Imperial College London

Imaging for experimental cardiovascular science

Daniel Stuckey

Cardiac Myogenesis, Death and Regeneration Group

National Heart and Lung Institute

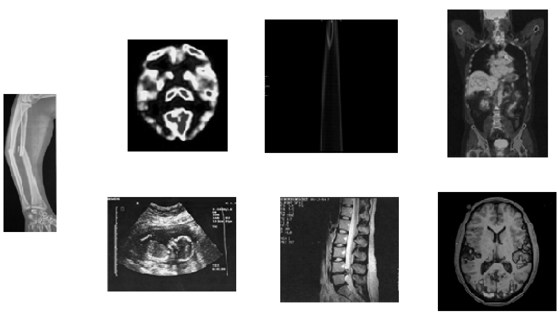
British Heart Foundation

How to see within

Rembrandt 1632
The Anatomy Lesson of Dr. Nicolaes Tulp

Wilhelm Roentgen 1895
The X-ray

Medical imaging

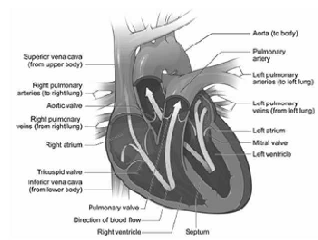


1900 1960 1980 2000

Applications of cardiac imaging

- Diagnosis
- Severity of disease
- Location of pathology
- Response to therapy

- Silent pathology
- Mechanisms of disease
- Location of grafts



Animal models of CV disease

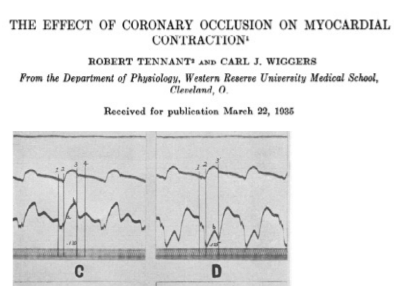
Myocardial infarction
- Permanent coronary occlusion
- Ischemia - reperfusion
- Cryoinjury

Hypertension and heart failure
- Trans aortic constriction
- Spontaneous hypertension
- Cardiotoxins

Congenital heart disease – HCM/DCM
- Genetic modification of contractile protein

Myocarditis

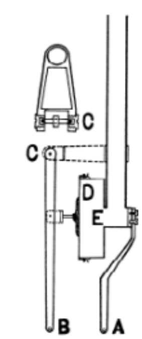
In vivo cardiac imaging modalities


THE EFFECT OF CORONARY OCCLUSION ON MYOCARDIAL CONTRACTION[1]

ROBERT TENNANT[2] AND CARL J. WIGGERS

From the Department of Physiology, Western Reserve University Medical School, Cleveland, O.

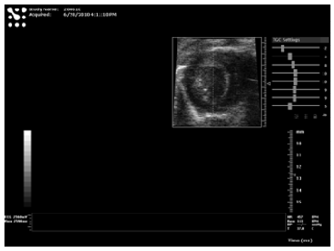
Received for publication March 22, 1935



US – Ultrasound / echocardiography

Method
- reflection of ultrasonic waves
- 1D, M-Mode imaging

Measure
- Cavity volume
- Contraction

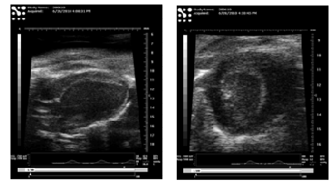


Visualsonics Vevo 770
Hammersmith Hospital
Imperial College

US – Ultrasound / echocardiography

Method
- reflection of ultrasonic waves
- 2D, B-Mode imaging

Measure
- Cavity volume
- Contraction



Visualsonics Vevo 770
Biological Imaging Centre
Imperial College

US – Ultrasound / echocardiography

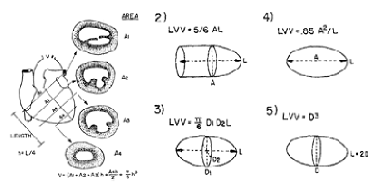
1D M-mode

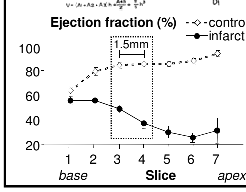
End diastolic dimension EDD	Cavity diameter at diastole
End systolic dimension ESD	Cavity diameter at systole
Fractional shortening	(EDD-ESD)/EDD
Wall thickness	

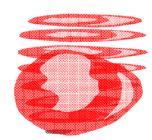
2D B-mode

End diastolic volume EDV	Cavity diameter at diastole
End systolic volume ESV	Cavity diameter at systole
Stroke volume SV	EDV-ESV
Ejection fraction EF	(EDV-ESV)/EDV
Cardiac output CO	SV x heart rate
Wall volume (LV mass)	

US – Ultrasound / echocardiography





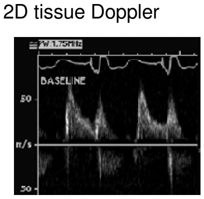


Stuckey, et al. NMR Biomed (2008) 21, 765

US – Doppler

Method
- Doppler effect on ultrasound
- 1D flow Doppler
- 2D tissue Doppler

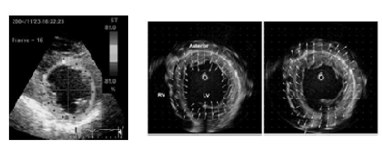
Measure
- mitral/aortic flow
- tissue movement



US - speckle tracking

Method
- motion of "speckles"
- 2D/3D image processing

Measure
- regional stress/strain

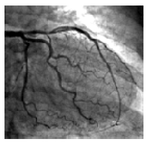
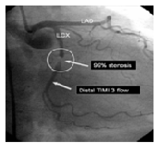


Suffoletto *et al. 2006. Circ* 113: 960

Angiography

Method
- Catheterization + X-ray
- Infusion of Iodine based CA

Measure
- Coronary occlusion
- Contraction

CT – Computed Tomography

Method
- Multiple 2D X-ray
- Iodine based CA
- Resolution 50 um

Measure
- Cavity volume
- Contraction

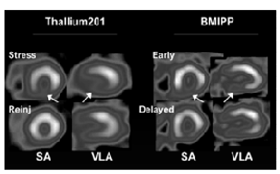
Siemens PET/SPECT/CT
Biological Imaging Centre
Imperial College


Dr W. Gsell, BIC Impérial College London

SPECT – *Single Photon Emission Computed Tomography*

Method
- Gamma emitting radioisotope
- Directly detected by CCD
- Resolution 2-3 mm

Measure
- Perfusion Technetium-^{99}
  Thallium-^{201}
- Metabolism BMIPP-I^{123}

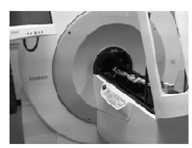
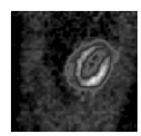
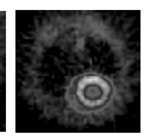



Mimosa *et al.* 2001 *Neuromusc Dis*

PET - Positron Emission Tomography

Method
- Positron emitting radioisotope
- Annihilation by e$^-$ collision
- Pair of photons produced
- Resolution 1.5-2 mm

Measure
- Perfusion ^{13}N ammonia
  ^{15}O water
- Metabolism ^{18}F FDG
- Plaques ^{18}F FDG

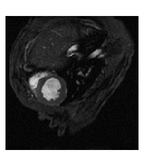
Dr W. Gsell, BIC Impérial College London

MRI – Magnetic Resonance Imaging

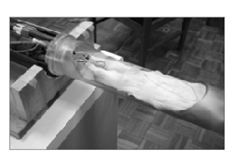
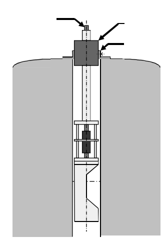
Method
- Magnetization of 1H
- Fourier transformed into 2D
- Resolution 100 um

Measure
- Cavity volume
- Contraction
- Myocardial viability
- Myocardial perfusion

11.7T MR system
CMRG, DPAG
University of Oxford

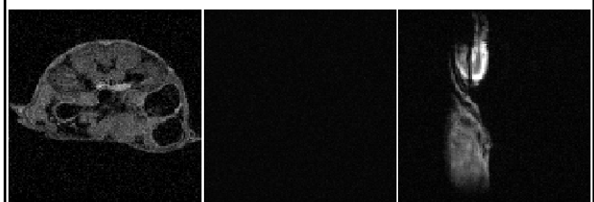



In vivo MRI of heart @11.7T

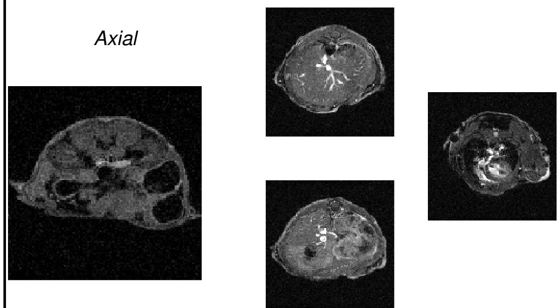
In vivo MRI

Axial *Coronal* *Sagital*



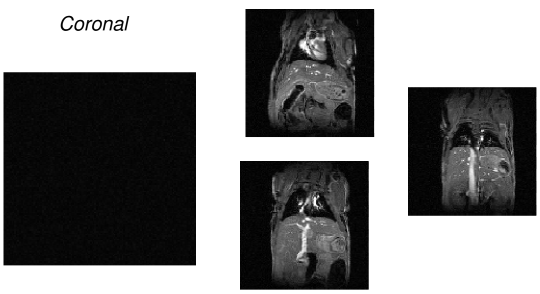
In vivo MRI

Axial



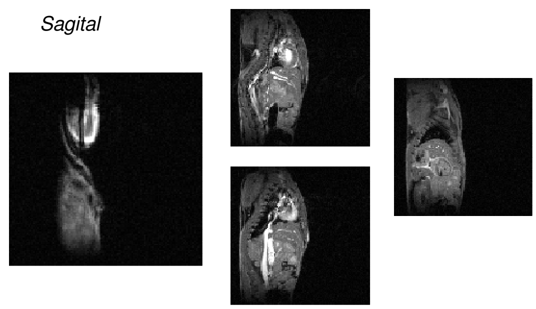
In vivo MRI

Coronal



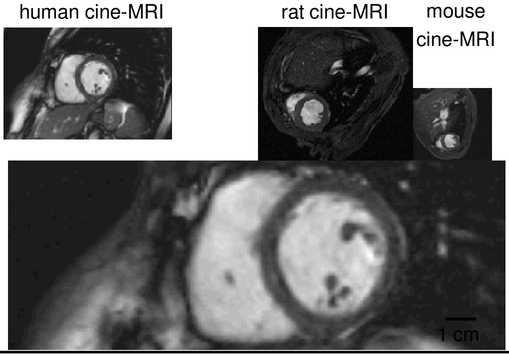
In vivo MRI

Sagital

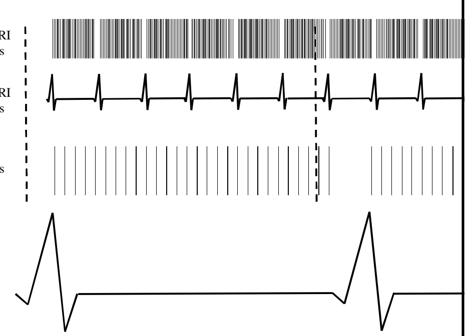


Small animal MRI - size

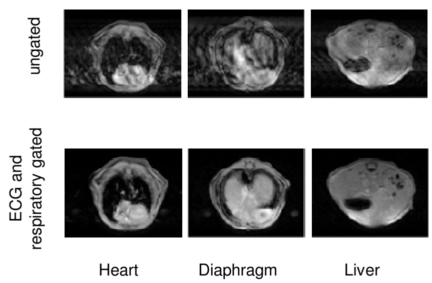
human cine-MRI rat cine-MRI mouse cine-MRI

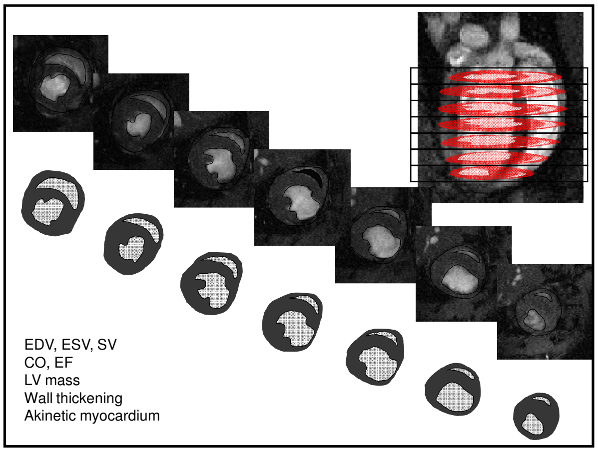


Small animal MRI - heart rates

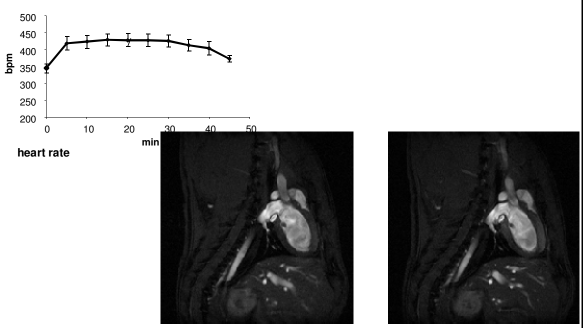


Small animal MRI - motion

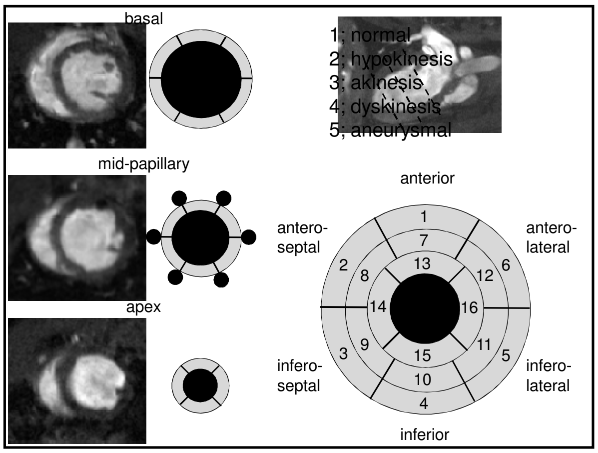




Dobutamine stress MRI

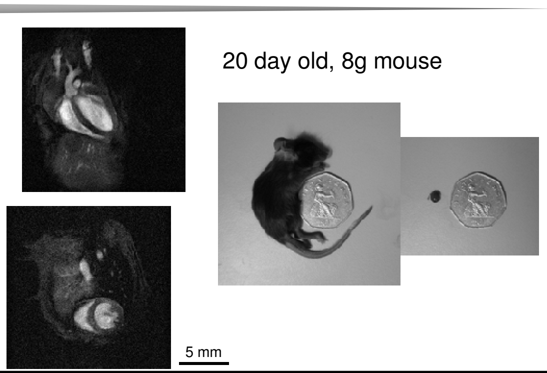


Pre Db (380 bpm) Post Db 460 bpm)

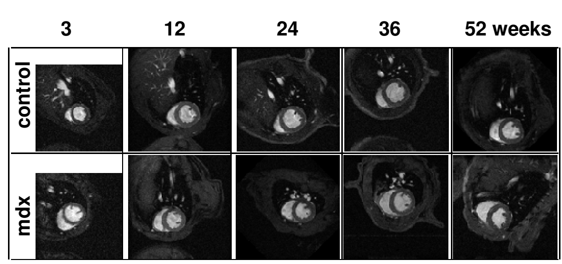


Serial imaging studies

20 day old, 8g mouse

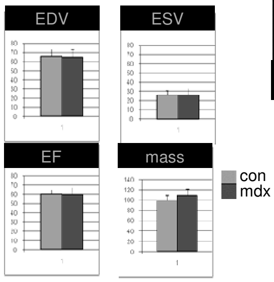


Cardiomyopathy in mdx mice

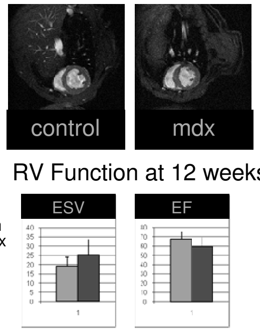


Cardiomyopathy in mdx mice

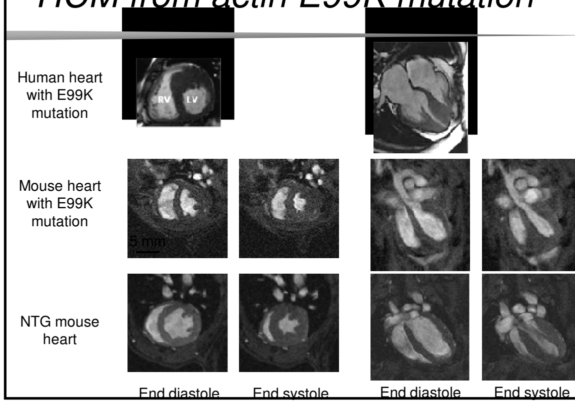
LV Function at 12 weeks



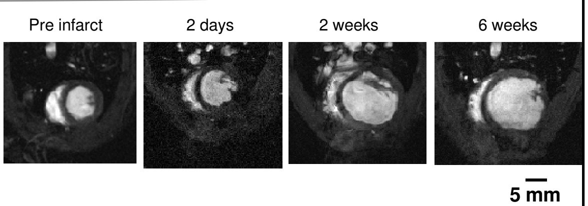
RV Function at 12 weeks



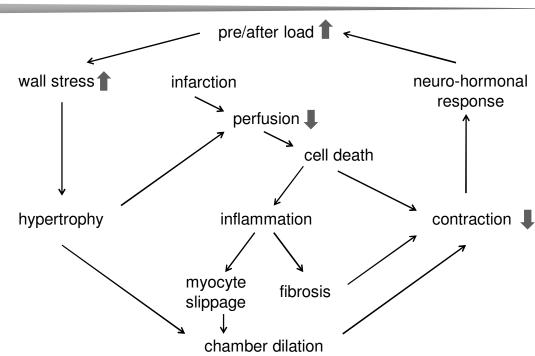
HCM from actin E99K mutation



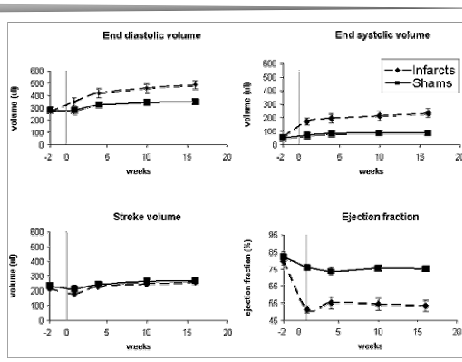
Myocardial infarction in rat



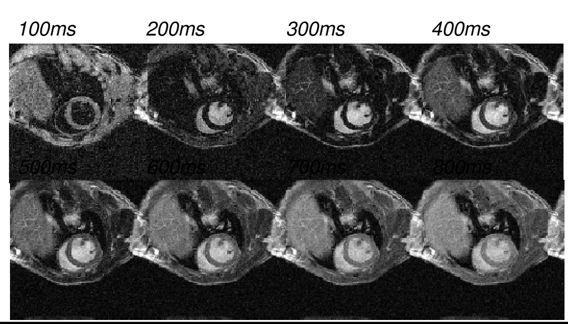
Cardiac remodelling



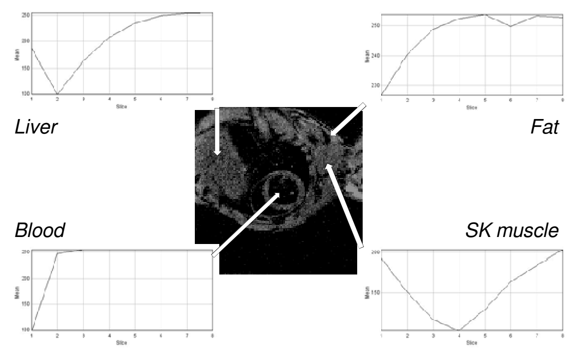
Myocardial infarction in rat



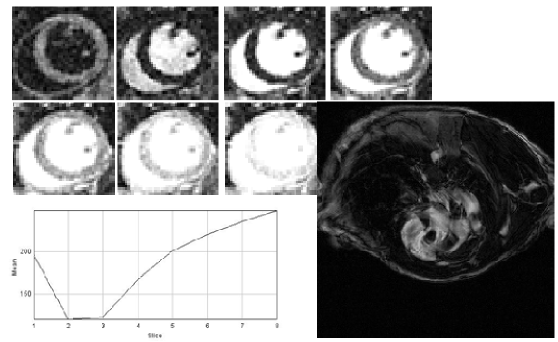
T1 relaxation



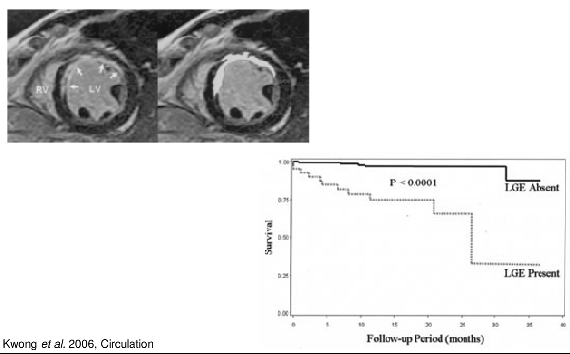
T1 relaxation



T1 relaxation

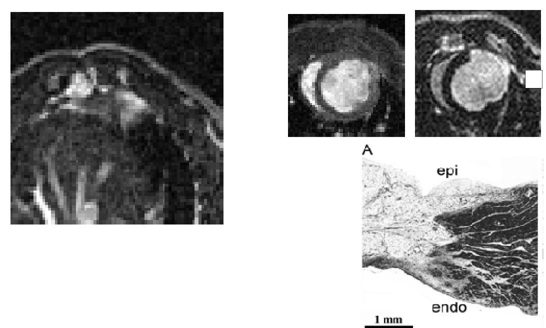


Delayed enhancement MRI (human)

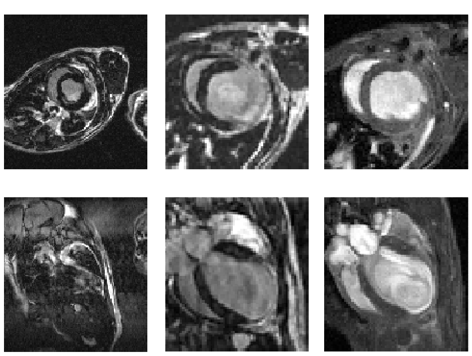


Kwong *et al.* 2006, Circulation

Delayed enhancement MRI (infarcted rat)

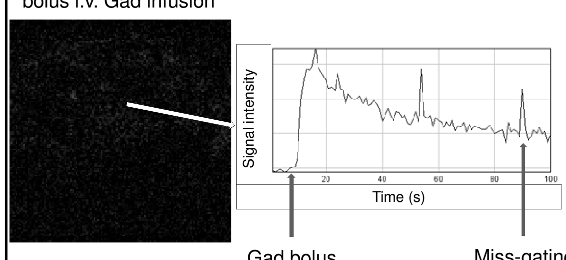


Delayed enhancement MRI (infarcted mouse)



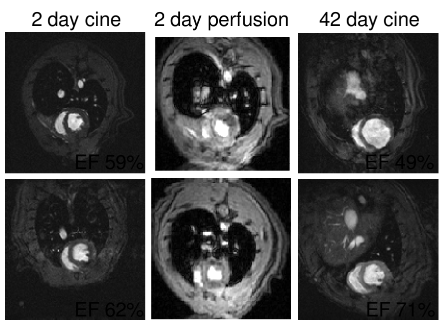
1st pass contrast enhanced MRI

1 image every respiratory cycle (~8 R-R) during bolus i.v. Gad infusion

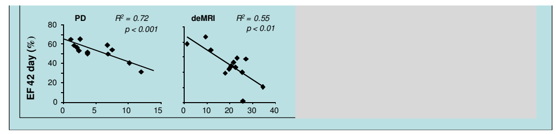


Stuckey *et al.* 2011 under review

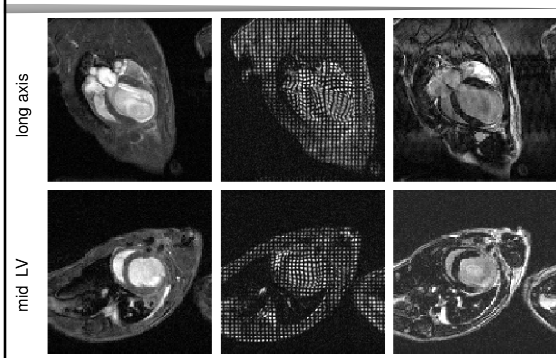
Imaging myocardial perfusion



Imaging myocardial perfusion



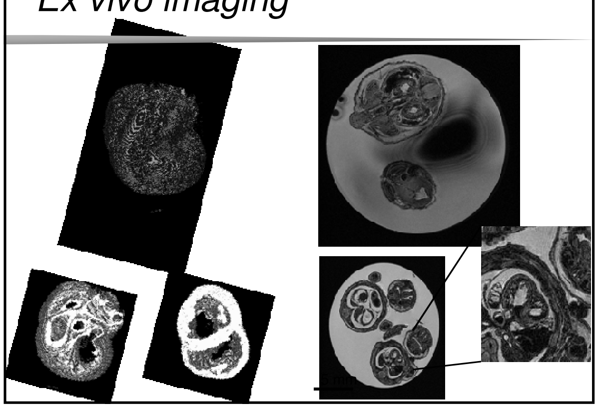
Tagged MRI *(infarcted mouse)*

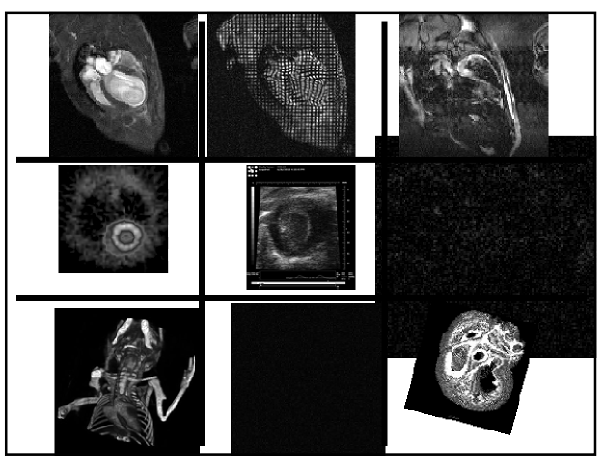


Other MR measurements

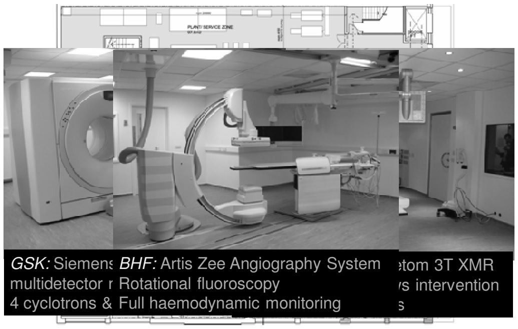
Arterial spin labelling	*myocardial perfusion*
T2 weighting	*edema/inflammation*
DTI	*myocardial ultrastructure*
MEMRI	*myocardial viability*
Molecular imaging	*arthrosclerosis*
	inflammation

Ex vivo imaging





ICL - Pre-Clinical Imaging Facility



In vivo cardiac imaging

Parameter	Information	Measurement	Method
Myocardial perfusion	Coronary circulation Tissue perfusion	inflow of contrast agent non-contrast agent based	Angiography US - microbubbles PET - ^{18}F FDG SPECT – ^{99}Te MRI – first pass MRI – Arterial spin labelling
Morphology	Chamber dilation Hypertrophy	End diastolic dimension/volume End systolic dimension/ volume LV mass Wall thickness	US - M-mode & 2D CineMRI CT
Global systolic function	Myocardial contraction	Fractional shortening ejection fraction, cardiac output,	US - M-mode & 2D US – 2D, CineMRI
Regional systolic function	Regional contraction	wall thickening tissue stress/strain/torsion	US – 2D, CineMRI US - speckle tracking MRI - tissue tagging./ Velocity encoding
Diastolic function	Myocardial relaxation	Myocardial compliance	US – Doppler
Myocardial viability	Dead or alive?	Glucose metabolism Uptake of contrast agent	PET - ^{18}F FDG MRI - Late enhancement
Metabolism	Energy production/ utilisation	Glucose metabolism Fatty acid metabolism ATP/PCr levels	PET - ^{18}F FDG SPECT – ^{18}I BMIPP MRI – spectroscopy
Ultrastructure	Fibre orientation	Water diffusion in direction of fibres	MRI – deffusion tensor imaging
Edema	Area at risk	Change in tissue's magnetic relaxation	MRI – T2 weighting
Molecular and cellular targets	Inflammation, apoptosis, cell grafting	Detection of a smart contrast agent that associates with the target	Antibody based smart contrast agents for PET/SPECT (radionuclides), MRI (iron oxide partices)and US (microbubbles)

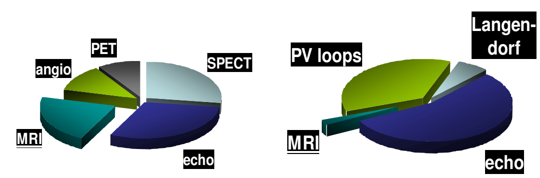
Clinical trials

Trial	Treatment effect	
Assmus 2002 TOPCARE-AMI(6)	6.0*	⇧
Fernandez 2004(13)	5.8*	⇧
Assmus 2006 TOPCARE-CHD(5)	2.0	⇔
Janssens 2006(19)	1.1	⇔
Lunde 2006 ASTAMI(23)	-3.1	⬇
Meyer 2006 BOOST(28)	2.8	⇔
Schachinger 2006 REPAIR(39)	2.5*	⇧
Mean	**2.4 ± 1.3**	?

Cardiac function post cell therapy

Human studies

Rodent studies



How to evaluate cell delivery methods

- Stem cell tracking
  - Label cells *in vitro* with "tracker"
  - Determine distribution of labelled cells after administration

Confirm successful delivery

Determine position of delivery

Quantify cell retention

Monitor cell function

The ideal cell tracking platform

Label

- Bio-compatible
- Not diluted with proliferation
- Specific to donor cell
- Long term retention within cell
- Information on cell status

Detection

- Non invasive
- Quantitative
- Spatial and serial distribution
- Single cell detection
- Cell proliferation rates

Cell tracking platforms

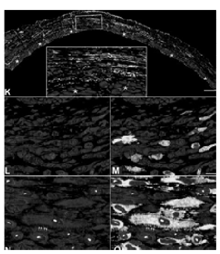
- Post-mortem histology with microscopy
- rtPCR
- Radio-labelling
- Reported gene imaging
  - Bio luminescence imaging (BLI)
  - PET
- MRI

Post-mortem histology with microscopy

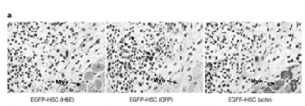
- Labelling
  - Fluorescent dye (DiI, DAPI, BrdU)
  - genetic marker (GFP, RFP, B-galactosidase, Y chromosome)
- Imaging
  - fluorescent / confocal / two photon microscopy
  - light microscopy (B-Gal, DAB)

Post-mortem histology with microscopy

Fluorescence



light microscopy



Kajstura: 2005, Circ Res, 96.127-137

Murry, 2004, Nature, 428, 664

Post-mortem histology with microscopy

Label

- Bio-compatible ?
- Not diluted with proliferation
- Specific to donor cell
- Long term retention within cell
- Information on cell status

Detection

- Non invasive ✘
- Quantitative
- Spatial and serial distribution ✘
- Single cell detection
- Cell proliferation rates ?

? *debatable*

✘ *impossible*

rtPCR

Deliver genetically distinct donor cells
-- male cells to female
-- human cells to mouse
Harvest organs
Perform rtPCR for
-- Y chromosome
-- SRY sequence
Quantity of PCR product relates to cell number

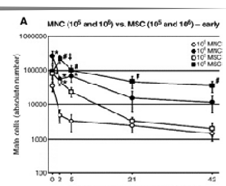
Müller-Ehmsen 2006 JMCC 41 876

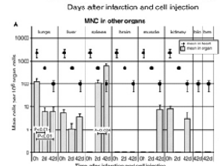
rtPCR

Müller-Ehmsen, 2006
i.m. Injection of...
-- 10^5 or 10^6
-- MNCs or MSCs at
-- 0 or 7 days
...into infarcted rat heart
Harvested organs at 0 hours and 2, 5, 21 and 42 days

$10^6 > 10^5$
MSC > MNC
0 days = 7 days

Donor cells in other organs





Müller-Ehmsen 2006 JMCC 41 876

rtPCR

Label

- Bio-compatible
- Not diluted with proliferation
- Specific to donor cell
- Long term retention within cell
- Information on cell status

Detection

- Non invasive
- Quantitative
- Spatial and serial distribution
- Single cell detection
- Cell proliferation rates

? *debatable*

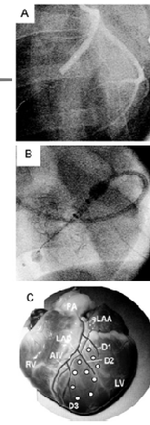
✗ *impossible*

Radio-labeling

Hou et al, 2005

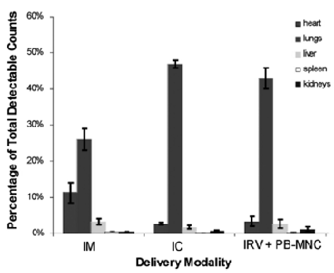
Comparison in pigs of …
- Trans-coronary-venous delivery
- Intra-coronary delivery
- Trans-epicardial delivery

LAD occlusion
In111 labelled MNC delivery
Organs harvested at 1 hour



Hou, 2005 Circulation;112:I

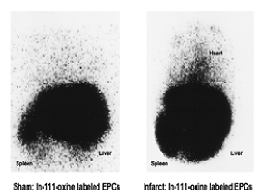
Radio-labeling



Hou, 2005 Circulation;112:I

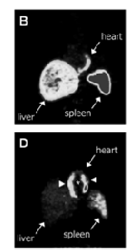
Radio-labeling

Indium-111 labelled i.v. rat



Scintigraphic pinhole images

18F-FDG labelled into LAD



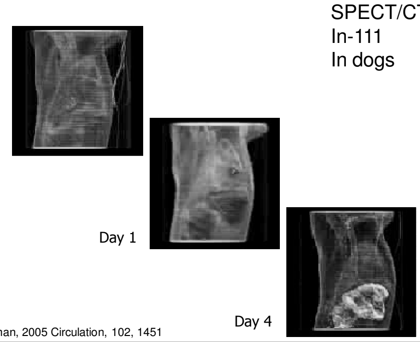
PET. In humans

Aicher et al Circulation. 2003;107:2134

Hofmann 2005: Circulation, 111.2198

Radio-labeling

SPECT/CT
In-111
In dogs



Day 0

Day 1

Day 4

Kraitchman, 2005 Circulation, 102, 1451

Radio-labeling

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✗ *impossible*

Imaging reporter genes

- Transfect cells with reporter genes which are subsequently detected in combination with an imaging probe

  Bioluminescence imaging of luciferase activity

  Positron emission tomography (PET) of thymidine kinase activity

Bioluminescence imaging

- Transfect cells with firefly luciferase
- Deliver cells to animal
- Use Xenogen Bioluminescence charged-coupled device camera.
- Infuse substrate (D-luciferin)
- low energy photons emitted from cells - attenuated within deep tissues.
- Detects 1000 cells in heart (sheiki/wuSCs)

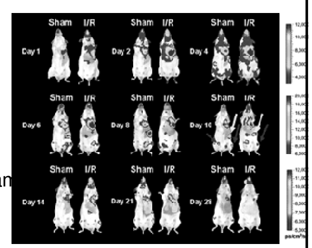
Bioluminescence imaging

Sheikh et al, 2007
Tail vein infusion of $5x10^6$ MNCs into sham or IR rat

Signal greatest in heart at 2 and 6 days post infusion

Signal greater in IR than sham

rtPCR used to confirm MNC retention at 4 weeks
MI = 2580 (0.05%)
Sham = 245 (0.005%)



Sheikh AY, 2007, *Stem Cells*.10,2677

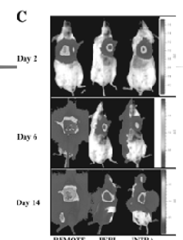
Bioluminescence imaging

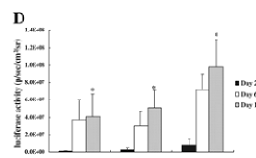
Hung et al 2008
Comparison of cell injection sites in infarcted mice

25 µL of $2.5x10^5$ ES cells injected into…
Scar tissue
Peri-infarct region
Remote region

Survival greatest in remote region

Teratomas formed
Cardiac function improved!!!





Hung et al 2008 circ cardiovasc imag, 1,6

Bioluminescence imaging

Label

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Detection

- Non invasive
- Quantitative
- Spatial and serial distribution ?
- Single cell detection ✘
- Cell proliferation rates

- Tissue penetration
- Only 2D

PET imaging of reported genes

- Nuclear imaging modality
- emission of high energy photons - deep tissue penetration.
- 18F-FDG (fluoro-deoxy-glucose) for heart structure, perfusion, metabolism, viability
- 18F-FHBG (fluoro-hydroxymethylbutyl-guanine) for cells

PET imaging of reported genes

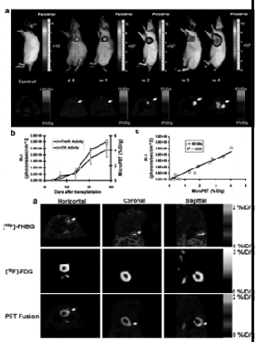
Cao et al 2006
Transfected ES cells with…
- luciferase
- thymidine kinase
- RFP

50 μL of 10^7 ES cells I.M.

BLI and PET signal increased

Teratomas formed

Transplanted cells killed by ganciclovir o.d. = suicide gene



Cao, F, 2006. Circulation. 113:1005

PET imaging of reported genes

Label

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- Not diluted with proliferation
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Detection

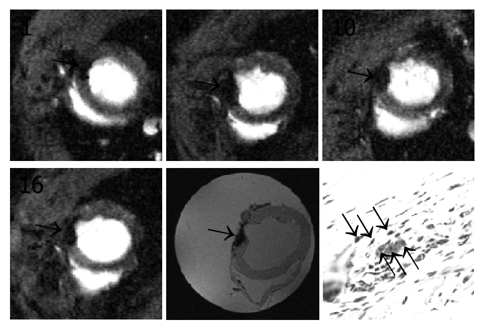
- Non invasive
- Quantitative
- Spatial and serial distribution ?
- Single cell detection ✘
- Cell proliferation rates

- Ionising radiation

Magnetic resonance imaging

- Nuclear imaging modality
- Anatomical image contrast without need for tracers
- Donor cells labelled *in vitro* with contrast agents
  - Iron oxide
  - Gadolinium
  - Fluorine

Tracking BMSCs over 16 weeks



Stuckey *et al.* 2006. Stem cells, 24;1968

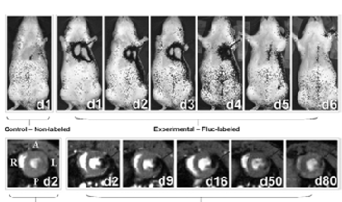
MRI

Chen et al 2008
BLI vs. MRI

Cell T1/2 faster with BLI

Injection of naked iron oxide gave persistent signal

Iron oxide phagocytosed



Chen, IY 2008, Molecular Imaging and Biology 1536-

MRI

Label

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