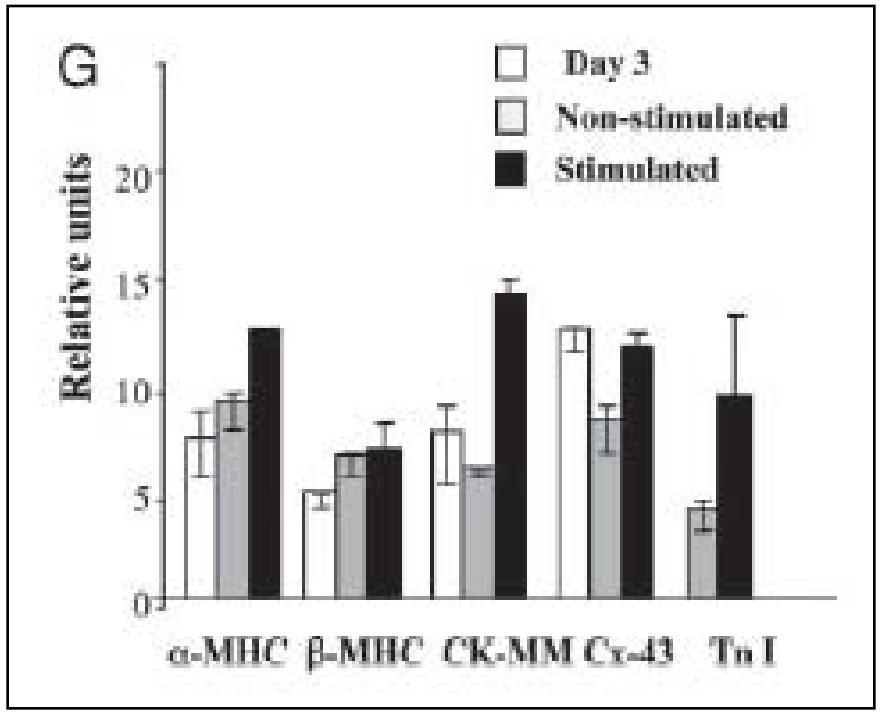
PNAS | December 28, 2004 | vol. 101 | no. 52 | 18129–18134

- **Hypotheses**
  - **To engineer myocardium, biophysical regulation of the cells needs to recapitulate multiple signals present in the native heart.**
  - **excitation–contraction coupling, critical for the development and function of a normal heart, determines the development and function of engineered myocardium.**
- **After only 8 days *in vitro*, electrical field stimulation**
  - **induced cell alignment and coupling,**
  - **increased the amplitude of synchronous construct contractions by a factor of 7, and**
  - **resulted in ultrastructural organization.**

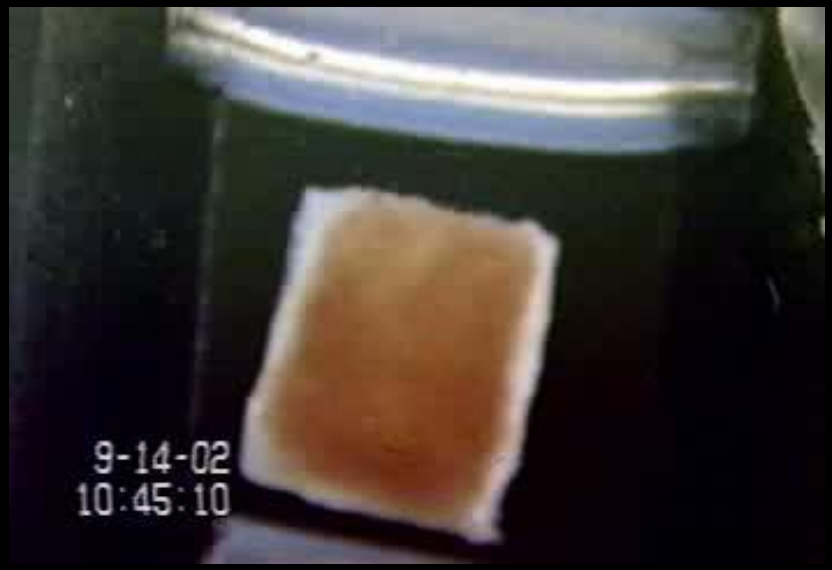
Electrical stimulation for synchronous beating

- Neonatal rat ventricular myocytes on Ultrafoam collagen sponges
- Electrical pulses (rectangular, 2 ms, 5 V/cm, 1 Hz) for 5 days
- Cardiac proteins:
  - –connexin-43 (Cx-43)
  - –cardiac troponin I (Tn-I)
  - – α and β isoforms of myosin heavy chain (MHC)
  - –creatine kinase-MM (CK-MM)





Electrical Stimulation for Synchronous Beating



Radisic, M. *PNAS* **101**(52): 18129-18134 (2004)
Day 3: Paced contractions ofa construct cultured for 3 days without electrical

Day 3: Paced contractions of a construct cultured for 3 days without electrical

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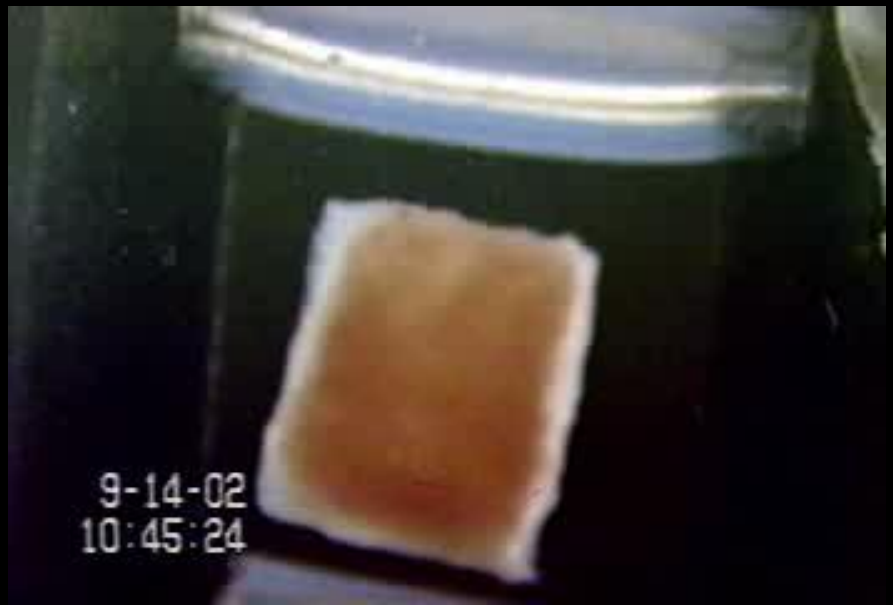
Day 8: Nonstimulated; Paced contractions of a construct cultured for 8 days

Day 8: Stimulated; Contractions of a construct cultured for 3 days (-) electrical stimulation and for 5 days (+) electrical stimulation.



Optimizing Engineered Heart Tissue for Therapeutic Applications as Surrogate Heart Muscle

Hiroshi Naito, Ivan Melnychenko, Michael Didié, Karin Schneiderbanger, Pia Schubert, Stephan Rosenkranz, Thomas Eschenhagen and Wolfram-Hubertus Zimmermann

Circulation 2006;114;I-72-I-78

- **Optimal myocardial structure and function depends not only on the cardiac myocyte fraction but also on non-myocytes, which compose 70% of the total cell content of a heart.**
- **While a serum-free cardiac tissue engineering approach is important with respect to future human applications, it has not been achieved because extracellular matrix from Engelbreth-Holm-Swarm tumors (also known as Matrigel) has been identified as an essential component in engineered heart tissue (EHT).**

Institute of Experimental and Clinical Pharmacology and Toxicology, University Medical Center Hamburg-Eppendorf, Germany.

- **Engineered heart tissue (EHT) can be improved by using:**
  - **mixed heart cell populations**
  - **culture in defined serum-free**
  - **Matrigel-free conditions**
  - **fusion of single-unit EHTs to multi-unit heart muscle surrogates.**

H. Naito, *et al.*, Circ 114:I-72 (2006)

EHT Construction

- **Solubilized type collagen I was mixed with concentrated culture medium.**
- **Matrigel was added.**
- **Cells were added to the reconstitution mixture, which was mixed before casting in circular molds**
  - **inner diameter, 8 mm**
  - **outer diameter, 16 mm**
  - **height, 5 mm.**
- **Within 3 to 7 days, EHTs coalesced to form spontaneously contracting circular structures and were transferred on automated stretch devices or flexible holders for continuous culture under chronic strain.**

H. Naito, *et al.*, Circ 114:I-72 (2006)

Methods and Results

[Text removed due to copyright restrictions.]

Conclusions

[Text removed due to copyright restrictions.]

H. Naito, *et al*., Circ 114:I-72 (2006)

Heart muscle engineering: An update on cardiac muscle replacement therapy

Wolfram-Hubertus Zimmermann *, Michael Didié, Stephan Döker, Ivan Melnychenko, Hiroshi Naito, Christina Rogge, Malte Tiburcy, Thomas Eschenhagen

Institute of Experimental and Clinical Pharmacology and Toxicology, University Medical Center Hamburg-Eppendorf, Martinistraße 52, 20246 Hamburg, Germany

Cardiovascular Research 71 (2006) 419 – 429

Slides of Figures 1, 2 and 3 removed due to copyright restrictions.

NATURE | VOL 410 | 5 APRIL 2001

Bone marrow cells regenerate infarcted myocardium

Donald Orlic†, Jan Kajstura*, Stefano Chimenti*, Igor Jakoniuk*, Stacie M. Anderson†, Baosheng Li*, James Pickel‡, Ronald McKay‡, Bernardo Nadal-Ginard*, David M. Bodine†, Annarosa Leri* & Piero Anversa*

Myocardial infarction leads to loss of tissue and impairment of cardiac performance. The remaining myocytes are unable to reconstitute the necrotic tissue, and the post-infarcted heart deteriorates with time[1]. Injury to a target organ is sensed by distant stem cells, which migrate to the site of damage and undergo alternate stem cell differentiation[2–5]; these events promote structural and functional repair[6–8]. This high degree of stem cell plasticity prompted us to test whether dead myocardium could be restored by transplanting bone marrow cells in infarcted mice. We sorted lineage-negative (Lin^-) bone marrow cells from transgenic mice expressing enhanced green fluorescent protein[9] by fluorescence-activated cell sorting on the basis of c-*kit* expression[10]. Shortly after coronary ligation, Lin^- c-kit^{POS} cells were injected in the contracting wall bordering the infarct. Here we report that newly formed myocardium occupied 68% of the infarcted portion of the ventricle 9 days after transplanting the bone marrow cells. The developing tissue comprised proliferating myocytes and vascular structures. Our studies indicate that locally delivered bone marrow cells can generate *de novo* myocardium, ameliorating the outcome of coronary artery disease.

MSCs and their potential as cardiac therapeutics

- MSCs: readily grown in culture, retains "stemness" with many passages
- Stem cells need to be functionally defined
- Allogeneic MSCs: inhibit T cell proliferation, available on demand
- Myogenic media containing DNA-methylating agent 5-azacytidine

Pittenger, MF. *Circ Res* **95**: 9-20 (2004).

K. Shu

MSCs and their potential as cardiac therapeutics

- Pittenger MF (2004):
  - Direct injection vs. intravenous injection of MSCs
  - Homing ability of MSCs
- Fukuda K (2001): MSCs treated with 5-azacytidine
  - 30% of the cells formed myotube-like structures
  - Spontaneous beating after 2 weeks
  - Phenotype was similar to fetal ventricular cardiomyocytes (contractile protein genes)
- Berry MF (2006): MSC injection after MI reduced the stiffness of the subsequent scar and attenuated postinfarction remodeling, preserving some cardiac function

Collagen-GAG scaffolds grafted on MIs in rats

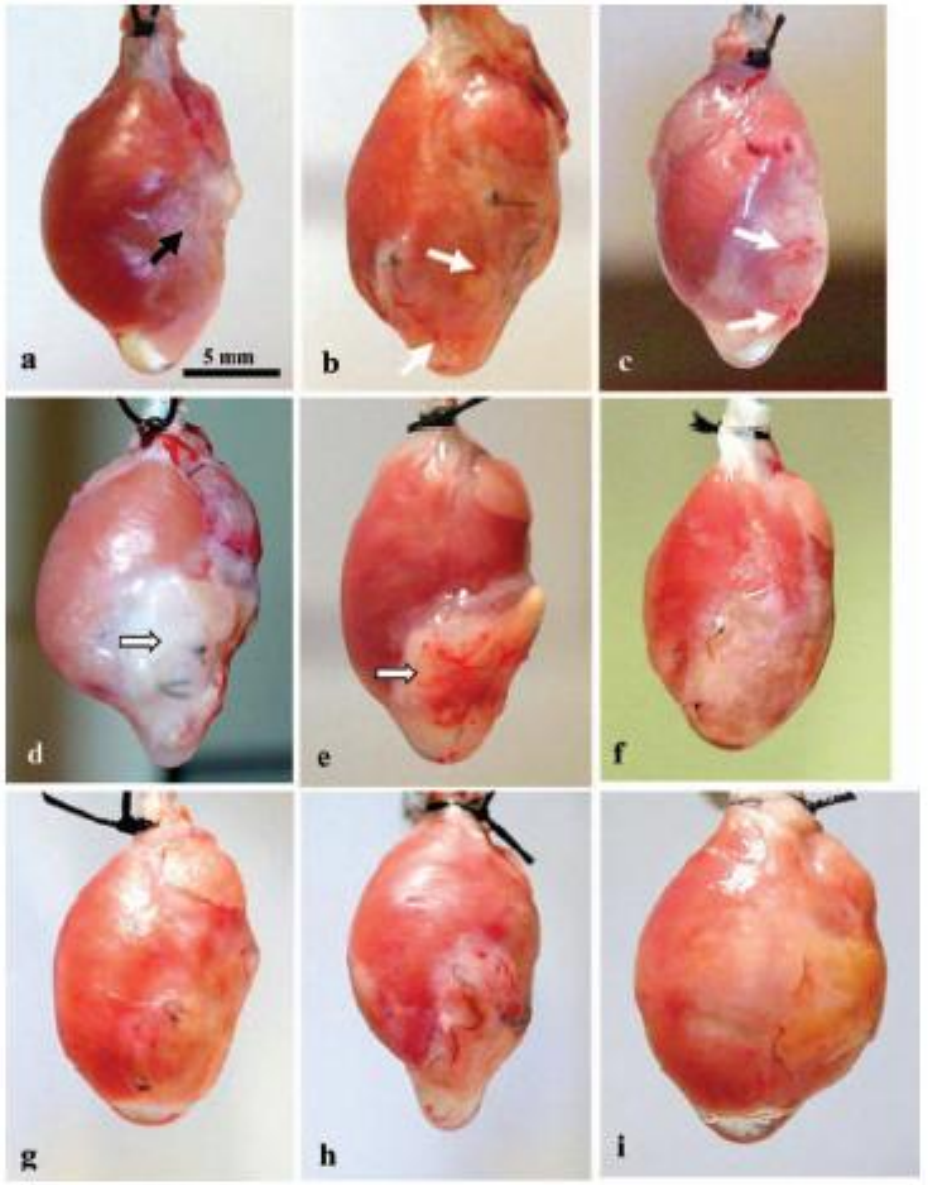
- Coronary ligation of main branch of left marginal artery for 60 min, then reperfusion
- Scaffolds (0.5wt% Type I collagen):
  1. DHT w/o cells
  2. EDAC w/o cells
  3. DHT with BrdU-labeled MSCs

Xiang Z. *Tiss. Engr.* **12**(9): 2467-2478 (2006)

K. Shu

Collagen-GAG scaffolds grafted on MIs in rats

- **(A)** Typical scar.
- **(B, C)** DHT group showing blood vessels in the scarred regions
- **(D, E)** EDAC group
- **(F-I)** Cell–scaffold group.



Xiang Z. *Tiss. Engr.* **12**(9): 2467-2478 (2006)

TABLE 2. MEASUREMENTS MADE ON THE HISTOLOGIC SECTIONS (MEAN ± STANDARD ERROR OF THE MEAN)

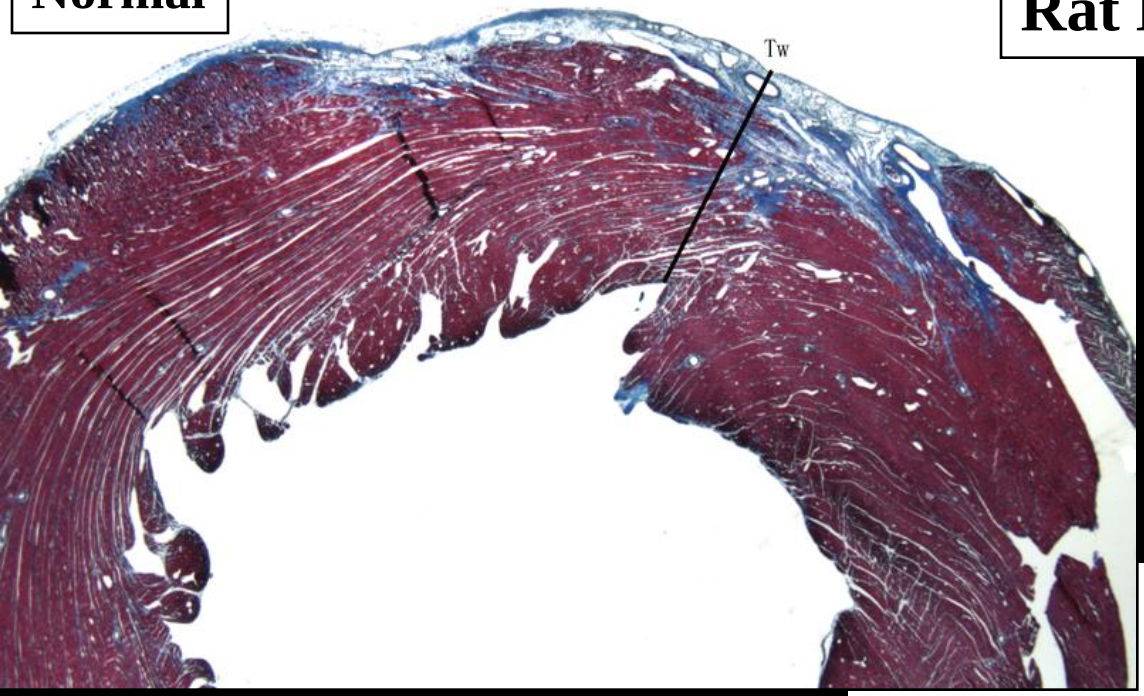
Group	n	T_w (*μm*)	D_c (*μm*)	T_m (*μm*)
Control	4	62 ± 29	182 ± 75	0
DHT	10	71 ± 15	116 ± 33	0
EDAC	8	50 ± 9	100 ± 28	56 ± 7
Cell-scaffold	8*	84 ± 16	92 ± 21	0

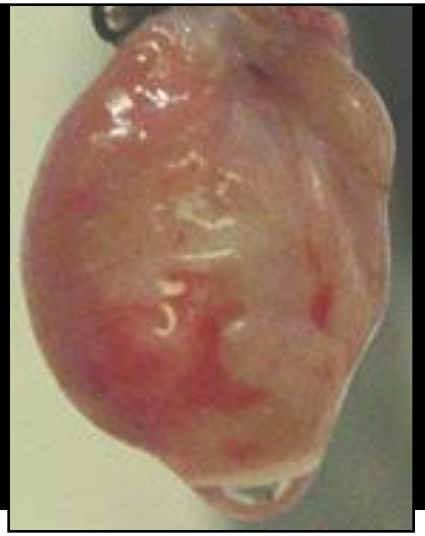
- Minimum heart wall thickness (T_w)
- Width of scar at the site of infarct as reflected in the minimum distance between cardiomyocytes (D_c)
- Minimum thickness of the residual collagen-GAG matrix (T_m)
- Relative numbers of macrophages and other mononuclear leukocytes

Xiang Z. *Tiss. Engr.* **12**(9): 2467-2478 (2006)

Rat Myocardial Infarct Model

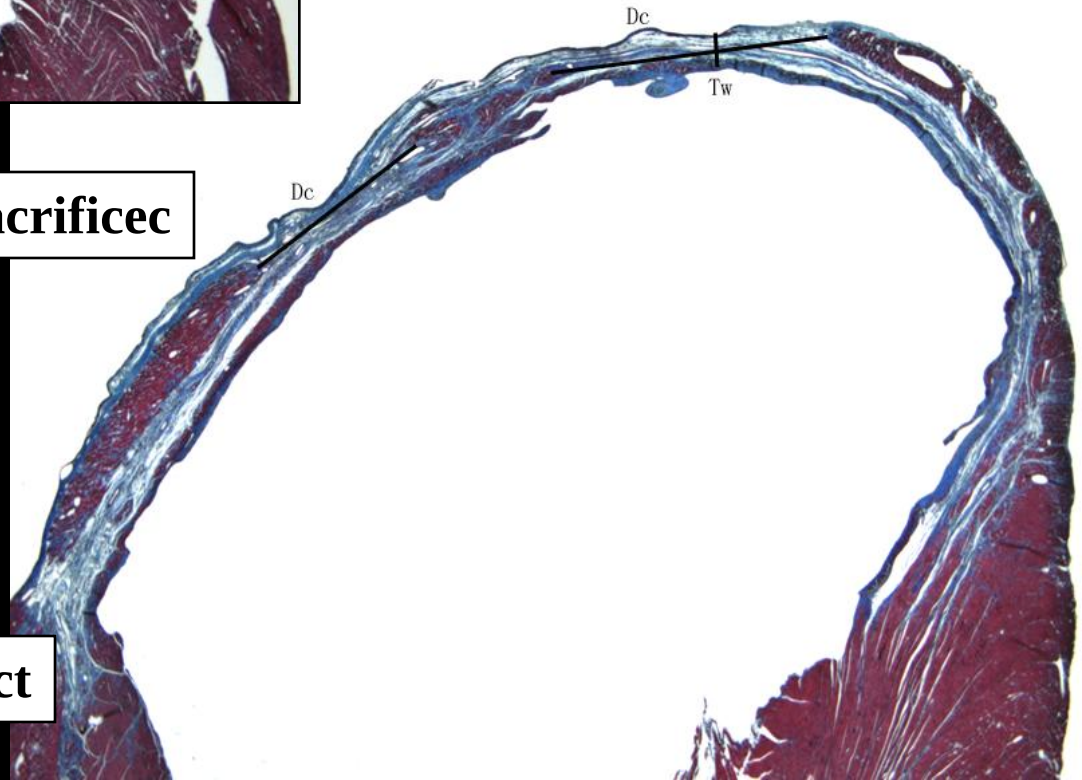
Normal





R. Liao, BU

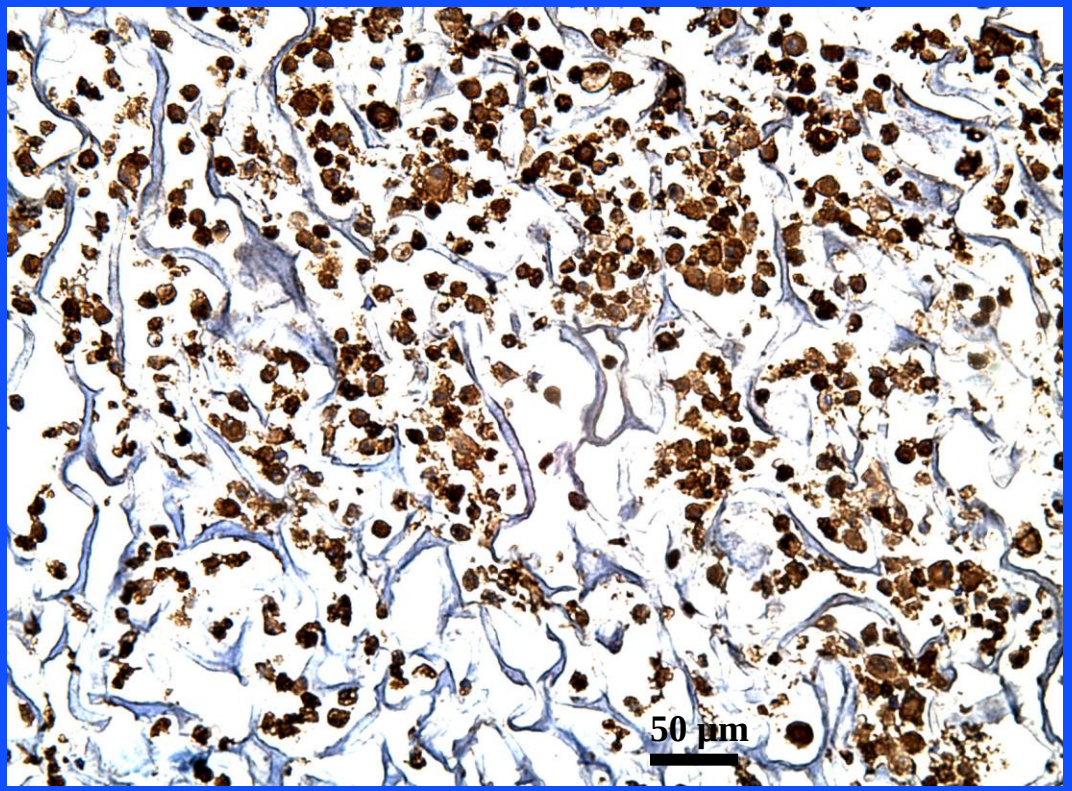
Infarct > 1wk, Implant > 3wks, Sacrificec



Sham Control, 4 weeks post-infarct

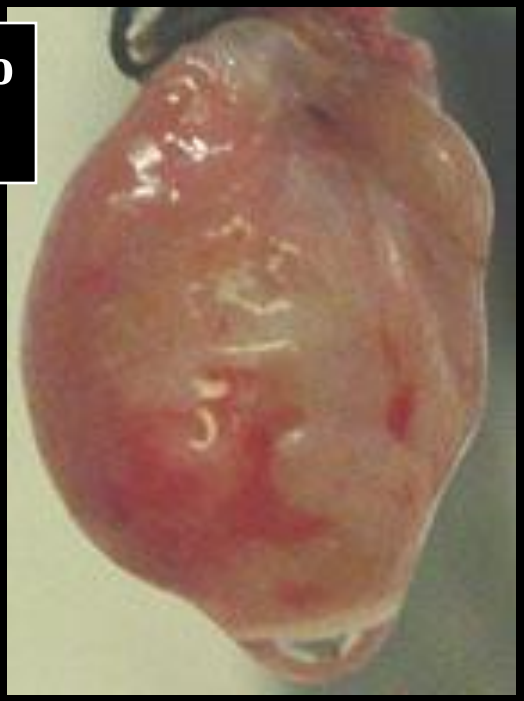
CELL-SEEDED SCAFFOLDS FOR GRAFTING TO THE RAT MYOCARDIUM

- **Collagen-GAG scaffolds as delivery vehicles for stem cells (Z. Xiang)**

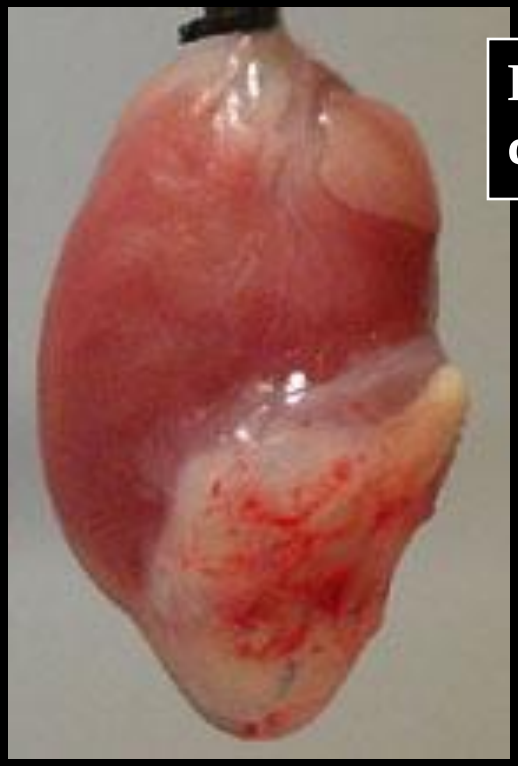


BrdU-labeled MSCs in a collagen-GAG scaffold

•Control, no implant

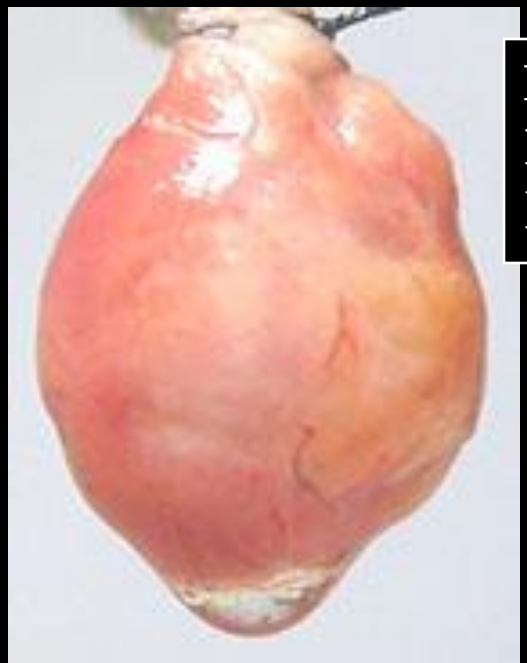


DHT + EDAC cross-linked



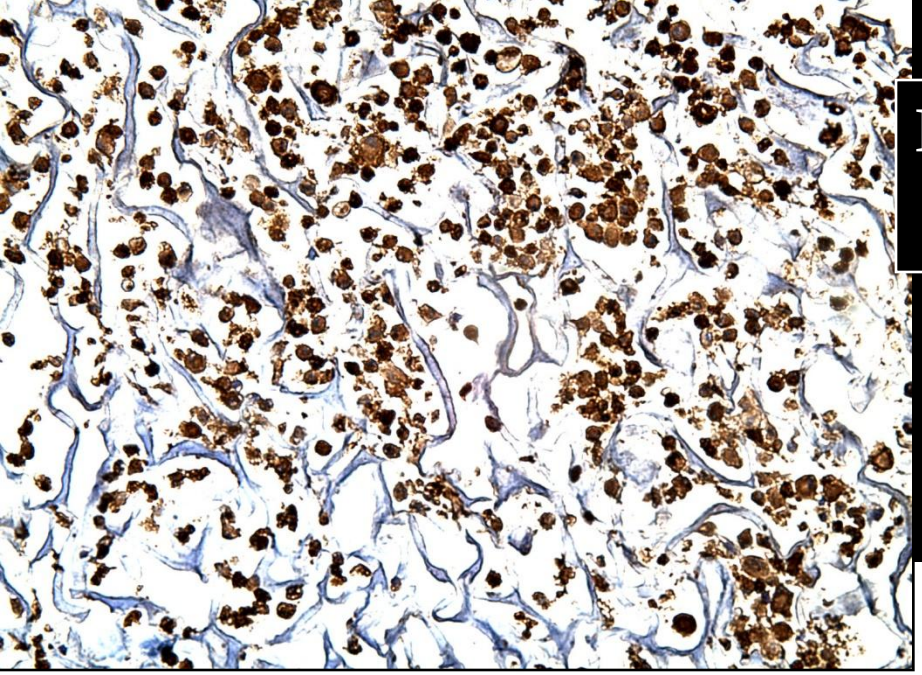
DHT cross-linked type I collagen-GAG implant

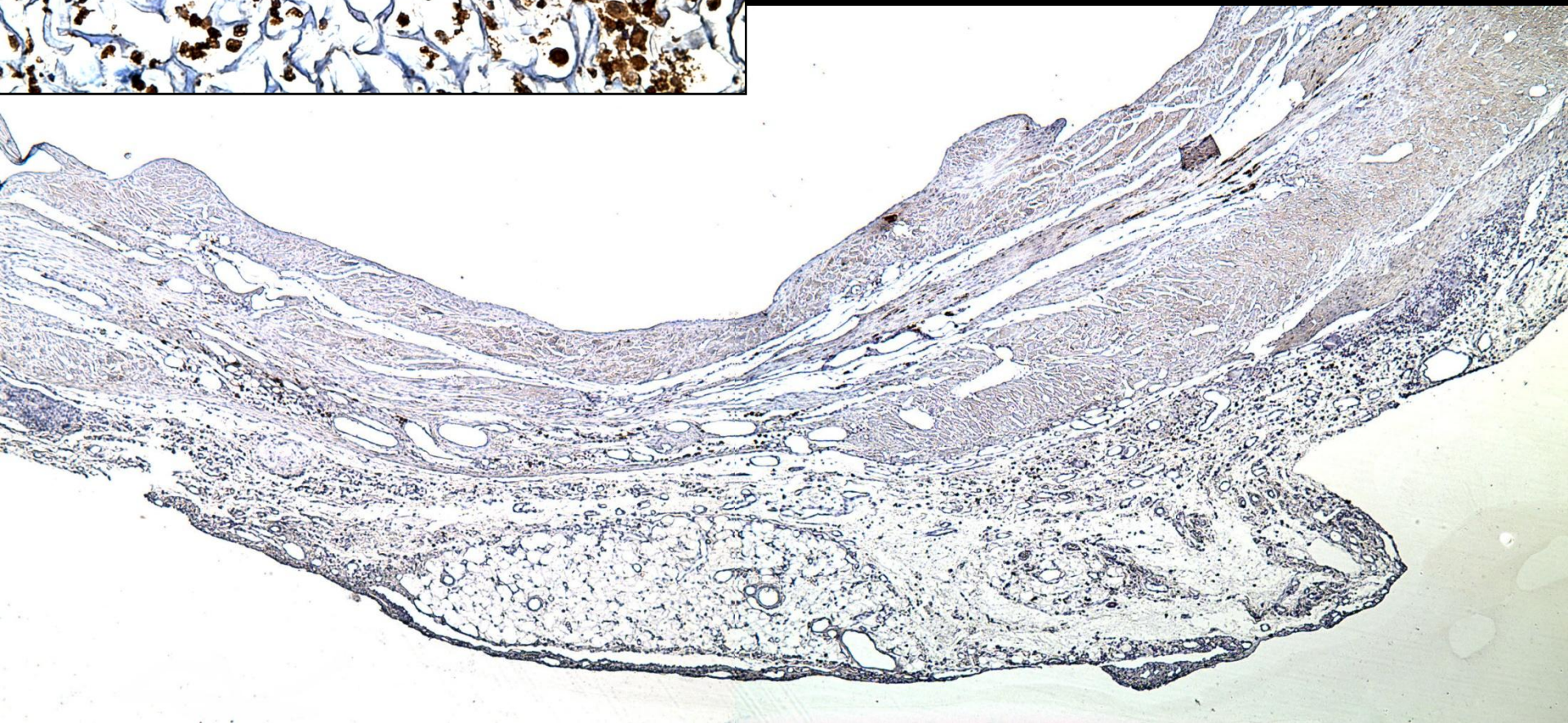
MSC-seeded DHT cross-linked



Z. Xiang

MSCs seeded DHT cross-linked type I collagen-GAG scaffold





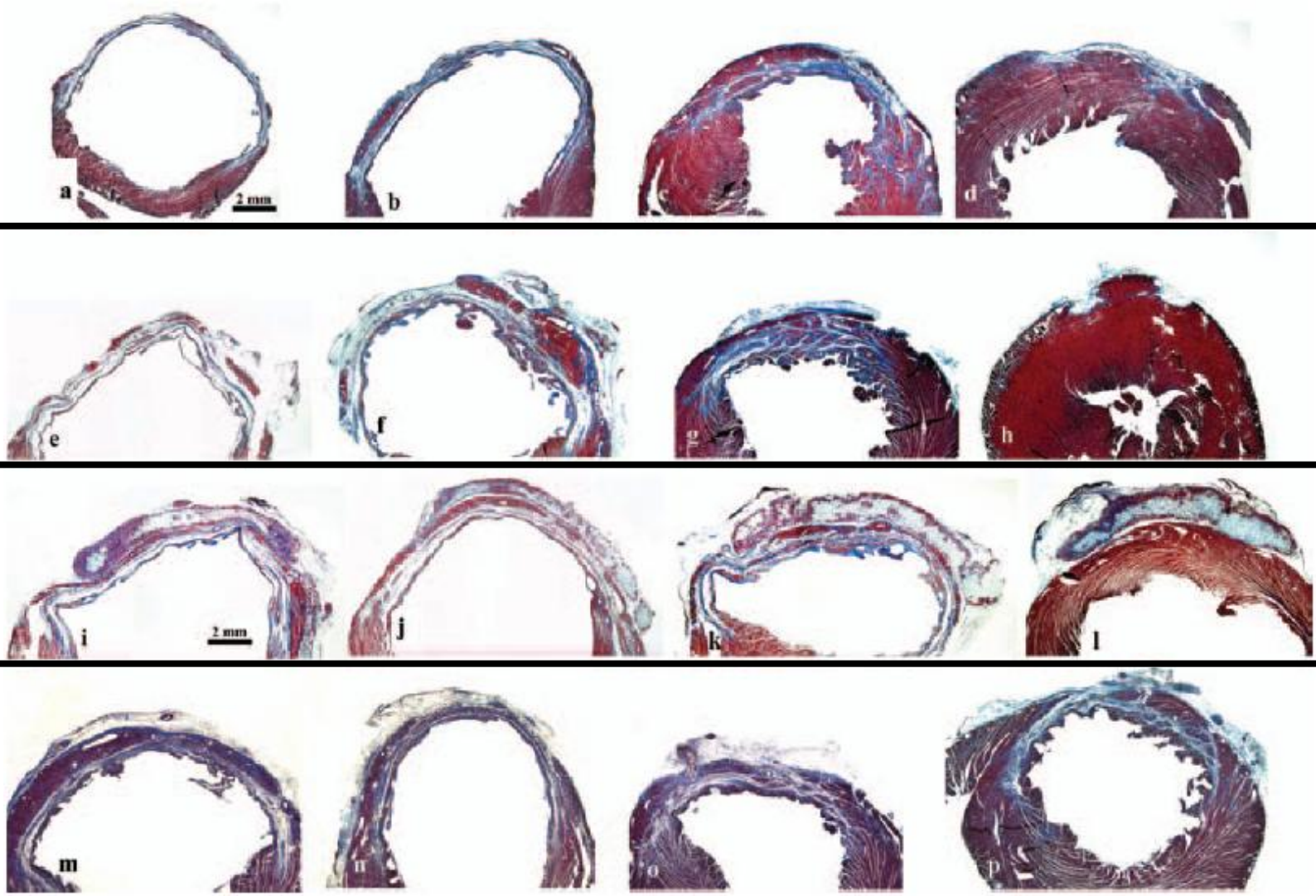
Collagen-GAG scaffolds grafted on MIs in rats

Control

DHT

EDAC

Cell-scaffold



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20.441J / 2.79J / 3.96J / HST.522J Biomaterials-Tissue Interactions

Fall 2009