Department of Pharmacology
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Πειραματικά μοντέλα στην έρευνα του καρδιαγγειακού

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Why we need pre-clinical models

The pre-clinical models are **simplified** simulations of human disease. They serve 2 main purposes:

1. Investigate the various etiologies and pathophysiology of the disease

2. Develop novel treatment strategies

- The aim of the study is very important for appropriate selection of the model (Fit-for-purpose)

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New Horizons in Translational Medicine (2014);2: 5–11

Criteria for Validation of Translational Capacity of Experimental Models

Proposed validity scoring system.

Criterion	Value	Score
Species	Human	4
	Non-human primate	3
	Non-human mammal	2
	Non-mammal	1
Disease simulation	True	4
	Complex	3
	Pharmacological	2
	No	1
Face validity	> 1 core symptom	4
	1 core symptom	3
	1 symptom	2
	No	1
Complexity	*In vivo*	4
	Tissue	3
	Cellular	2
	Sub-cellular/molecular	1
Predictivity	Graded for all pharmacology principles	4
	Graded for certain pharmacology principles	3
	All or none for certain pharmacology principles	2
	No or not shown	1

New Horizons in Translational Medicine (2014);2: 5–11

Preclinical Models of Myocardial Infarction

Goal: Simulate myocardial injury and cardiac remodeling after myocardial infarction

- ❑ In vivo model of coronary artery ligation (mouse, rat, pigs)
- ❑ Zero flow, global ischemia in isolated rat heart (Langendorff)
- ❑ Simulation of Ischemia in isolated cardiac cells
- ❑ Others (isoproterenol, Cryoinjury etc)

Pathophysiology of Myocardial Ischemia

Low Oxygen and Energy Substates

↓

Decrease in ATP

↓

Disrupted Ion Homeostasis

↓

Intracellular Increase in Sodium, Calcium, H^+

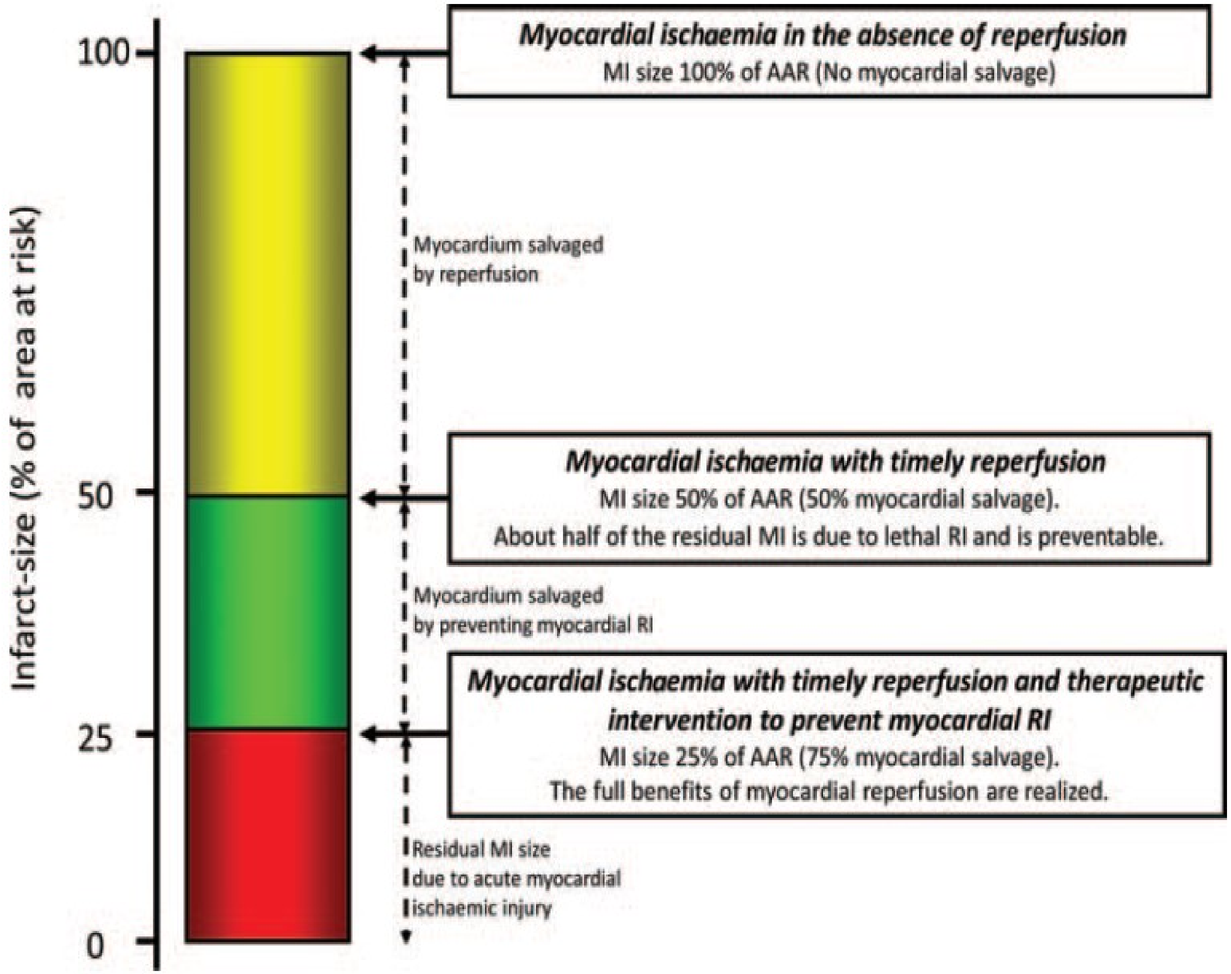
↓

Damage in molecules and organelles

↓

Cell swelling and death

Myocardial reperfusion injury: looking beyond primary PCI



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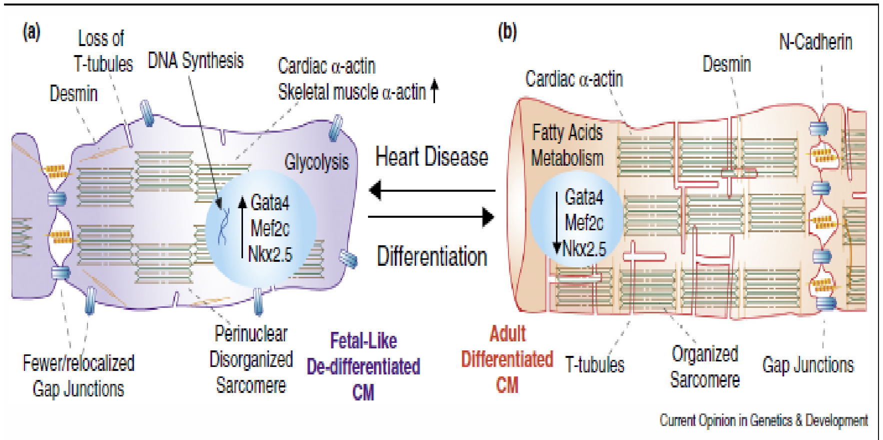
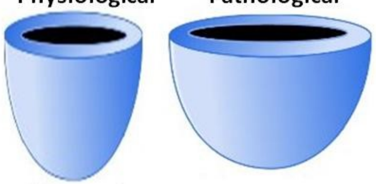
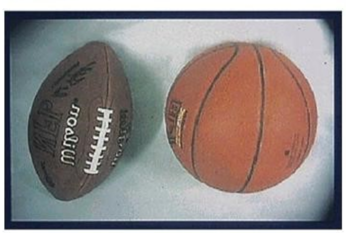
Eur Heart Journal 2013

Cardiac Remodeling after MI



Hypertrophy and changes in LV geometry





Fetal reprogramming

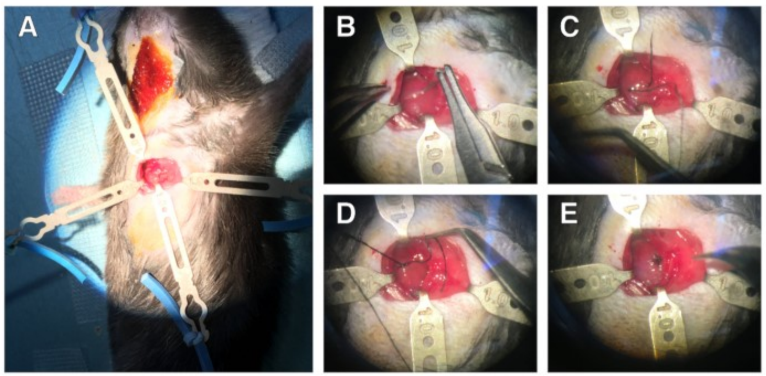
	Hypertrophy
Contractile proteins	
α-MHC	↓
β-MHC	↑
Cardiac α-actin	↓
Skeletal α-actin	↑
Ion pumps	
α_2 Na/K-ATPase	↓
SERCA 2a	↓
Metabolic proteins	
GLUT4	↓
GLUT1	=
Muscle CPT-1	↓
Liver CPT-1	=
mCK	↓
PPARα	↓
PDK4	↓
MCD	↓
UCP2	↓
UCP3	↓

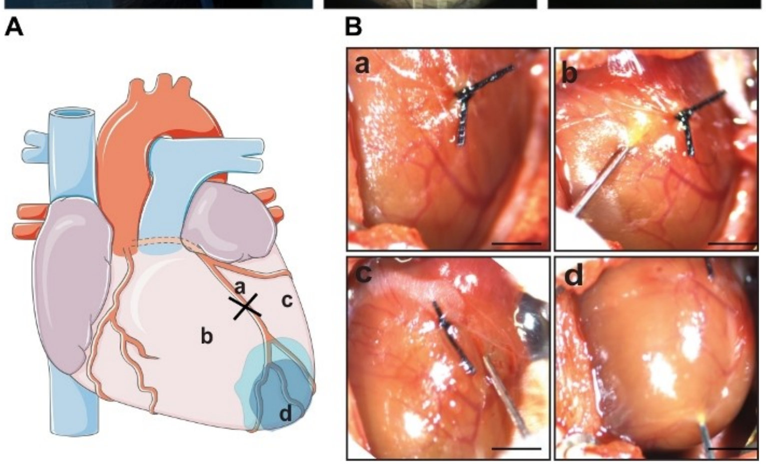
- Changes in LV geometry
- Fetal reprograming
- Dedifferentiation
- Fibrosis

Bloomekatz J et al 2016

Model of in vivo ischemia/reperfusion by temporary ligation of LAD

Purpose: Simulate Acute Myocardial Infarction and reperfusion in humans





	Sham (n=10)	I/R (n=11)
LVIDd (mm)	6.5 (0.06)	7.0 (0.21)
LVIDs (mm)	3.7 (0.1)	4.5 (0.30)*
LVEF%	75.7 (1.6)	50.0 (1.6)*
SVPW (mm/sec)	38.4 (1.4)	32.0 (2.1)*
Scar Area (mm^2)	-------	82 (5.4)

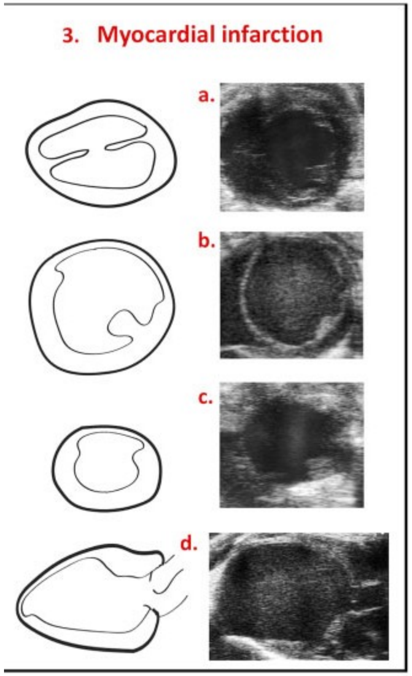
Limitations:
Intubation, surgical technique, time-consuming procedure, variation in infarct size

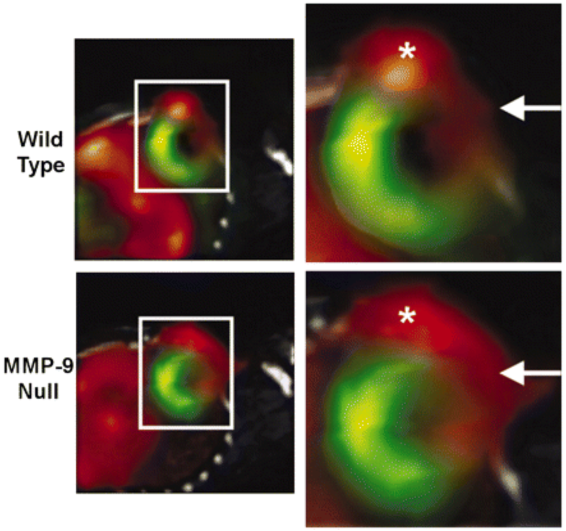
Model of in vivo ischemia/reperfusion by temporary ligation of LAD

Advantages

Imaging Modalities to assess Cardiac Function and injury in vivo

- Echocardiography
- Micro-Computed Tomography
- Cardiac Magnetic Resonance
- micro-PET
- micro-SPECT

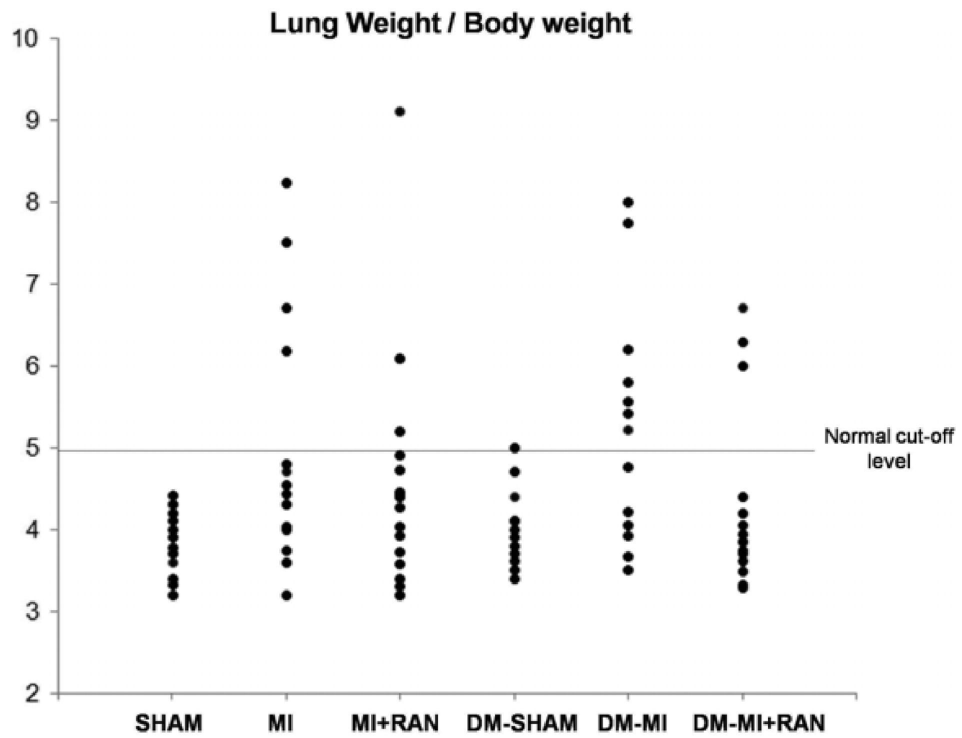




Model of in vivo ischemia/reperfusion by temporary ligation of LAD

Advantages

Assessment of mortality and clinical signs such as pulmonary congestion



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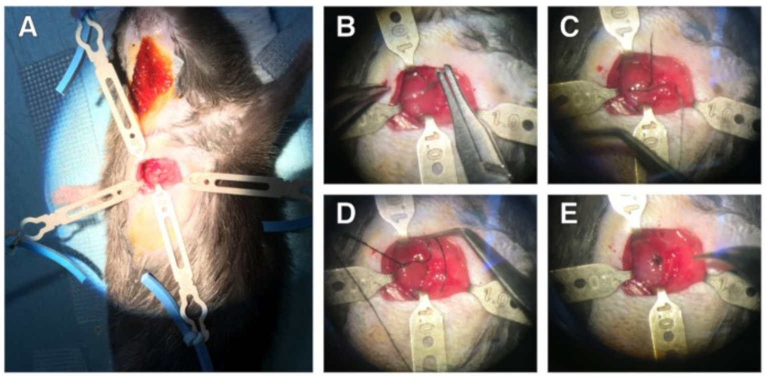
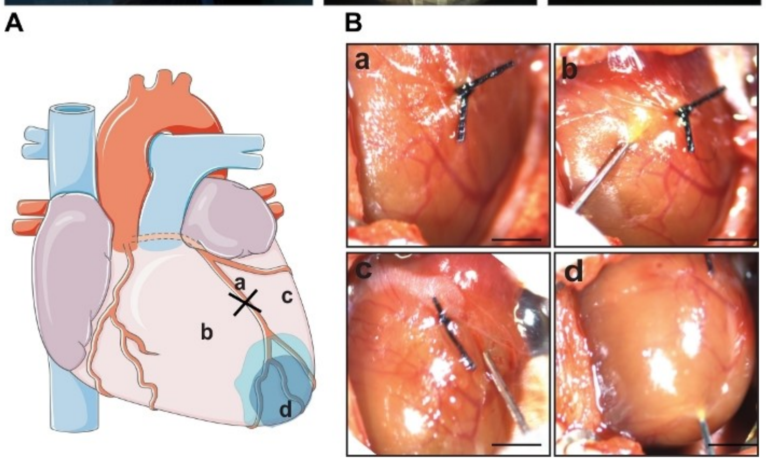
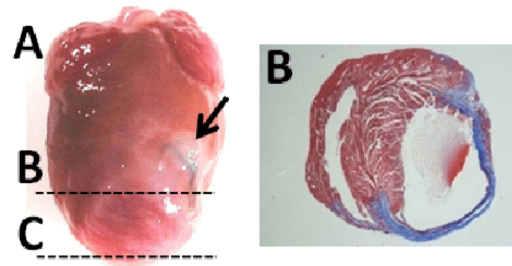
Mourouzis I et al , Journal of Cardiovascular Pharmacology and Therapeutics,

Model of in vivo ischemia/reperfusion by permanent ligation of LAD

Purpose: Simulate Chronic Heart Failure with reduced systolic function in humans







	Sham (n=10)	AMI(n=11)
LVIDd (mm)	6.9(0.2)	9.6(0.3)*
LVIDs (mm)	4.2(0.2)	8.5(0.4)*
LVEF%	73.8(1.8)	30(2.1)*
SVPW (mm/sec)	36.4 (1.5)	26.0 (2.8)*
Scar Area (mm^2)	-------	82 (5.4)

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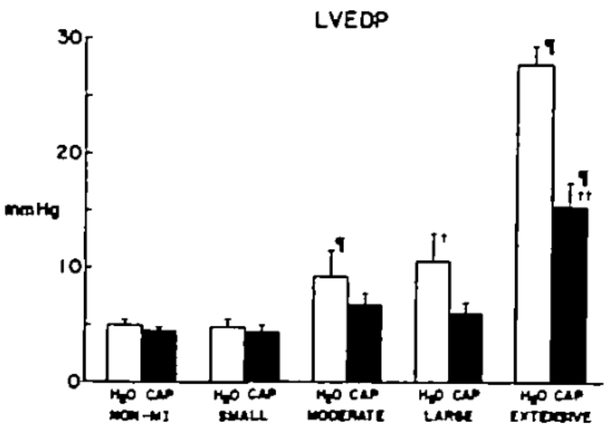
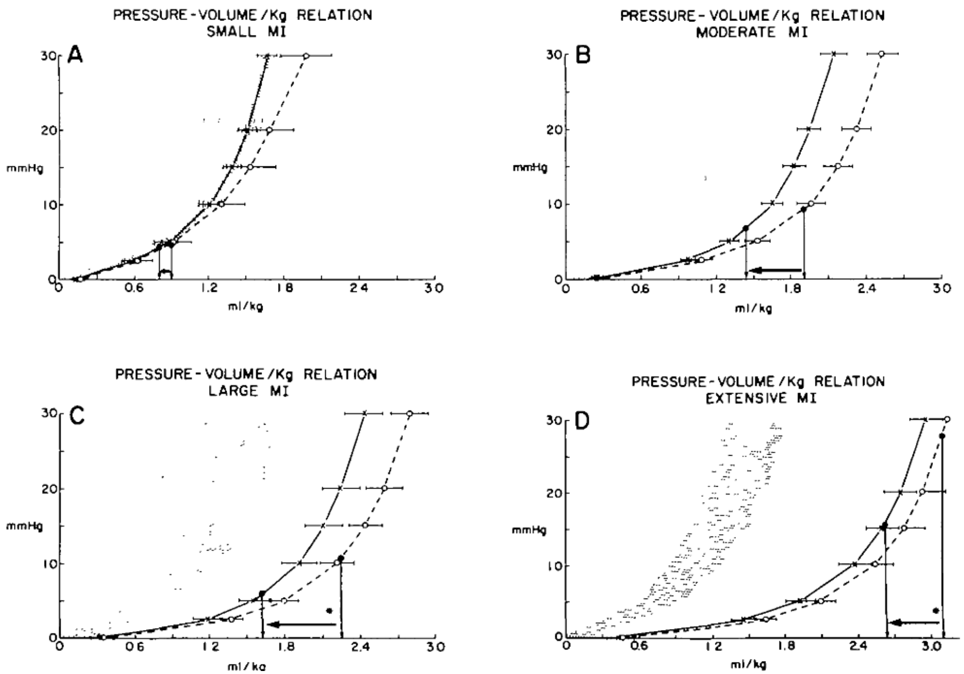
Model of in vivo ischemia/reperfusion by temporary ligation of LAD

First experimental evidence that ACE inhibitors are beneficial after MI





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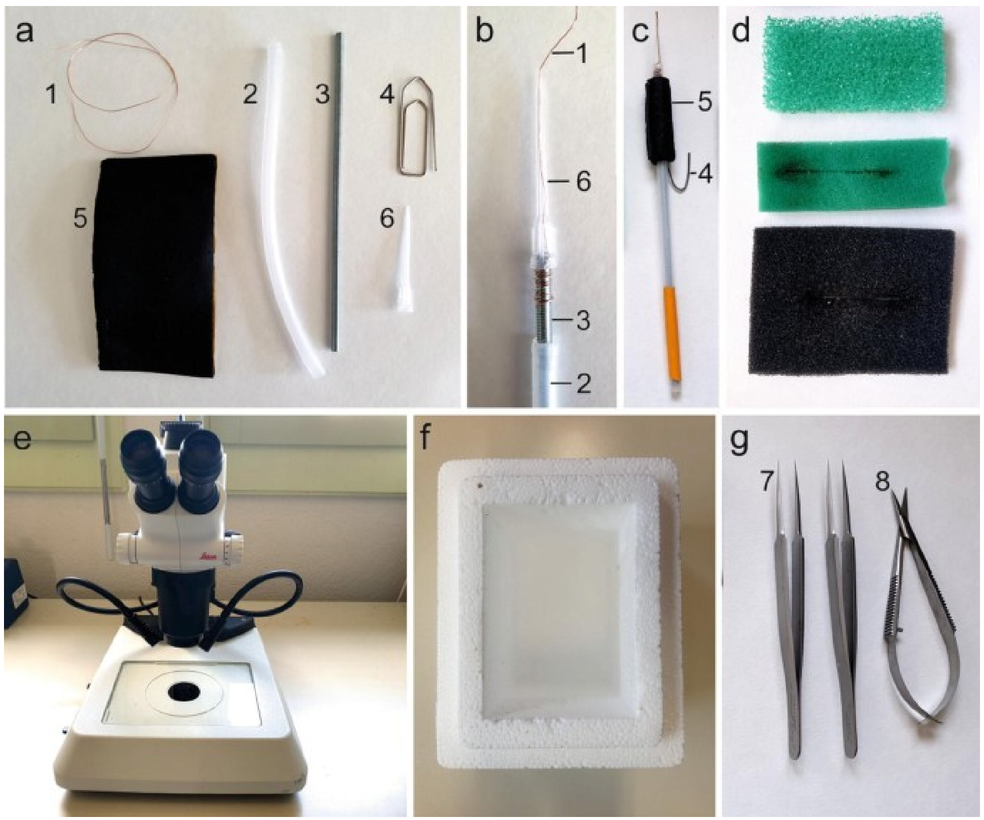
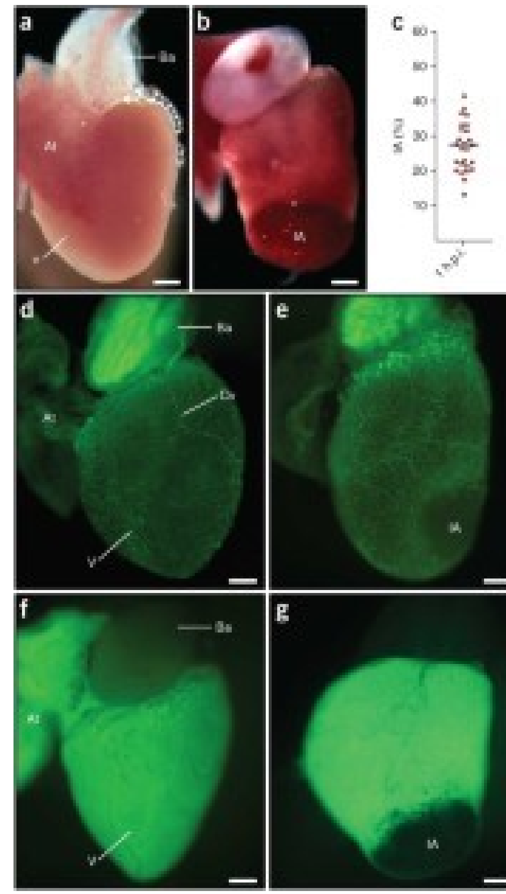
Pfeffer JM, Pfeffer MA, Braunwald E. Influence of chronic captopril therapy on the infarcted left ventricle of the rat. Circ Res. 1985 Jul;57(1):84-95.

Cryoinjury as a myocardial infarction model for the study of cardiac regeneration

Purpose: Studies of myocardial regeneration



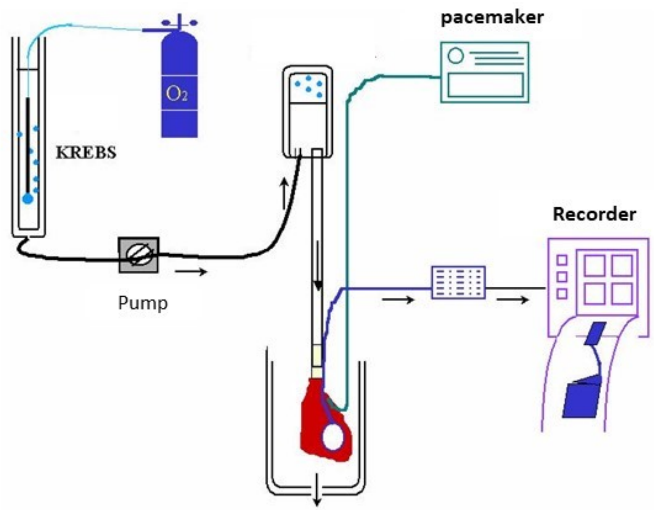


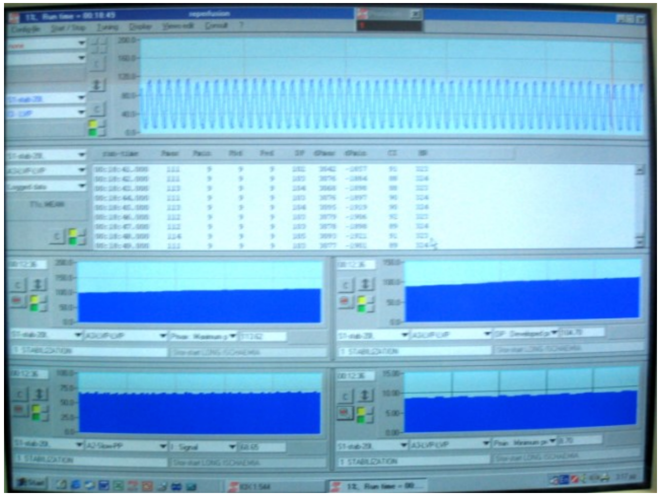
Nat Protoc **7**, 782–788 (2012)

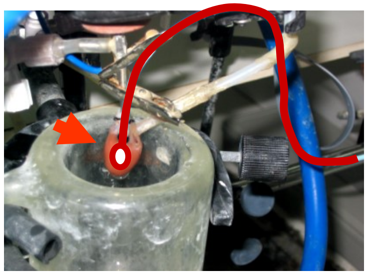
Rodent Models of Myocardial Ischemia/Reperfusion

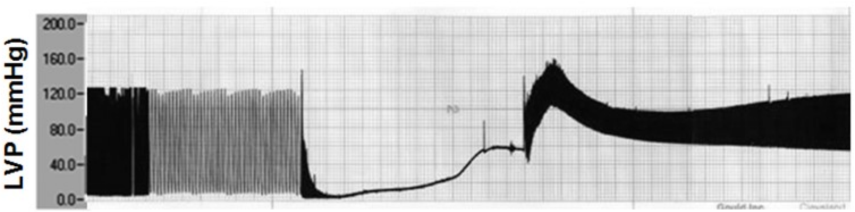
Model of Isolated Mouse/Rat Heart (Langendorff)

Purpose: Simulate Acute Myocardial Ischemia/Reperfusion









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Model of Ischemia/Reperfusion in Isolated Mouse/Rat Heart

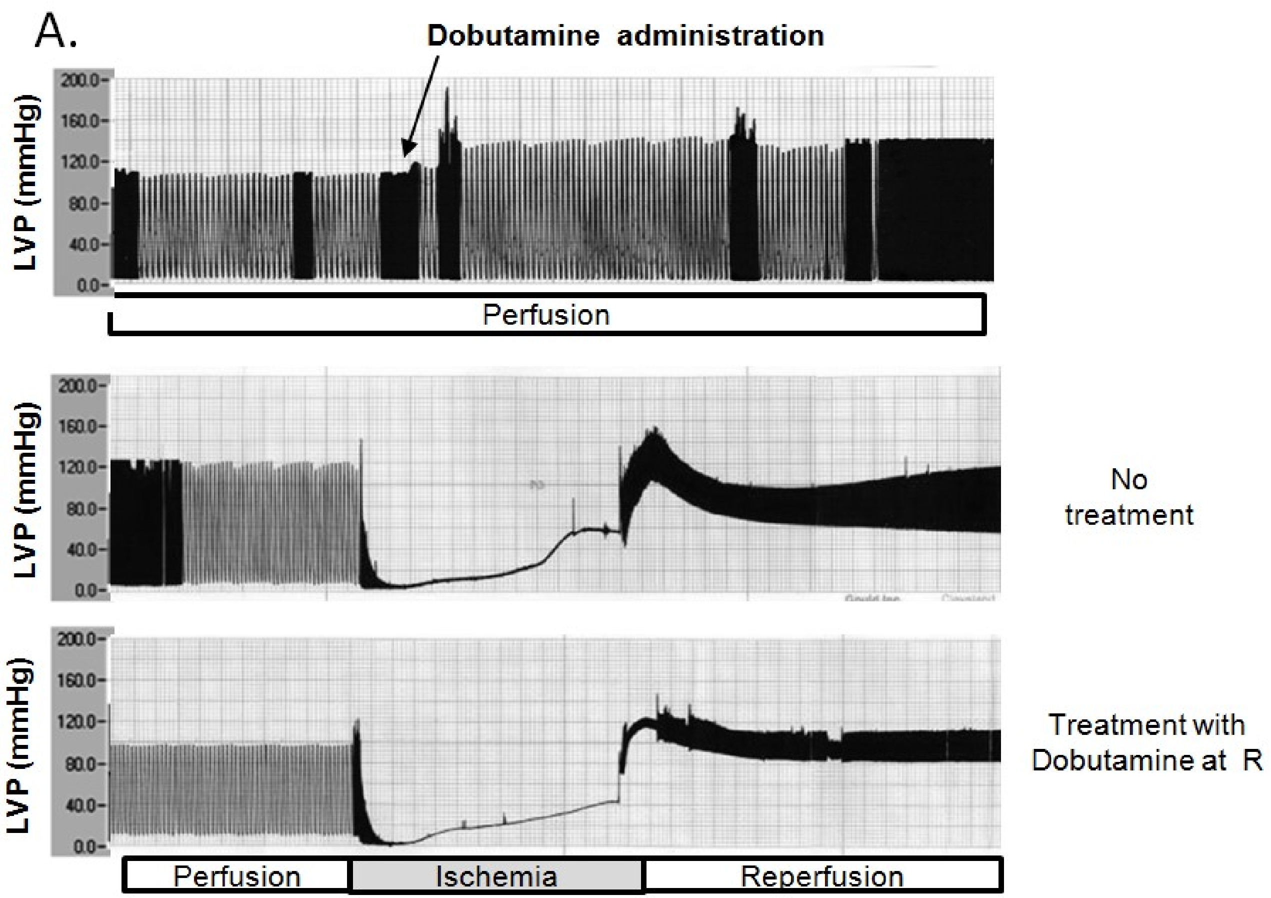
Disadvantages

- ❑ Changes in Metabolism (dependence on Glucose)
- ❑ Retrograde perfusion
- ❑ Hypoxic Environment
- ❑ No inflammatory component
- ❑ Global Ischemia

Advantages

- ❑ Control on Heart Rate
- ❑ Control on preload and afterload
- ❑ Investigation of direct action on the heart (mechanistic studies)
- ❑ Accurate Assessment of cardiac function and injury

The effect of Sympathomimetics in Reperfusion Injury



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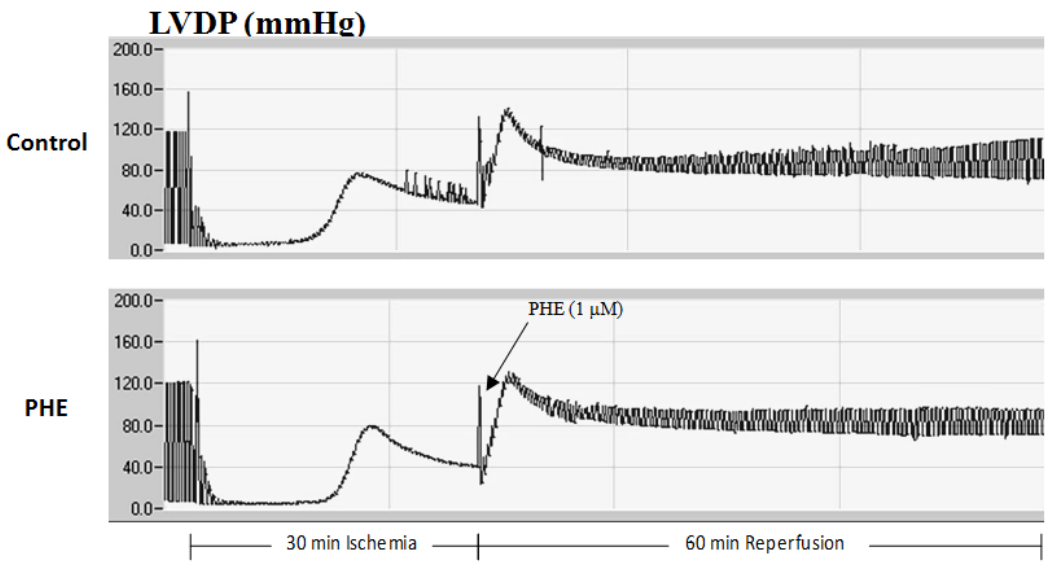
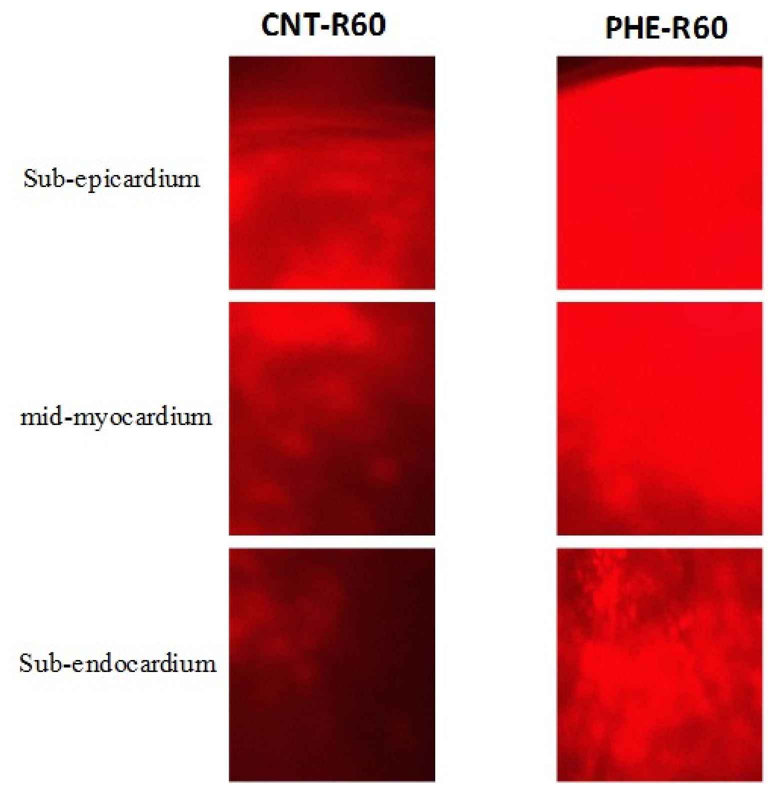
The effect of Sympathomimetics in Reperfusion Injury

Function



Apoptosis



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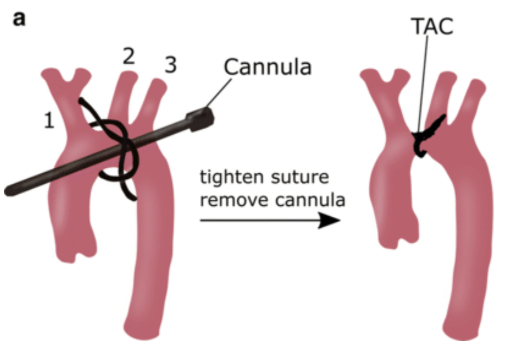
Mourouzis et al. Ann Card Anaesth. 2014 Jul-Sep;17(3):200-9

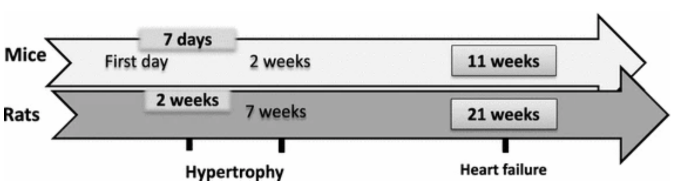
Rodent Models of Heart Failure (HFrEF)

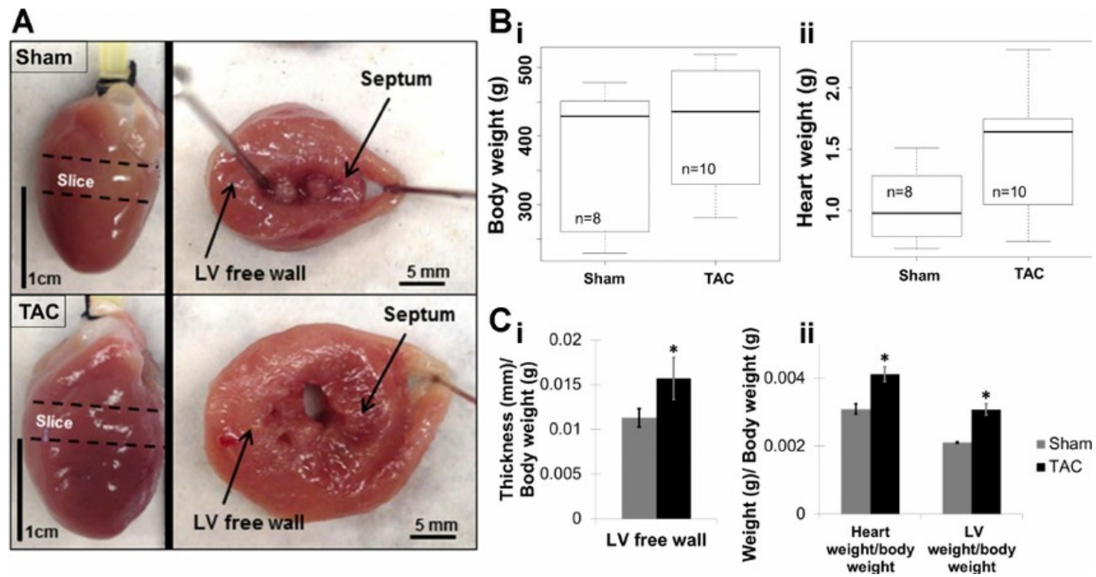
Model of in vivo transverse aortic constriction (TAC)

Purpose: Simulate cardiac hypertrophy and Heart Failure due to Aortic valve stenosis in humans



For TAC model, the band is placed on the aortic arch between the innominate and left carotid arteries.





Limitations:
Does not reflect the gradual progression of AoV stenosis in patients

Rodent Models of Cardiomyopathy

Genetically Engineered Models of cardiomyopathy in mice

Purpose: Simulate dilated or hypertrophic cardiomyopathy in humans

Cytoskeletal and sarcomeric proteins: Muscle LIM protein (MLP), β-MHC, troponin T, MyBP-C, Desmin, actin, Dystrophin

Calcium-regulating proteins: L-type Ca^{2+} channels, SERCA2A, Calsequestrin, S100A1, RyR

Neurohumoral receptors : α1B-AR, α2A or α2C-AR, β1-AR, β2-AR, β-ARK1, AT-1, TNF-α, IL-6 and gp130 (receptor)

Cellular signalling proteins: Gaq, Gas, Gi, Ras, Rho, Rac1, PKC, PKA, CaMKII, Calmodulin, Calcineurin

ECM proteins : β1-integrin, MMP-2, MMP-9, TIMP-1

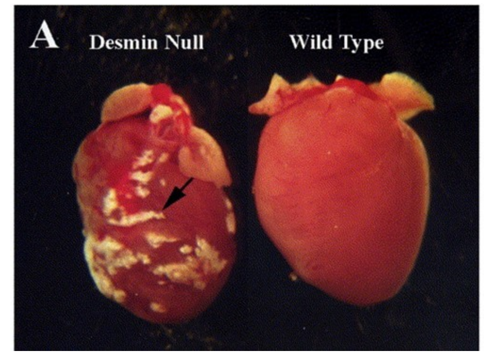
Heart Fail Rev (2013) 18:219–249

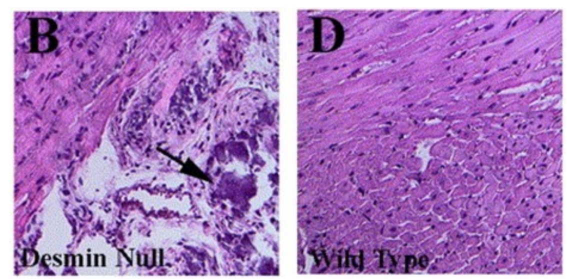
Rodent Models of Cardiomyopathy

Genetically Engineered Models of cardiomyopathy in mice

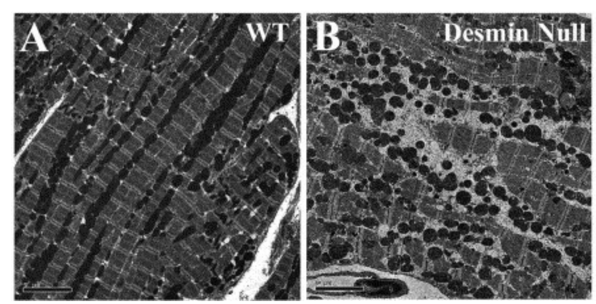
The Desmin Null mouse

	Wild-type (n=8)	DesKO(n=8)
Body weight (BW in g)	19.8(1.0)	16.0 (0.5)
Left Ventricular Weight (LVW in mg)	61.5(2.4)	58.4 (3.8)
LVW/BW	3.1 (0.12)	3.6 (0.18)#
LVEDD/BW (mm/g)	$14.7*10^{-3}$ $(0.7*10^{-3})$	$22.3*10^{-3}$ $(0.7*10^{-3})$ #
LVESD/BW (mm/g)	$8.2*10^{-3}$ $(0.6*10^{-3})$	$14.7*10^{-3}$ $(1.1*10^{-3})$ #
Ejection Fraction (EF%)	87% (1.2)	58% (1.9) #
SVPW (mm/sec)	3.12(0.15)	2.47(0.14) #





Left ventricle Ventricle



Right Ventricle

Rodent Models of Cardiomyopathy

Genetically Engineered Models of cardiomyopathy in mice

The Tg197 Rheumatoid Arthritis mouse model

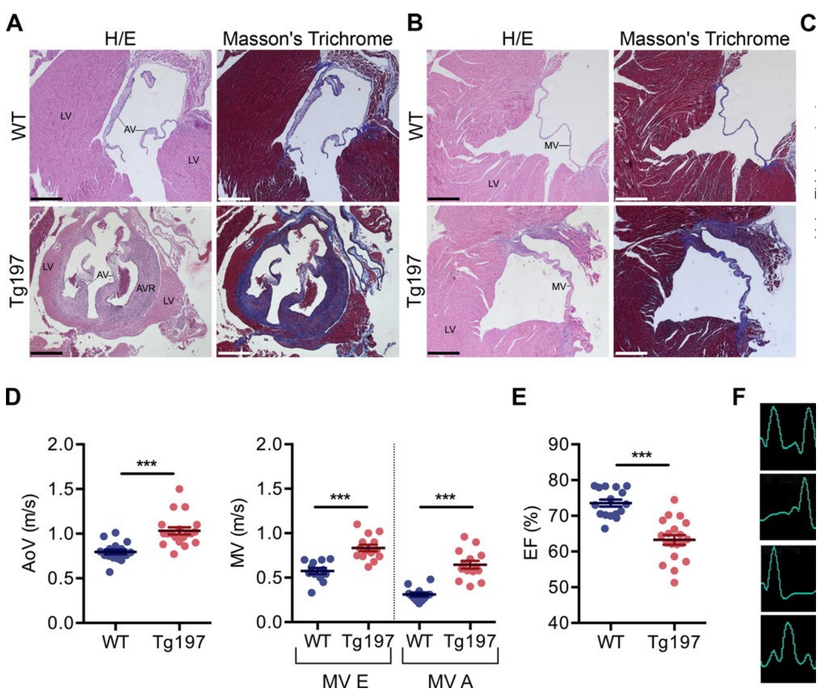


Table 1 Echocardiographic parameters in Tg197 and WT mice at 12 weeks of age

	WT (n=16)	Tg197 (n=19)	P value
Body weight (g)	26.25±1.06	16.73±0.091	<0.0001
Heart weight (mg)	107.10±3.39	83.00±5.31	<0.0001
HW/BW (mg/g)	4.11±0.08	5.05±0.27	0.0054
LVEDd (mm/BW)	0.14±0.01	0.22±0.01	<0.0001
LVEDs (mm/BW)	0.08±0.01	0.14±0.01	<0.0001
LVLd (mm/BW)	0.28±0.01	0.38±0.01	<0.0001
LVPWd (mm/BW)	0.026±0.001	0.038±0.002	<0.0001
IVSd (mm/BW)	0.026±0.001	0.038±0.002	<0.0001
LA (mm/BW)	0.083±0.003	0.132±0.006	<0.0001
SVPW (cm/s)	3.02±0.08	2.14±0.07	<0.0001
E/A ratio	1.87±0.13	1.33±0.09	0.0009

Values were normalised with the body weight (except for SVPW), as indicated in the table. Data were expressed as mean±SEM.
E/A ratio, ratio between E (peak early diastolic flow) and A (peak late diastolic flow); HW/BW, heart weight-to-body weight ratio; IVSd, end-diastolic interventricular septal thickness; LA, left atrium; LVEDd, left ventricular end-diastolic diameter; LVEDs, left ventricular end-systolic diameter; LVLd, left ventricular length in diastole; LVPWd, left ventricular end-diastolic posterior wall thickness; SVPW: systolic velocity of the posterior wall.

Ntari L, Sakkou M, Chouvardas P, et al. Ann Rheum Dis 2018;**77**:926–934.

Rodent Models of Cardiomyopathy

Drug induced Cardiomyopathy

Purpose: Simulate dilated cardiomyopathy or pulmonary hypertension and RV failure in humans

Doxorubicin: Anti-cancer drug, produces ROS, affects endothelial function and mitochondrial function and develops dilated cardiomyopathy	**Limitations:** Systemic toxic effects, high non-cardiac mortality
Isoproterenol: β-adrenergic agonist, in high doses causes cardiomyocyte apoptosis and fibrosis.	**Limitations:** Reproducibility, mortality due to arrhythmias, little translational value
Monocrotaline: plan toxin, causes endothelial injury and increases pulmonary resistance, leads to pulmonary hypertension and RV failure	**Limitations:** Toxicity of other organs (liver and kidney)

Rodent Models of Hypertension

Model of Dahl salt/sensitive rats and Spontaneous Hypertensive Rats

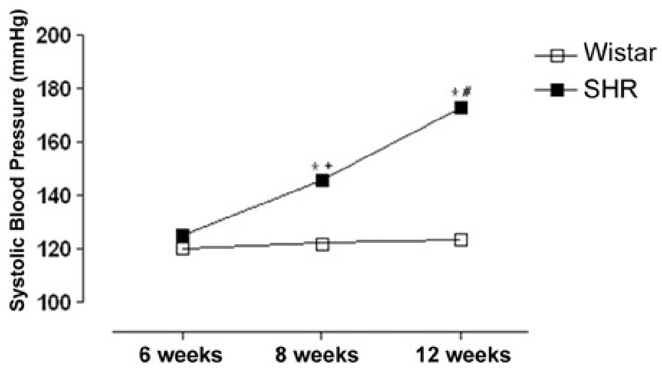
Purpose: Simulate hypertension, cardiac hypertrophy and Heart Failure in humans

Dahl salt/sensitive rats:

- Inbred strain of Sprague-Dawley rats susceptible to hypertension following a high-salt diet
- Slow progression of hypertension and HF

SHR rats:

- Inbred strain of Wistar-Kyoto rats with hypertension
- Slow progression of hypertension (after 8 wk) and HF (after 18–24 months)

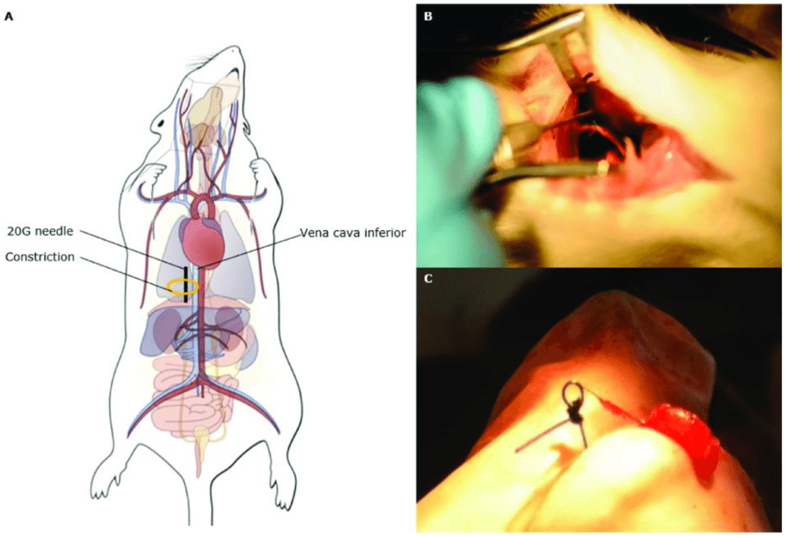


	CFY^{76w}	$SHR\text{-}C^{76w}$
EF (%)	71.07 ± 1.89	52.41 ± 1.85*
FS (%)	41.9 ± 1.71	28.08 ± 1.23*
EDD (mm)	7.1 ± 0.13	8.23 ± 0.2*
ESD (mm)	4.08 ± 0.06	5.92 ± 0.18*
LVEDV (mL)	278.5 ± 5.94	368.49 ± 7.13*
LVESV (mL)	86.03 ± 11.37	175.41 ± 12.29*

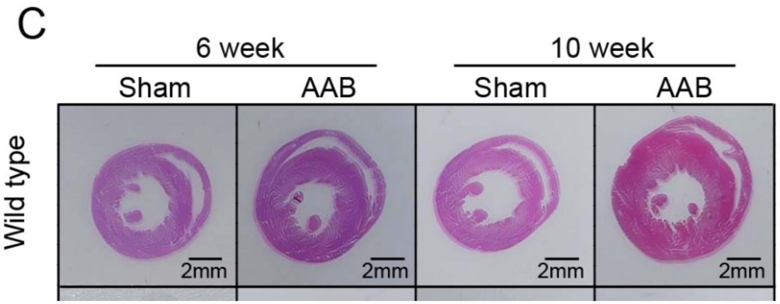
Rodent Models of Hypertension

Model of in vivo abdominal aortic banding

Purpose: Simulate hypertension and cardiac hypertrophy in humans



Constriction in abdominal aorta above the renal arteries



Hypertension by renal hypoperfusion and concomitantly LV hypertrophy
HF does not develop

Rodent Models of Diabetic Cardiomyopathy

Purpose: Simulate Chronic Heart Failure with preserved systolic function in humans (HFpEF)

Streptozotocin: Pancreatic beta-cell toxin, easy model of type 1 diabetes

Limitations:
Type-1 diabetes is rare

Zucker Diabetic Fatty rats: dysfunctional leptin receptor, hyperphagia, hyperlipidemia, hyperglycemia. Type 2 diabetes similar to patients. Multiorgan dysfunctions

Limitations:
Difficult to breed, high costs

Db/db mice: leptin receptor deficiency, hyperphagia, hyperlipidemia, hyperglycemia. Type 2 diabetes similar to patients. Multiorgan dysfunctions

Limitations:
Difficult to breed, high costs

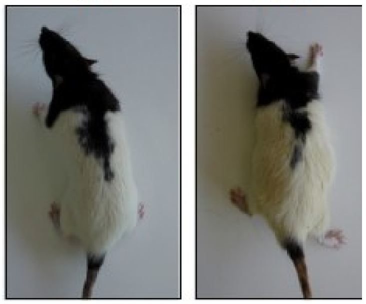
Rodent Models of Diabetic Cardiomyopathy

Purpose: Simulate Chronic Heart Failure with preserved systolic function in humans (HFpEF)

ZDF rats

A

ZL ZDF

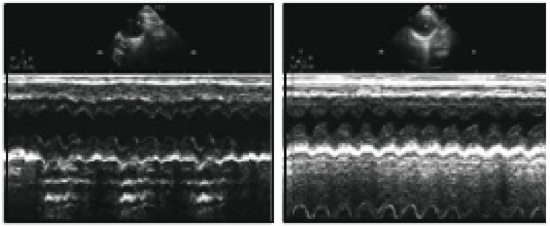


	ZL	ZDF
Plasma:		
Aldosterone (pg/ml)	180±57.19	430±192.63*
Glucose (mg/dl)	194.55±38.2	666.66±102.6:
Insulin (ng/mL)	1.08±0.11	1.83±0.24*
Ch (mg/dl)	61.11±8.35	187.16±14.13'
TAG (mg/dl)	39.66±8.36	397.66±166.8:
FFA (mM)	1.61±1.15	5.17±1.19**
HDL (mg/dl)	19.77±2.33	76.5±10.36**
Ch non-HDL (mg/dl)	41.33±6.53	112.33±14.05'
Weight (g)	431.9±26.73	341.14±38.7:
HW/FL	0.41±0.03	0.55±0.06*

B

ZL ZDF

Echo-Doppler



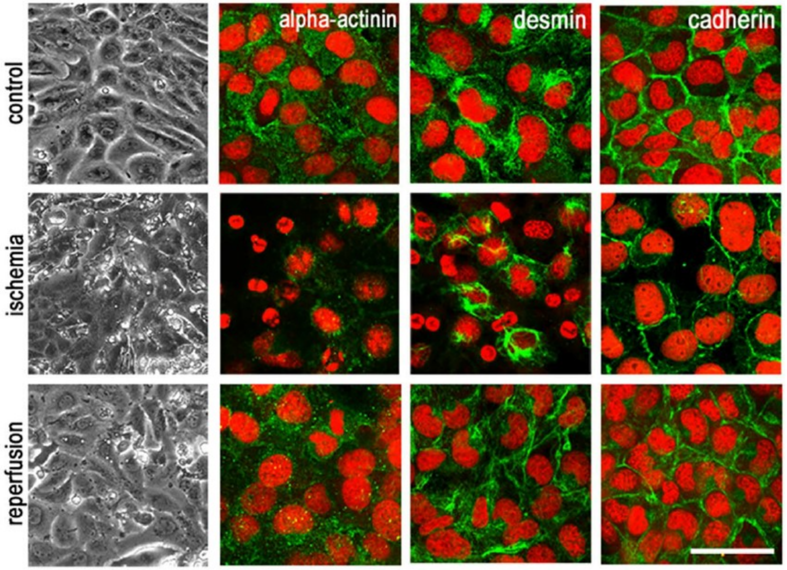
	ZL	ZDF
LVPW (cm)	0.21±0.036	0.23±0.034
IVS (cm)	0.18±0.029	0.24±0.015**
LVDD (cm)	0.71±0.10	0.51±0.115*
LVSD (cm)	0.32±0.097	0.21±0.076*
EF (Teich)	0.88±0.07	0.9±0.07
Dec. T (s)	0.036±0.001	0.04±0.003*
E/A ratio	1.22±0.03	1.05±0.03*

Ramírez et al. Cardiovascular Diabetology 2013, 12:1

Cell Culture Models

Purpose: Mechanistic Studies, Screening Studies

- ❑ Cardiac Myoblasts H9C2
- ❑ Immortalized Cardiac Cells
- ❑ Isolated Neonatal Cardiomyocytes
- ❑ Isolated Adult Cardiomyocytes



Simulation of Ischemia/Reperfusion

- Absence of glucose and substrates
- Acidosis
- Low oxygen chamber
- Incubator with N2
- H2O2

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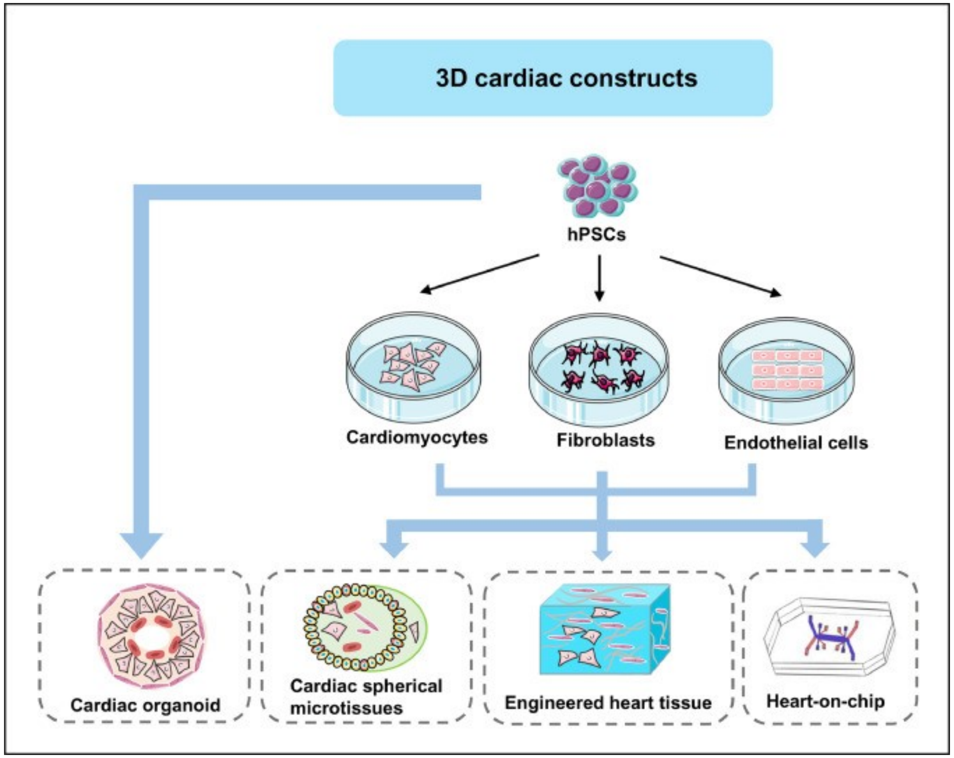
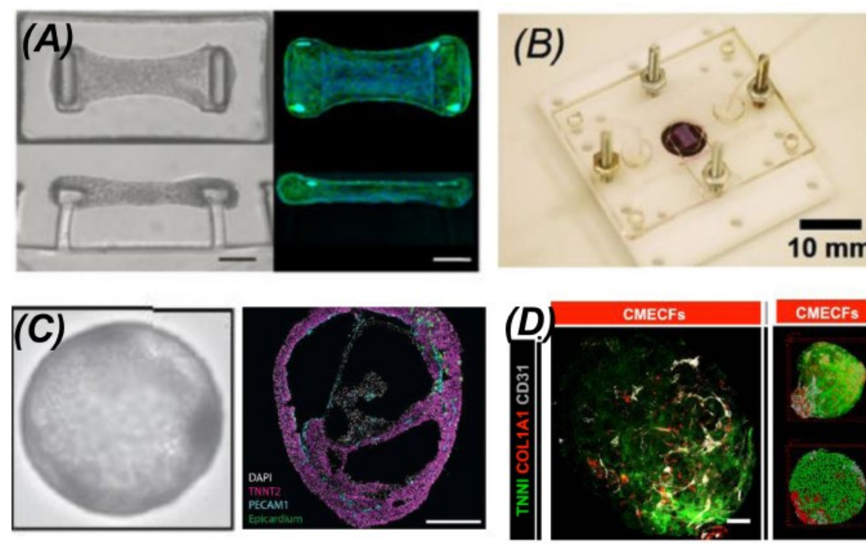
Problems in Cell Culture Models

- 3D structure and geometry is very important for cardiac function
- Cardiomyocytes contract in conjunction and synchronously
- Heart Rate – mechanical stress
- Different types of cell populations (CMs, fibroblasts, endothelial cells, inflammatory cells)
- Difficulty in induction of injury

Advanced Cell Culture Models





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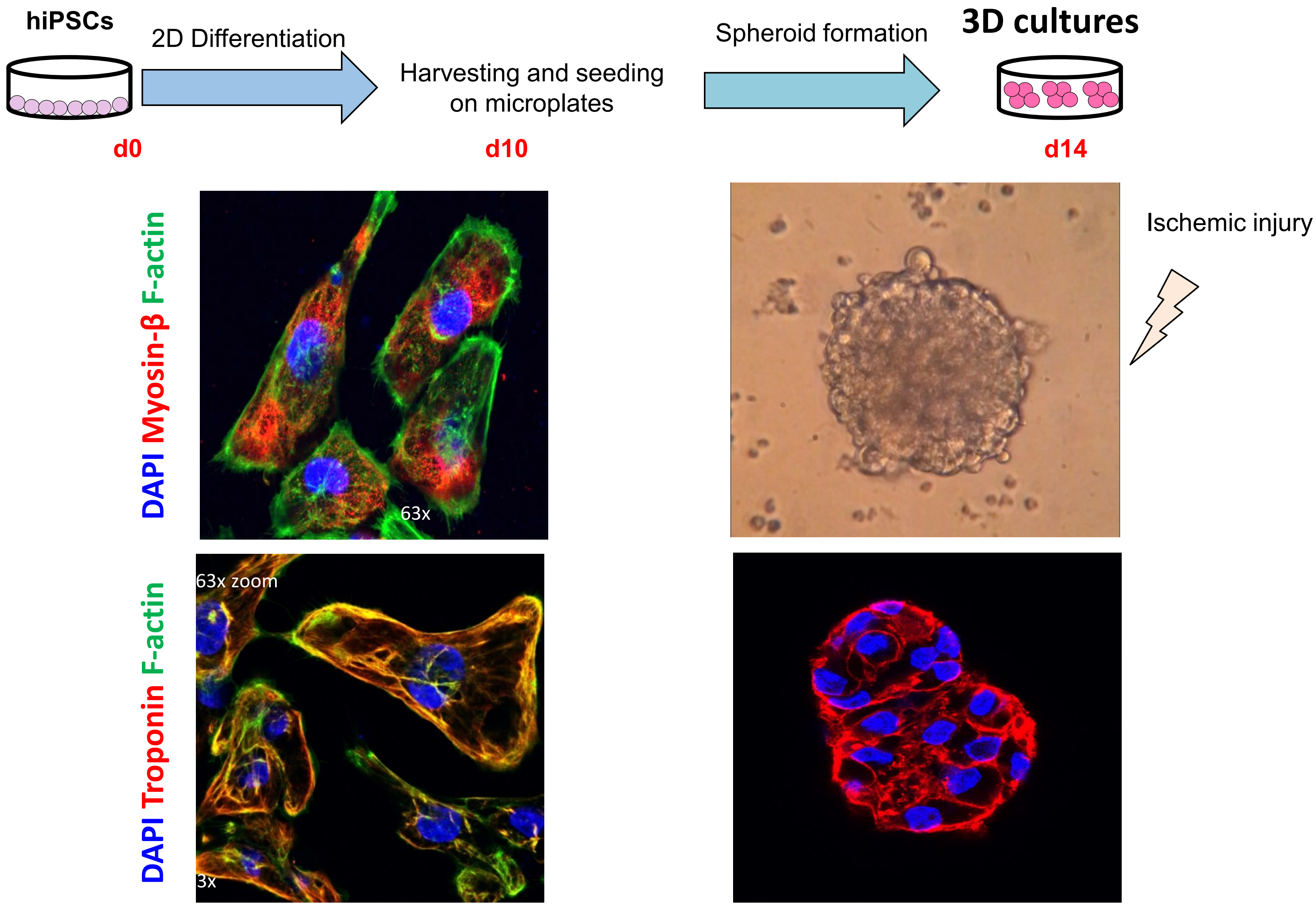
eBioMEdicine, DOI:https://doi.org/10.1016/j.ebiom.2022.103813

Cardiac Organoids from hiPSC



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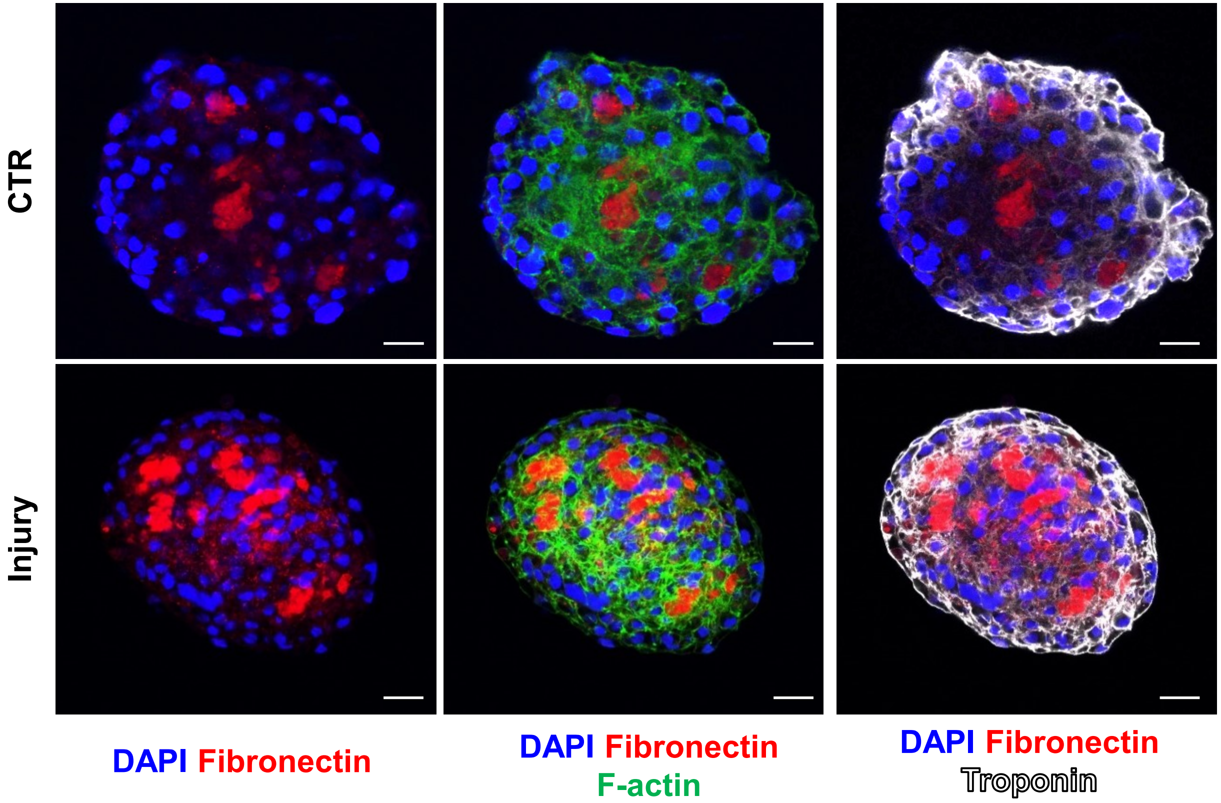
Cardiac Organoids from hiPSC



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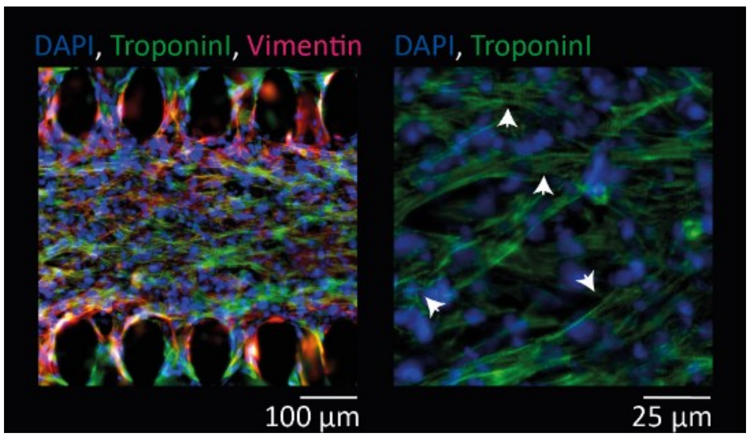
Heart on a chip

uHeart is a miniaturized model of a functional and beating human heart on a chip



culturing cardiomyocytes with supportive fibroblasts for 7 days in 3D cultures

BiomimX_uHeart.mp4.mp4

BiomimX, https://www.biomimx.com/uheart/

Thank you