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Cardiac Fibroblasts

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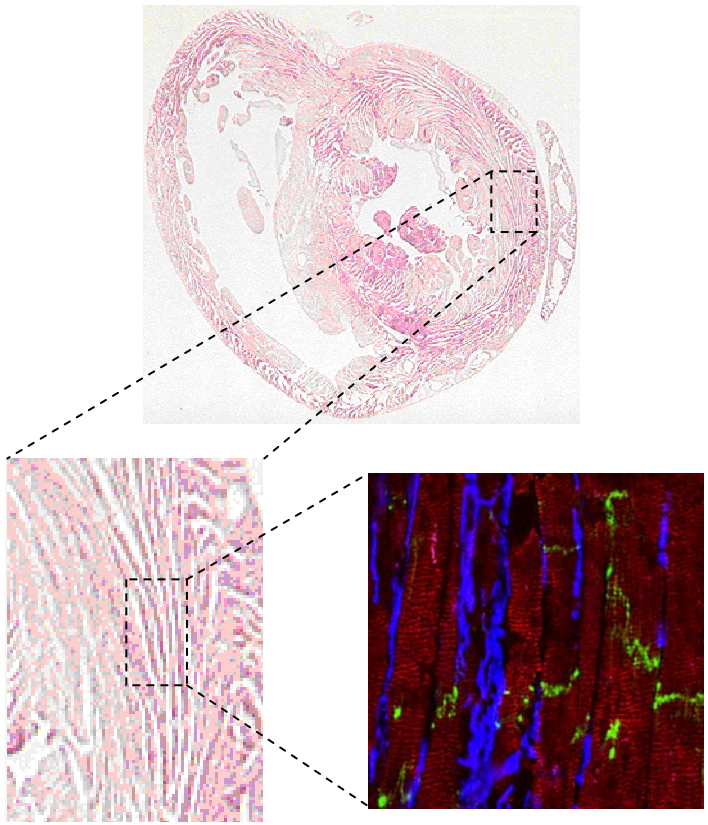
Introduction

Heart = Muscle = Myocytes (M) that are structurally and functionally coupled.

M are responsible for generating the contractile force enabling the heart to beat.

M are not the only cell type in the heart.

Cardiac cell numbers:
30% Myocytes
70% Non-myocytes
- 64% Fibroblasts (F)
- 6% Endothelial & VSMC



F are the largest cell population in the heart and form a dense network surrounding M clusters (each M 'in touch' with 1-6 F).

F number varies with stage of development, in different regions, with age, and in physiological vs pathological conditions.

Camelliti P. *et al*. Cardiovasc Res 2005/**65**:40-51.

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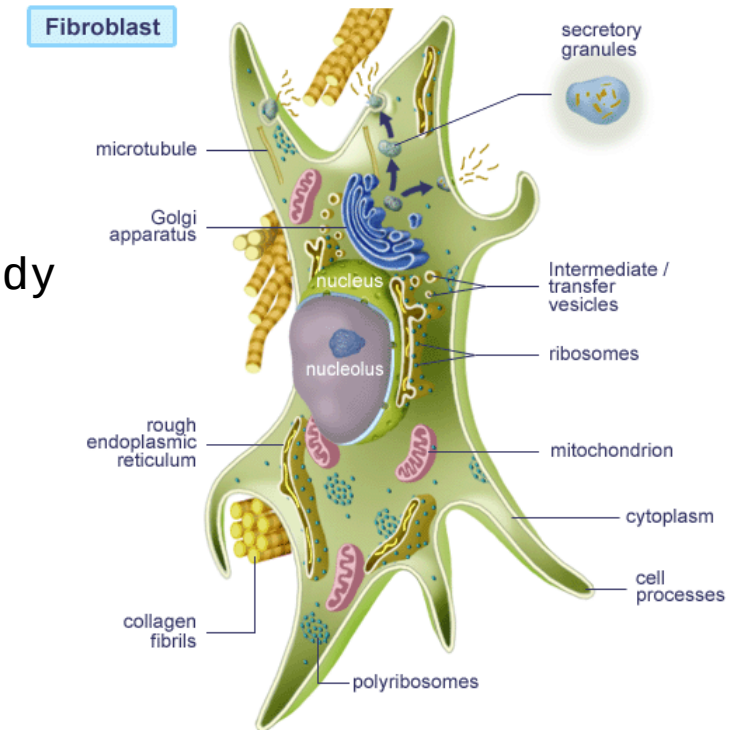
What is a Fibroblast?

A type of cell that produces the extracellular matrix, the structural framework for animal tissues – present in all vertebrate organisms and most organs (skin, liver, kidney, lung, heart…)

Cell classification based on morphological characteristics and/or proliferative potential.

Morphology:

- flat, spindle-shaped cells
- multiple processes originating from cell body
- lack a basement membrane
- one elliptical nucleus (with 1 or 2 nucleoli)
- extensive rough endoplasmic reticulum
- prominent Golgi apparatus
- abundant cytoplasmic granular material



Heterogeneous population: F from different regions have distinct phenotypes and gene expression patterns.

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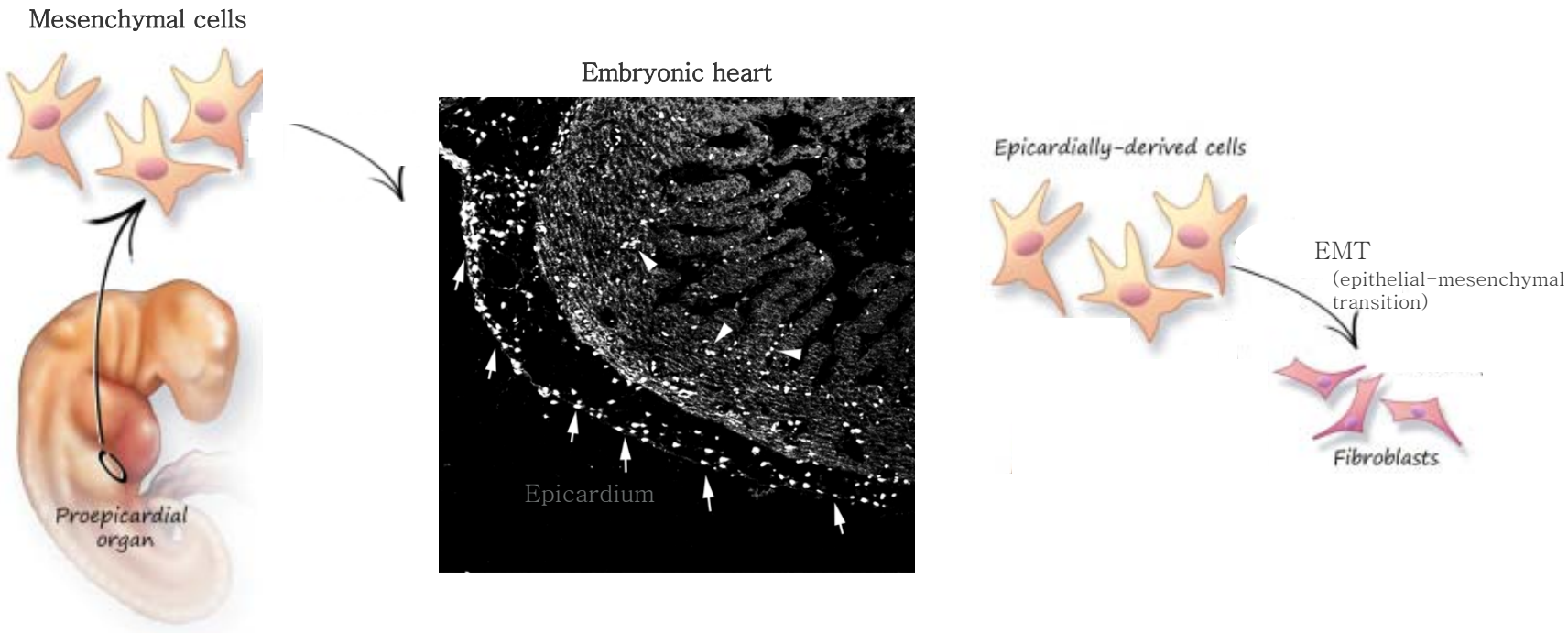
Origin of Cardiac Fibroblasts

Embryonic development



Norris, R.A. *et al.* Ann N Y Acad Sci 2008; 1123:30-40.

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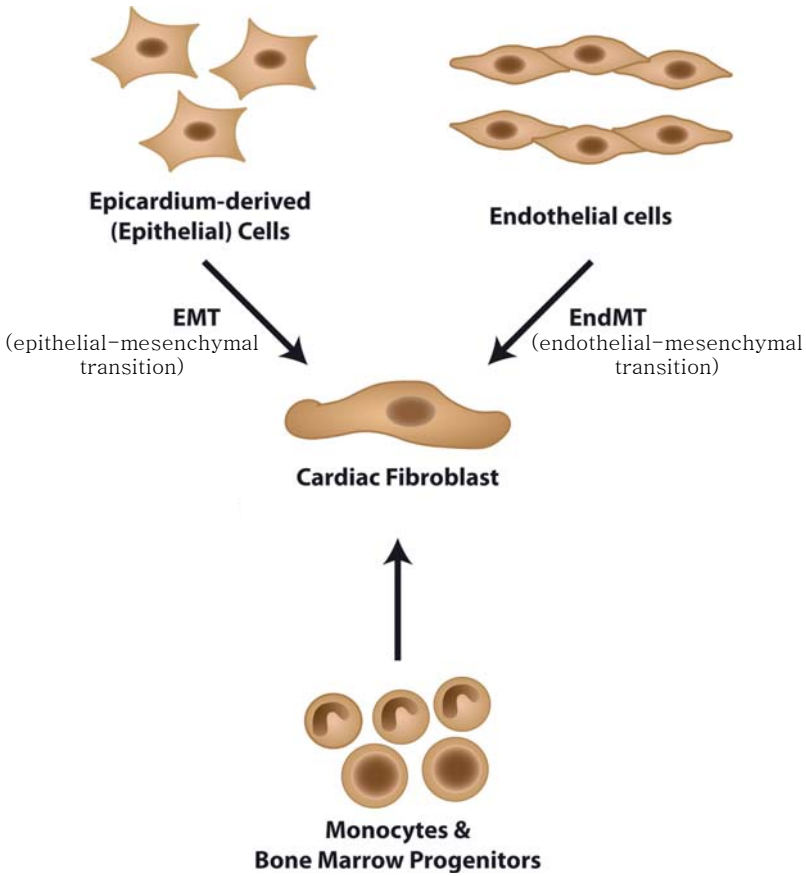
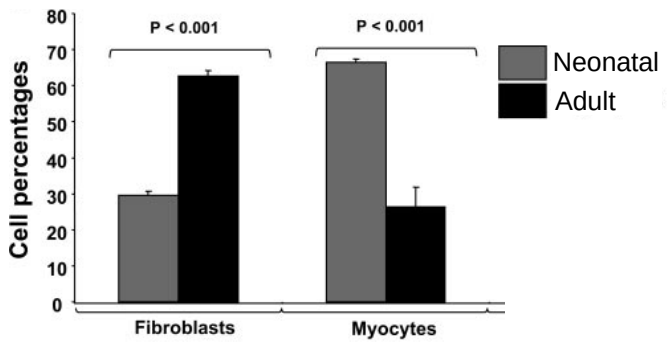
Origin of Cardiac Fibroblasts

Neonatal and adult heart





Benerjee I. *et al.* Am J Physiol 2007; 293:1883-91.

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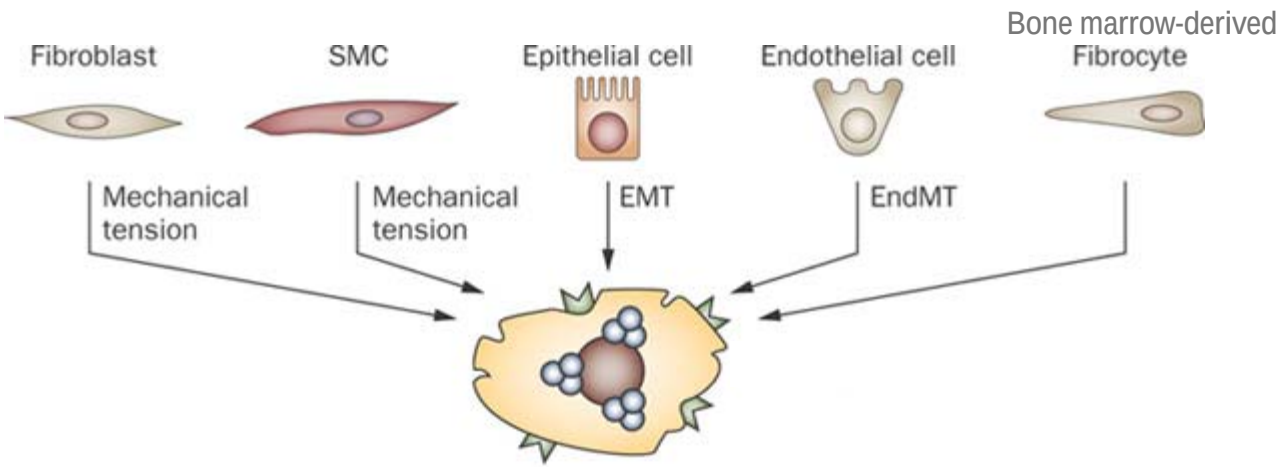
Origin of Cardiac Fibroblasts

Adult heart: Pathology



Myofibroblasts / Active F

- produce ECM
- express contractile proteins – α-smooth muscle actin
- proliferate, migrate and secrete bioactive molecules
- migrate, proliferate and deposit new ECM at the injury site
- replace the damaged and lost cardiomyocytes and form a scar (infarct healing)

EMT= epithelial-mesenchymal transition
EndMT= endothelial-mesenchymal transition

Van den Borne, S.W.M. *et al.* Nat Rev Cardiol 2009; 293:1883-91.

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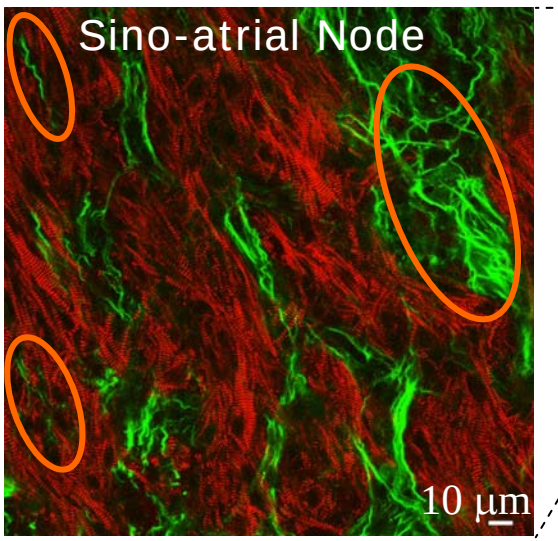
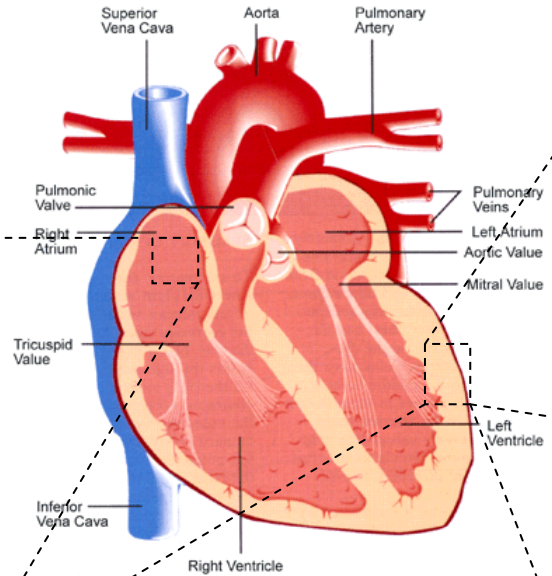
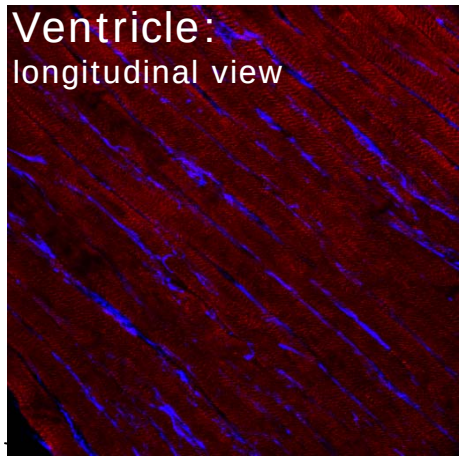
Organisation of Cardiac Fibroblasts: Physiology

Regional differences in the organisation and content of F

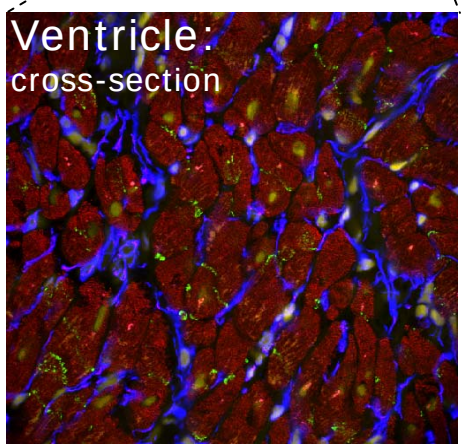


- Higher F content than ventricle
- F & M less regularly organised
- F interspersed with M
- F only islands





- M & F arranged in highly oriented layers
- F surround M clusters (2-4 M)



F form abundant contacts with M, which may be site of structural and function coupling between the 2 cell types.

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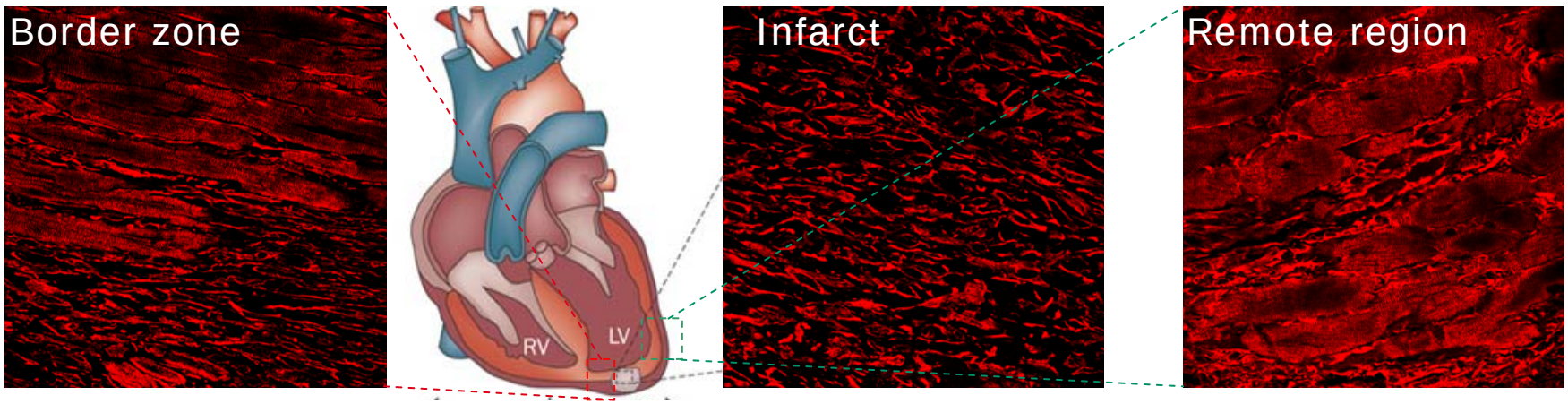
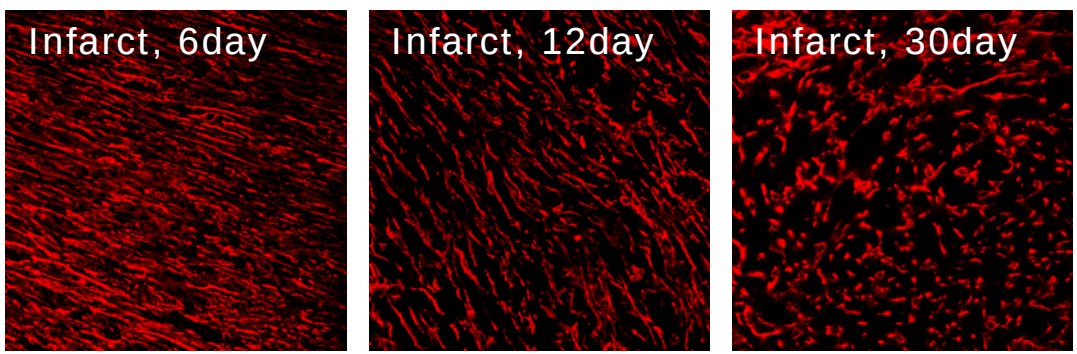
Organisation of Cardiac Fibroblasts: Pathology

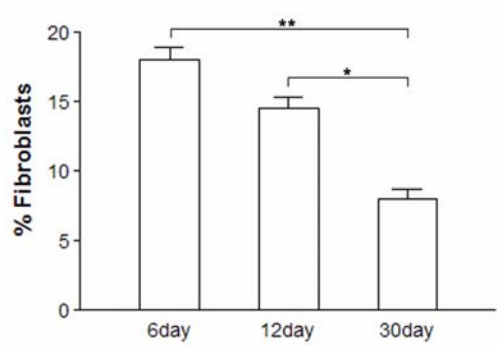
F content is increased in pathological conditions:
diffuse (fibrosis), local (scarring), or combined (e.g. post-infarct).

Sheep myocardial infarct model



F number *vs* infarct time





Camelliti P. *et al.* Cardiovasc Res 2004; 62:415-25.

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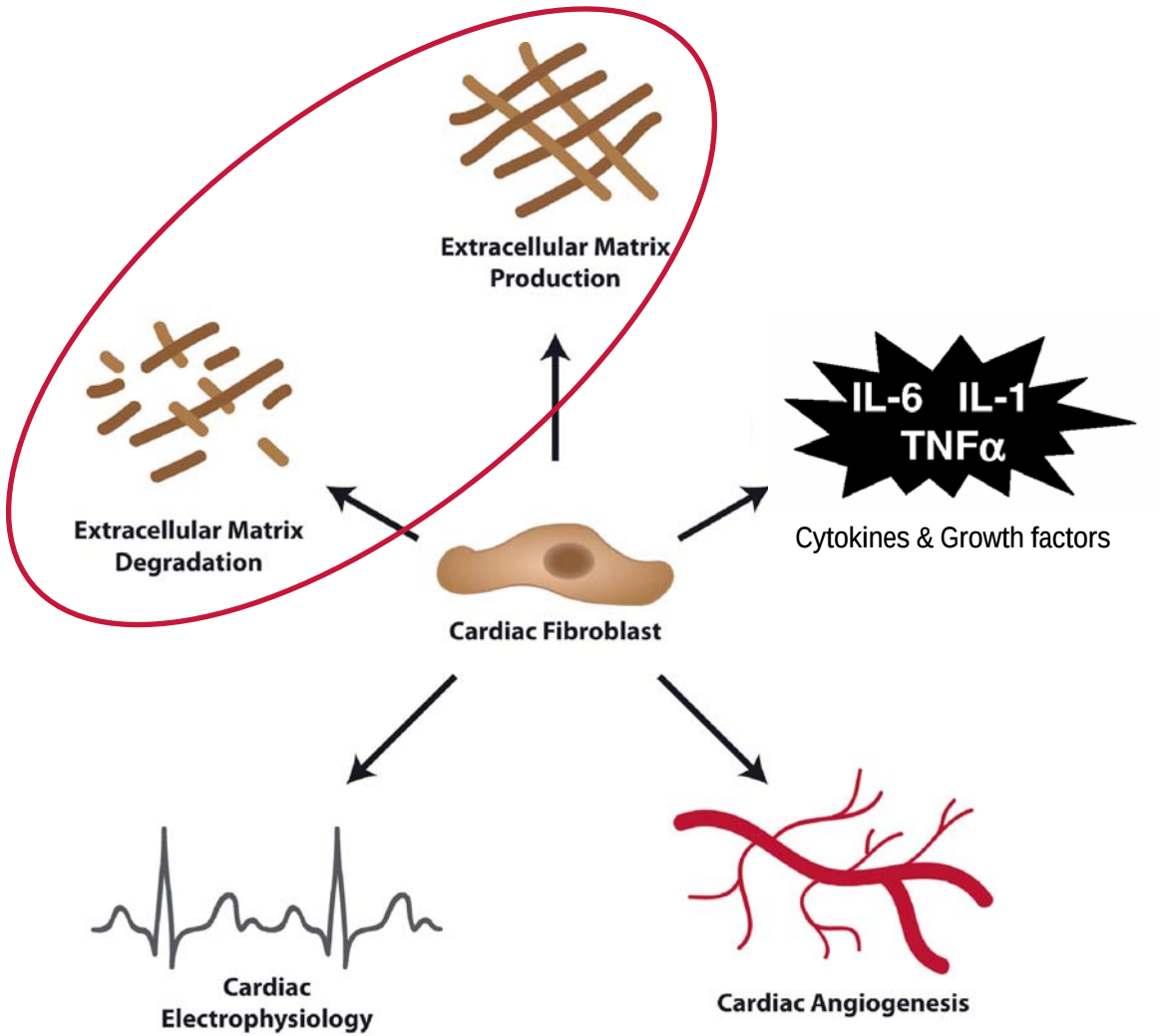
Cardiac Fibroblast Functions



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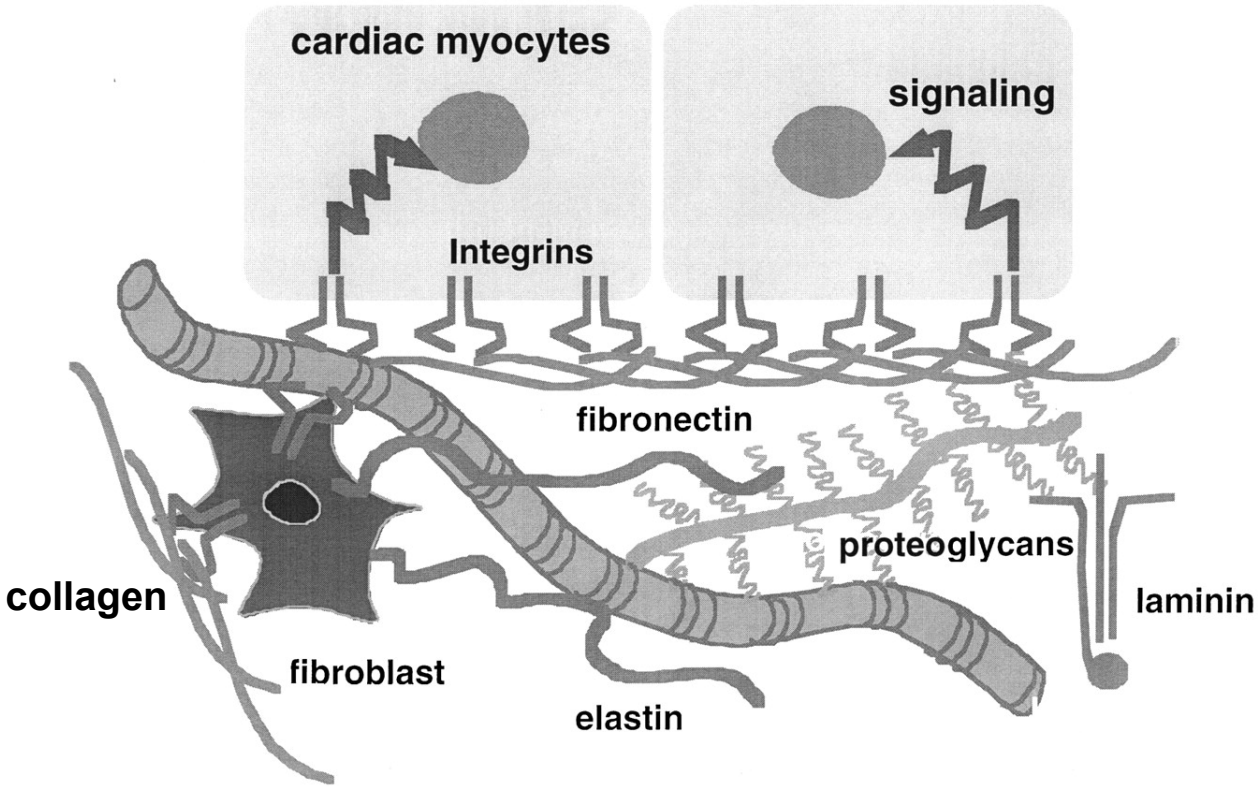
Extracellular Matrix (ECM)



- Provides support for the cardiac cells
- Distributes mechanical forces throughout the cardiac tissue
- Conveys mechanical signals to individual cells via cell surface ECM receptors

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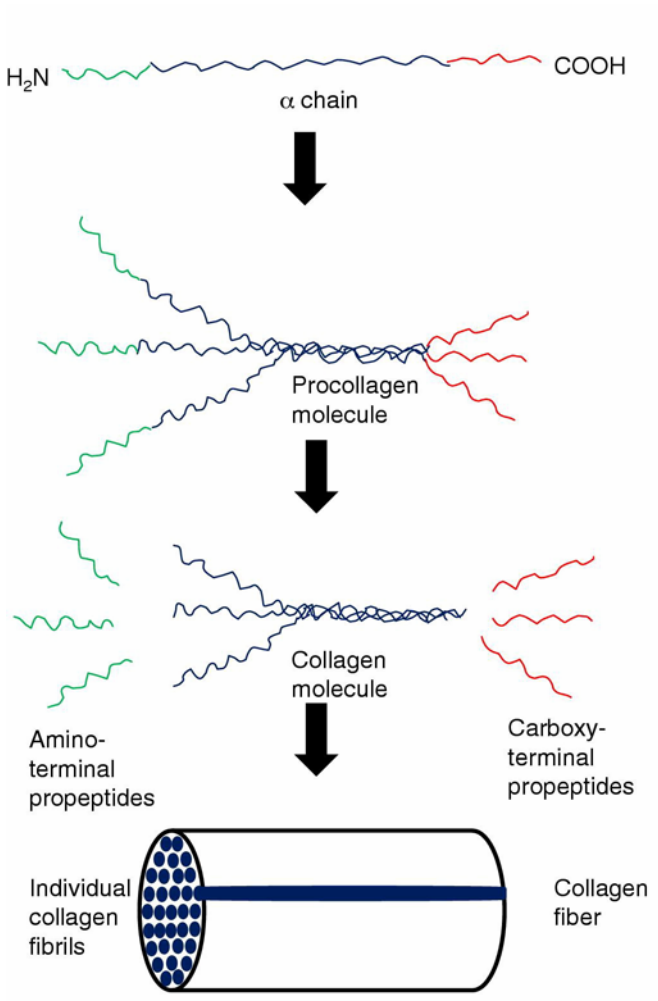
ECM: Collagen Formation



Intracellular

1. α chains synthesised within the endoplasmic reticulum with amino- (*green*) and carboxy-terminal (*red*) propeptides in situ

2. Self assembly of three α chains into procollagen triple helix

Extracellular

3. Secretion of the procollagen molecule into the extracellular space where propeptides are cleaved by procollagen N- and C-proteinases to enable collagen molecule formation

4. Self-assembly of collagen molecules into collagen fibrils which are strengthened by cross-links within and between molecules. Fibrils then aggregate to form collagen fibers.

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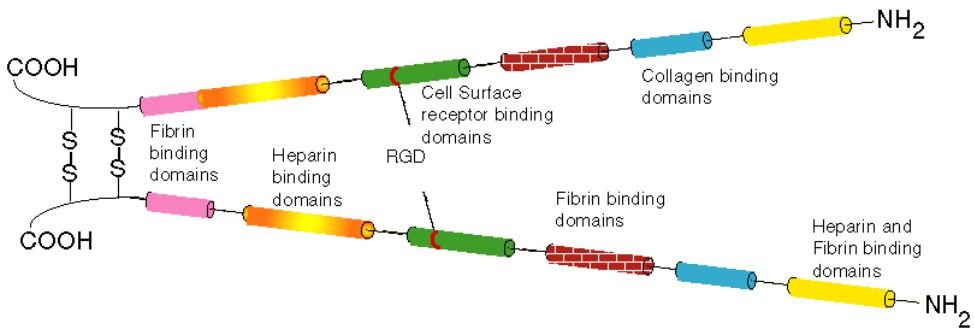
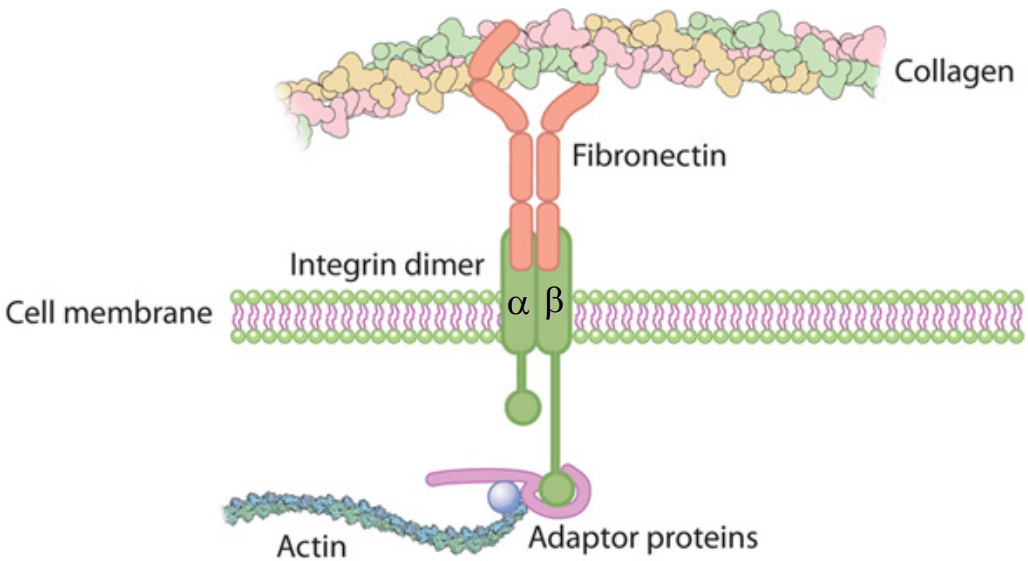
ECM: Other Components



Fibronectin

- Protein dimer: 2 nearly identical monomers linked by a pair of disulfide bonds.
- Each monomer contains binding domains to cell surface integrins, collagen and other fibronectin.
- Function: cell adhesion and migration by simultaneous binding to cells and other ECM components.



Integrins

- Cell surface receptors (expressed by both M & F).
- Heterodimers: α and β subunit.
- Bind to the ECM and anchor the intracellular cytoskeletal proteins to the surrounding ECM.

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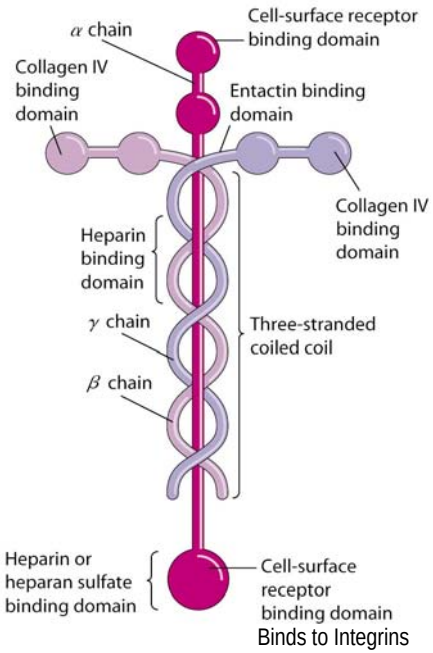
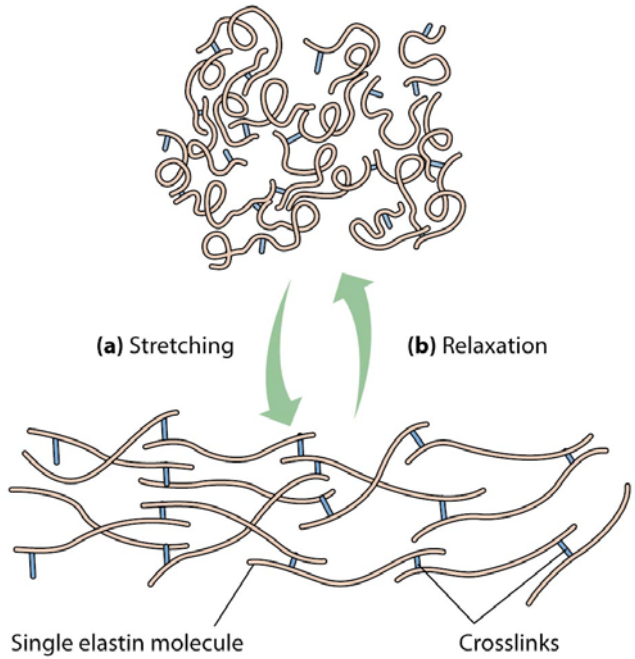
ECM: Other Components





Laminin

- Trimeric protein: 3 similar chains (α, β & γ).
- Binds to cell membranes through integrins and to other ECM proteins including collagen type IV and other laminin.
- Function: cell adhesion and differentiation, cell shape and migration.

Elastin

- Made by crosslinking of elastin molecules to form a coil structure.
- During stretch elastin molecules acquire an elongated and linear conformation.
- During relaxation they return to the more stable random-coil structure - like a rubber band.

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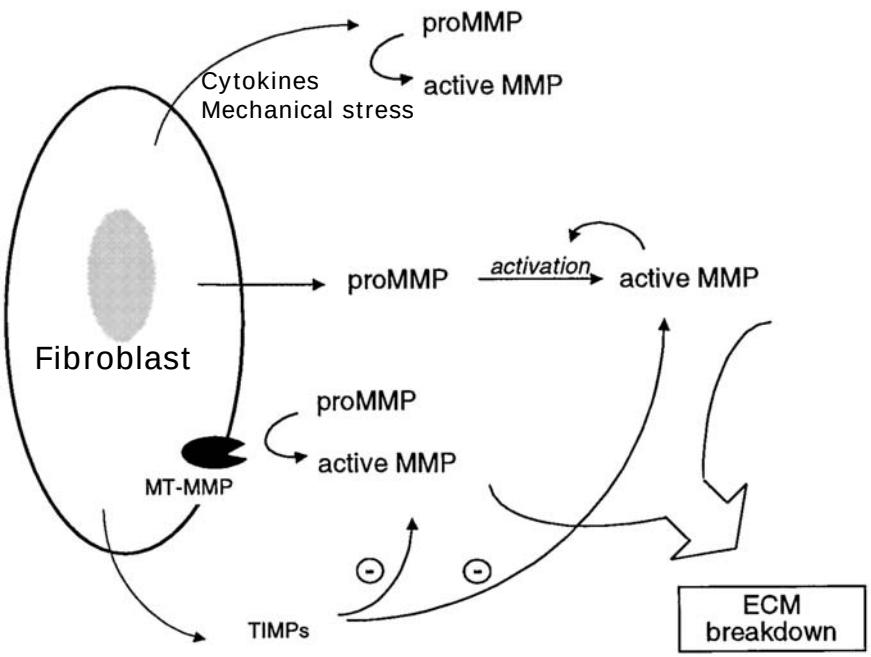
Regulation of ECM Turnover

ECM is a *dynamic* structure: components are maintained by a finely controlled balance between synthesis and degradation.

Fibroblasts regulate ECM turnover by synthesis and deposition of:

- **matrix metallo-proteinases** (**MMPs**): enzymes that degrade all ECM protein components; >20 enzymes; 2 types: secreted as latent proenzymes and membrane-bound.
- **tissue inhibitors of MMPs** (**TIMPs**): inhibit the activity of MMPs.



In *healthy* heart:
- low MMPs levels
- MMPs/TIMPs balance tightly regulated

Creemers *et al*., *Circ Res* 2001/**89**:201-10.

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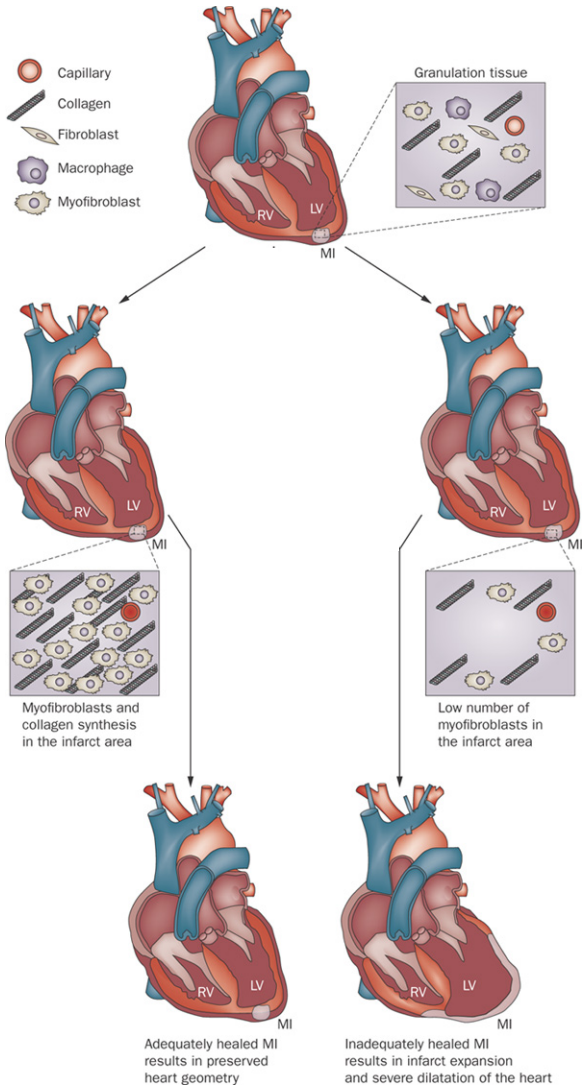
Regulation of ECM Turnover



In *diseased* heart:
- MMPs expression and activity ⇑
- TIMPs decreased
- ECM degradation
- Inflammatory cells and F migration
- F proliferation and new ECM deposition
- Wound healing and scar formation

- If MMPs ⇑ activity persist:
excessive ECM degradation, impairment of infarct healing and potentially cardiac rupture

Creemers *et al*., *Circ Res* 2001/**89**:201-10.

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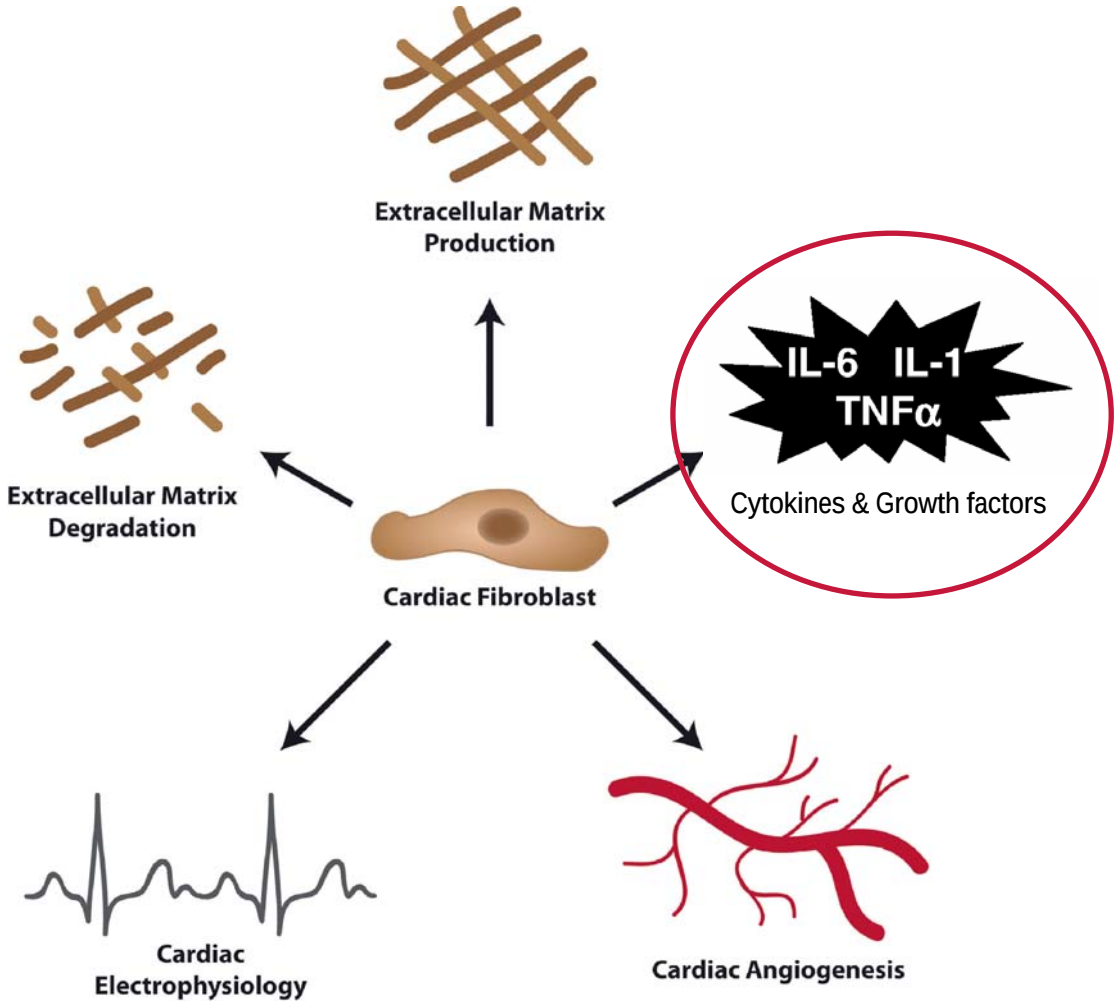
Cardiac Fibroblast Functions



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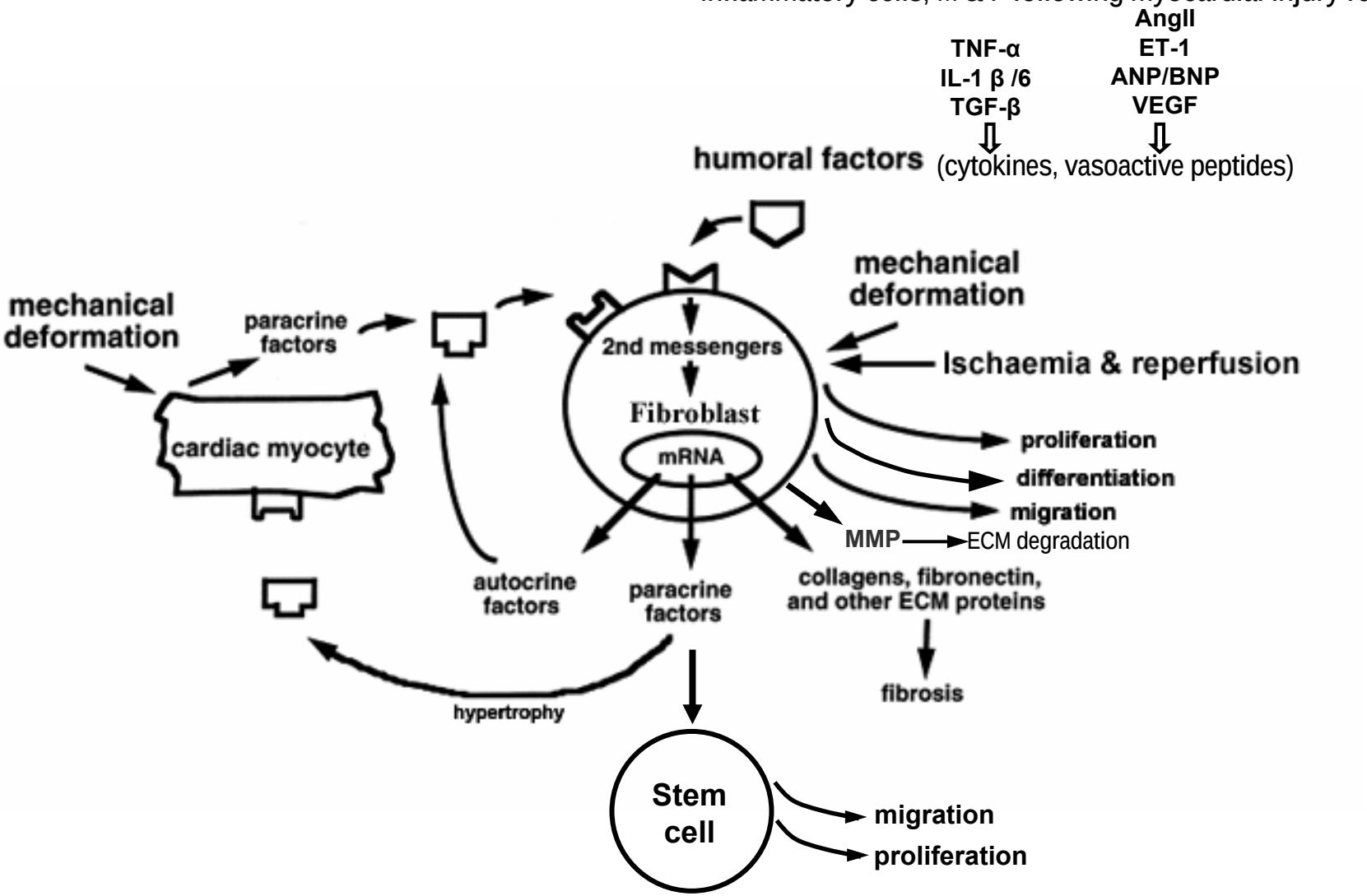
Biochemical Function



Modified from MacKenna *et al*., *Cardiovasc Res* 2000/**46**:257-63.

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Biochemical Function

Cytokines:

- **Tumor necrosis factor alpha** (**TNFα**) – pro-inflammatory cytokine
- **Interleukin-1β** (**IL-1β**) – pro-inflammatory cytokine
- **Interleukin-6** (**IL-6**) – pro-inflammatory cytokine
- **Transforming growth factor-beta** (**TGF-β**) – pro-fibrotic cytokine

Vasoactive peptides:

- **Angiotensin II** (**Ang II**) – regulate blood pressure and volume
- **Endothelin-1** (**ET-1**) – pro-fibrotic
- **Natriuretic peptides** (**ANP & BNP**) – regulate blood pressure
- **Vascular endothelial growth factor** (**VEGF**) – acts primarily on vascular endothelial cells and stimulates angiogenesis

Porter & Turner, *Pharmacology & Therapeutics* 2009/123:25-78.

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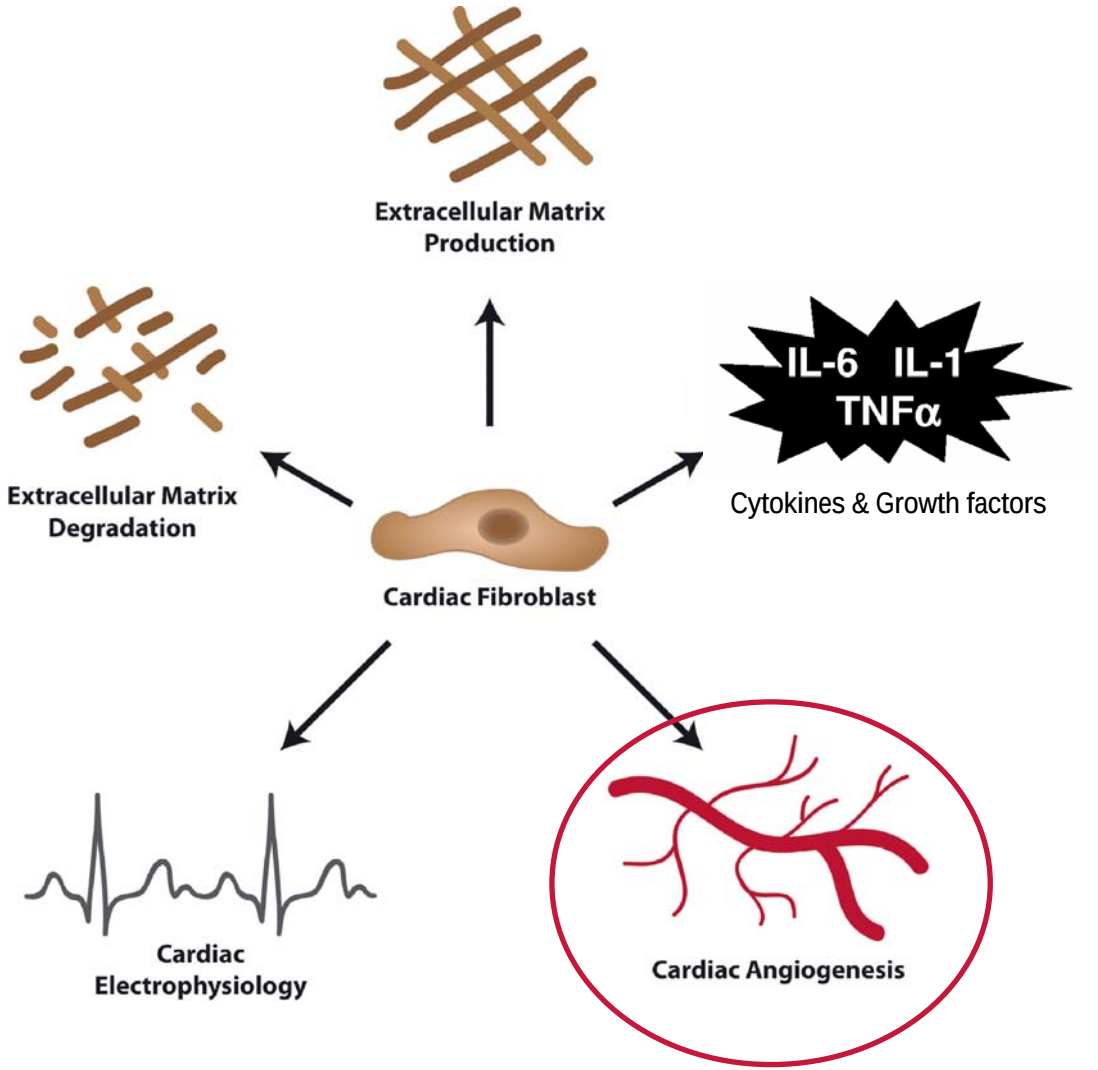
Cardiac Fibroblast Functions



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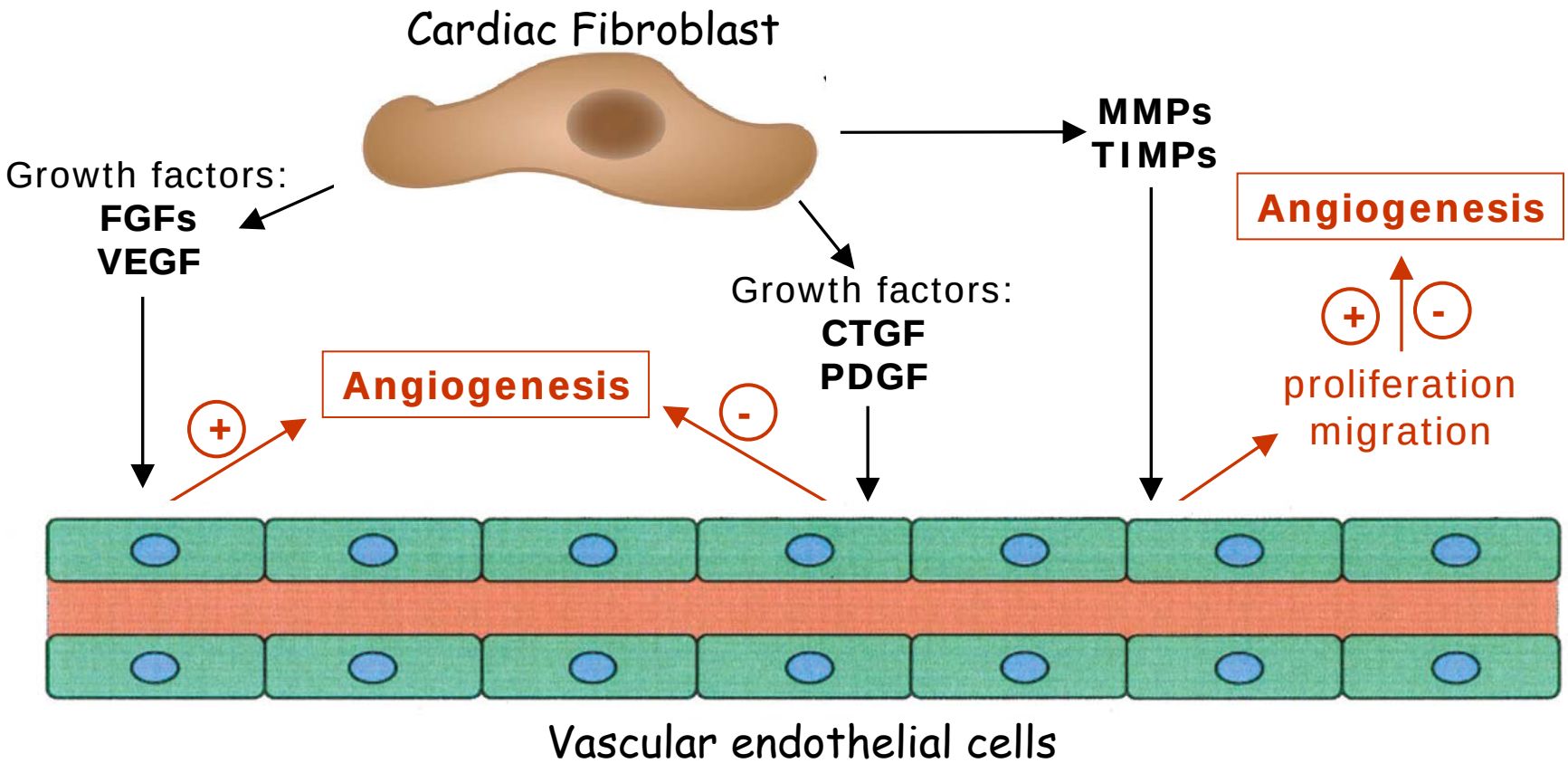
Angiogenesis

Angiogenesis= formation of capillaries from pre-existing blood vessels.

Cardiac fibroblasts interact with vascular endothelial cells during angiogenesis.

Fibroblasts can *induce* or *inhibit* formation of new blood vessels.



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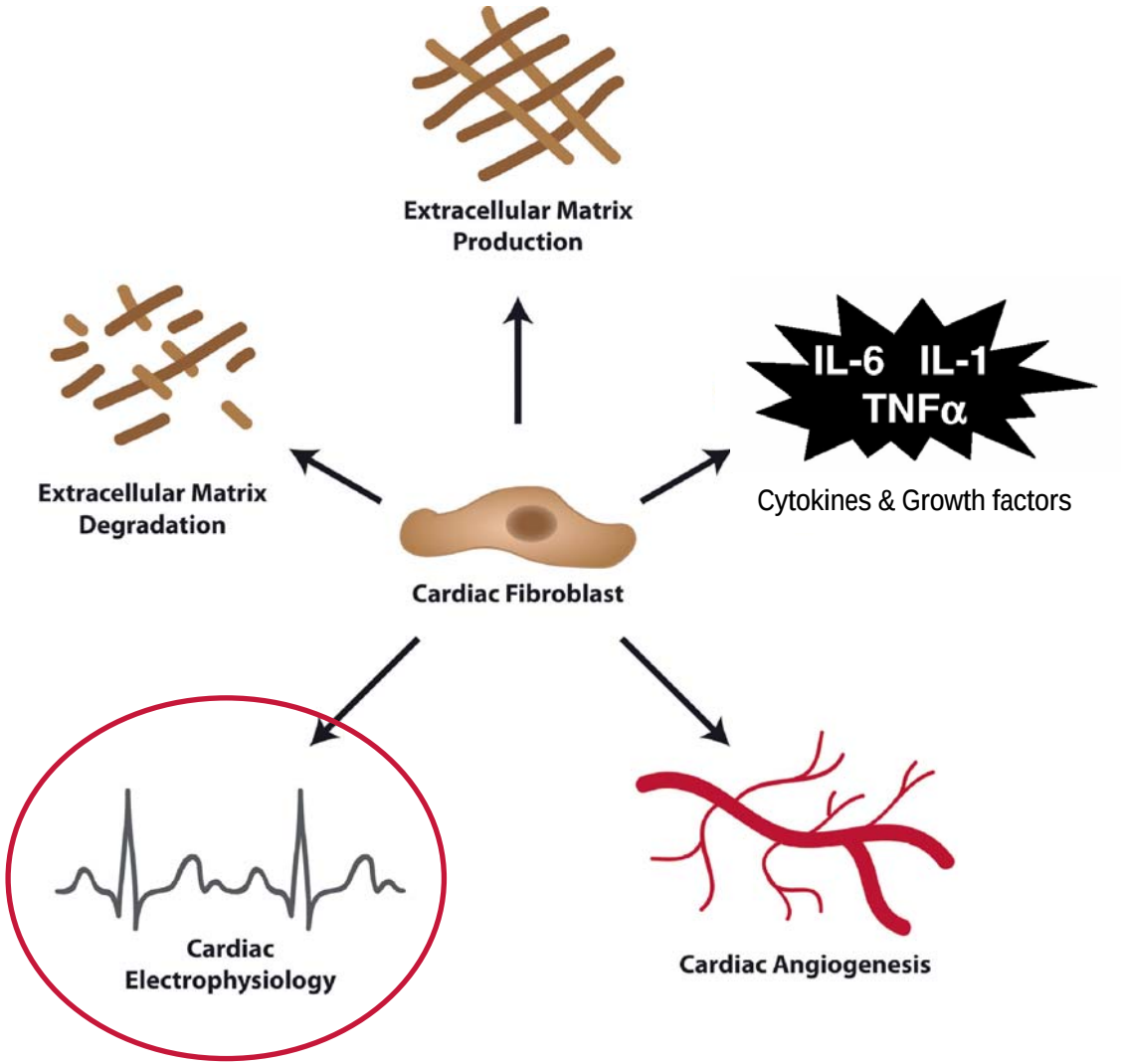
Cardiac Fibroblast Functions



Electrophysiological Function

Electrophysiological role?

F can affect cardiac electrophysiology by

- ***Direct gap-junctional coupling with M and other F***
- ***Paracrine signalling***

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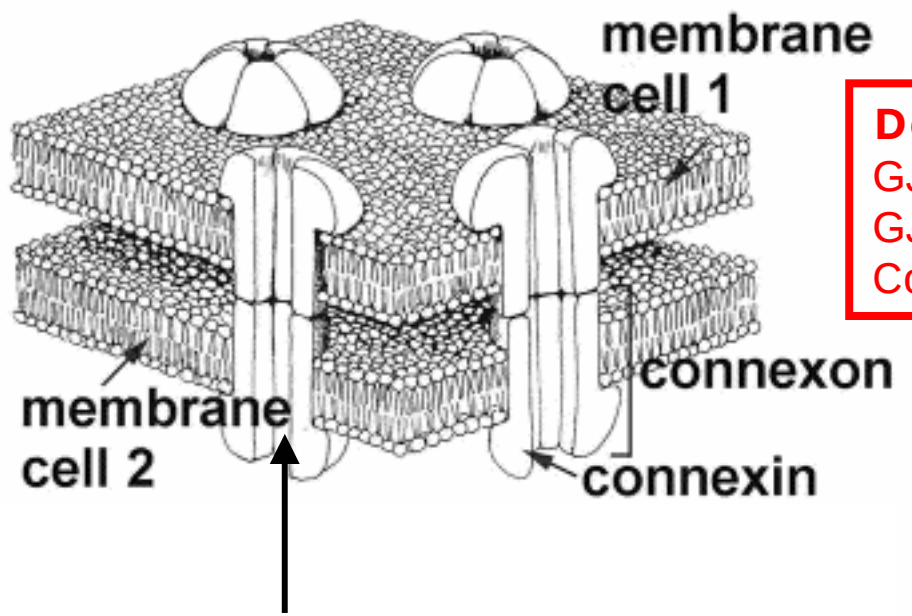
Gap Junctions

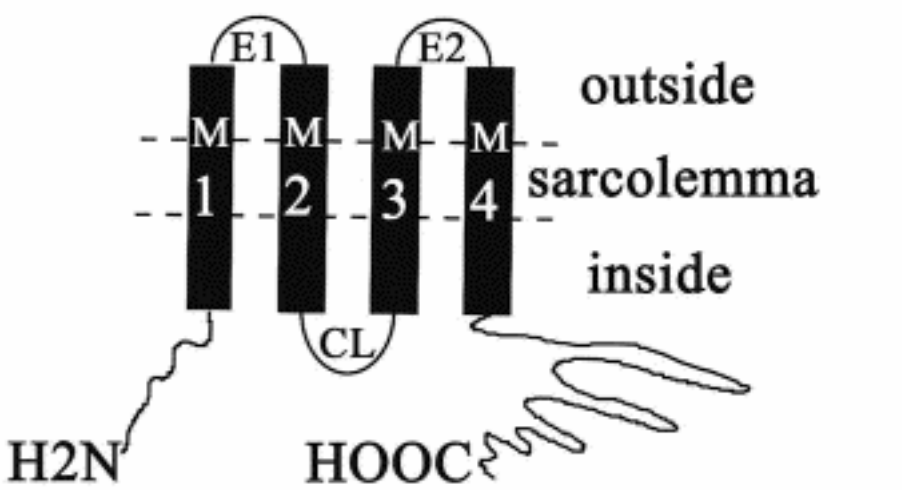


Gap junctional channel

Gap junction = cluster of channels

Dogma:
GJ only in M
GJ couple M
Coupled M form a network isolated from F

Connexin



Gap junctions in Heart:
Cx43, Cx40, Cx45

van der Velden & Jongsma, Cardiovasc Res 2002/**54**:270-9.

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Fibroblast Electrophysiology

- Electrically non-excitable cells
- Mechano-electric transducers (stretch activated ion channels)
- Express ion channels
  - I_{Kir} inward rectifier K^+ current
  - IK_{DR} delayed rectifier K^+ current
  - I_{to} transient outward K^+ current
  - voltage-activated proton current
  - BK_{Ca} Ca^{2+}-activated K^+ current
  - I_{Clvol} volume-sensitive chloride current
  - voltage-gated Na^+ current
  - cation nonselective mechano-sensitive current
- Resting MP: 0…-50 mV
- Membrane resistance: 10^9…10^{10} Ω ⇛ good conductors
- Could actively affect cardiac electrophysiology IF coupled to M via gap junctions

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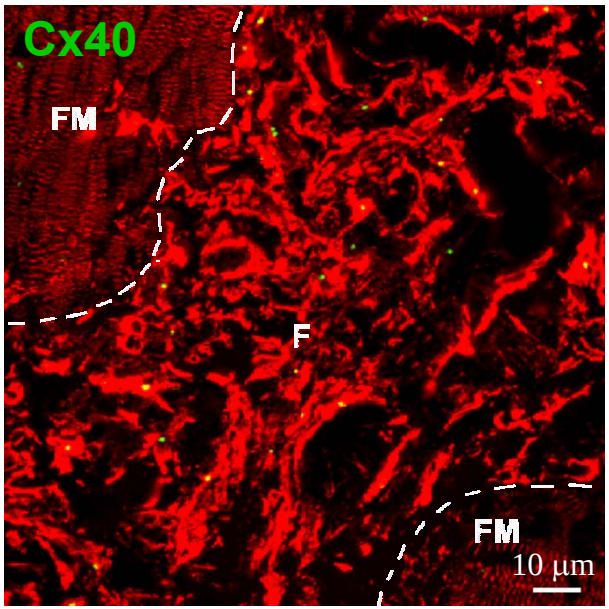
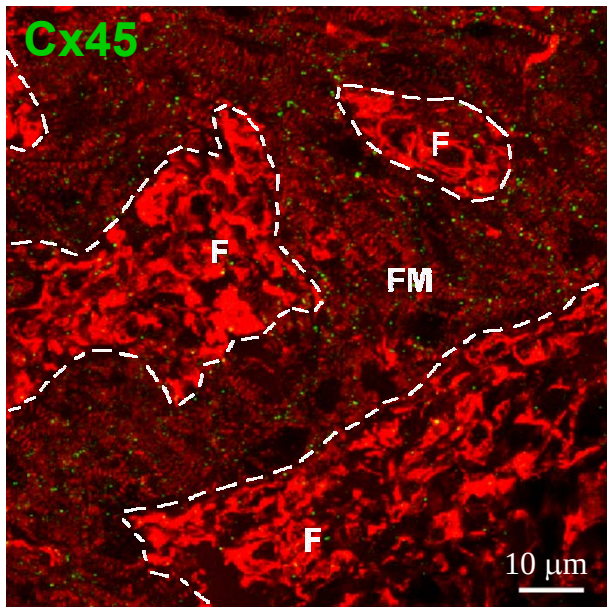
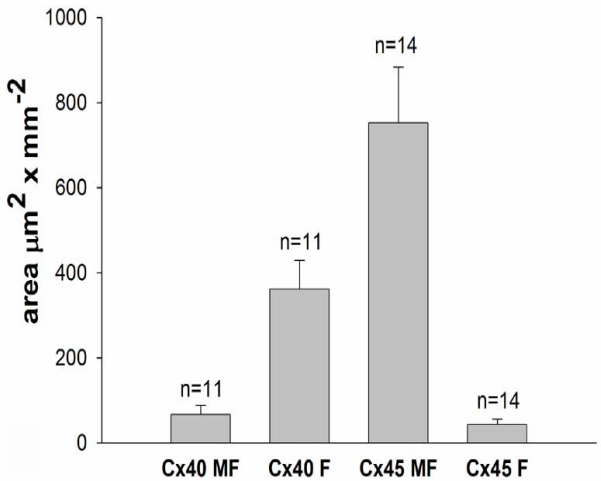
F-M and F-F Coupling *in situ*: Cx40 and Cx45 in Sino-Atrial Node







Cx40 is predominantly located in F areas, Cx45 in MF.

Camelliti P. *et al*., Circ Res 2004/**94**:828-835.

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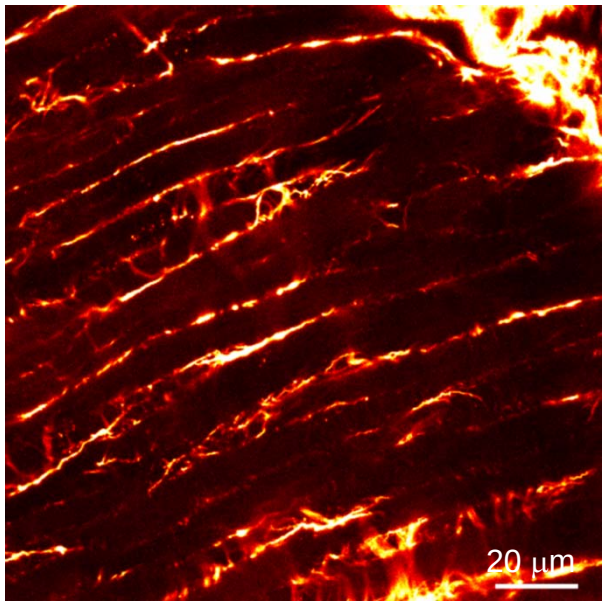
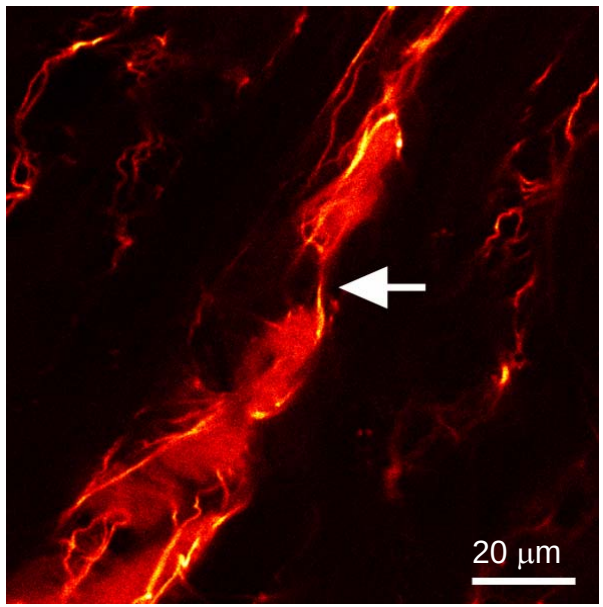
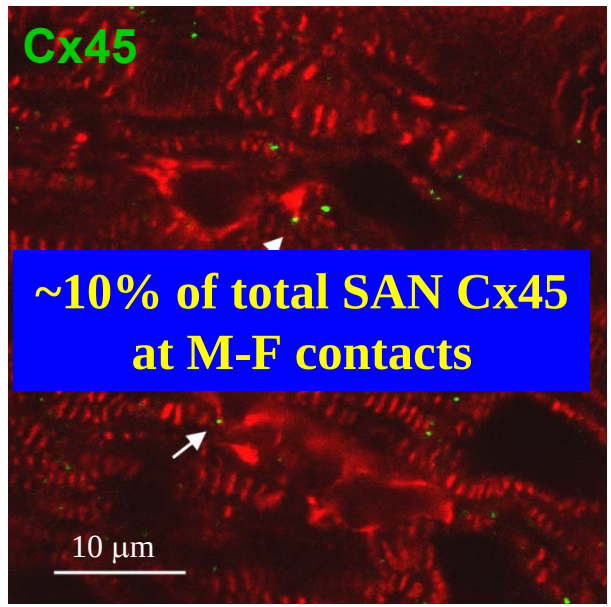
F-M and F-F Coupling *in situ*: Functional coupling in Sino-Atrial Node



Preferential *Lucifer Yellow* dye spread through F



Groups of *Lucifer Yellow* loaded M interconnected via loaded F



Cx45 at point of M-F contact.

There is F-F and M-F functional coupling in rabbit SAN.

Camelliti P. *et al*., Circ Res 2004/**94**:828-835.

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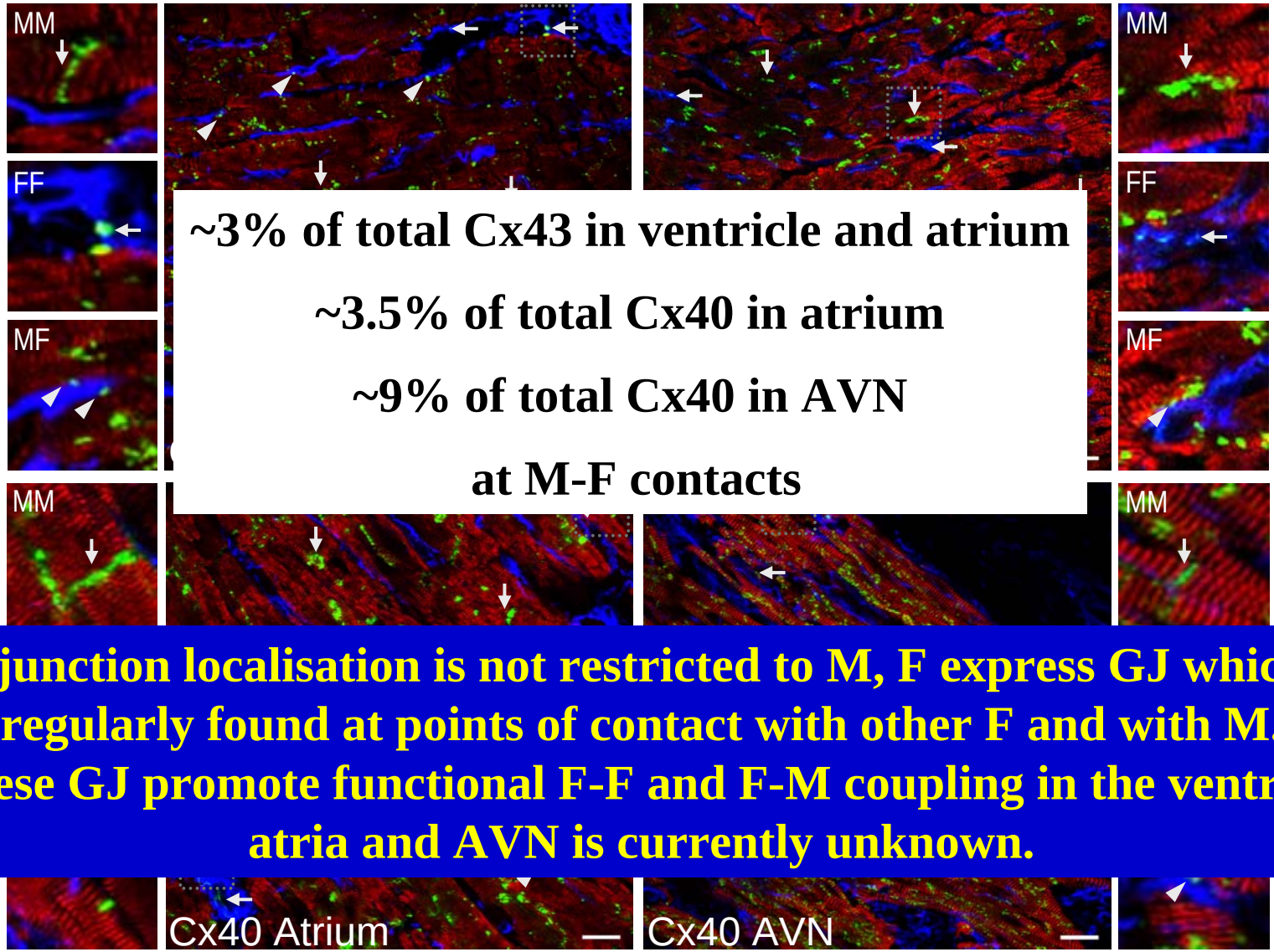
F-M and F-F Coupling *in situ*: Rabbit Ventricle/Atrium/AVN – Cx43 & Cx40



~3% of total Cx43 in ventricle and atrium

~3.5% of total Cx40 in atrium

~9% of total Cx40 in AVN

at M-F contacts

Gap junction localisation is not restricted to M, F express GJ which are regularly found at points of contact with other F and with M. If these GJ promote functional F-F and F-M coupling in the ventricles, atria and AVN is currently unknown.

Camelliti P. *et al*., Heart Rhythm 2011 (in press).

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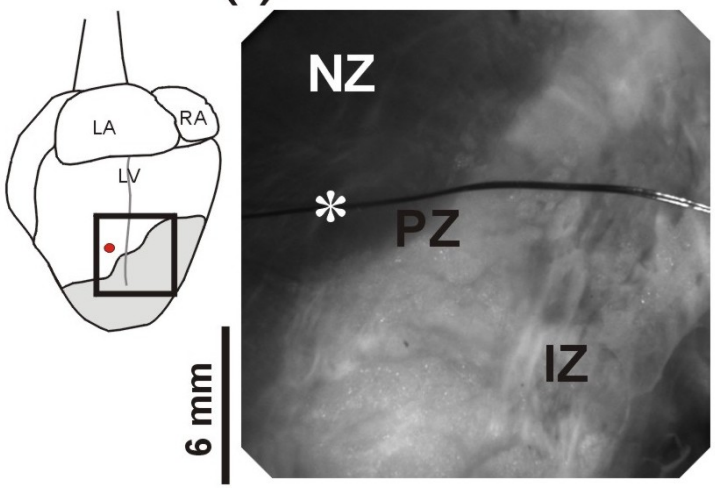
F-M and F-F Coupling *in situ*: Rabbit post-MI model – Collective F & M Recordings

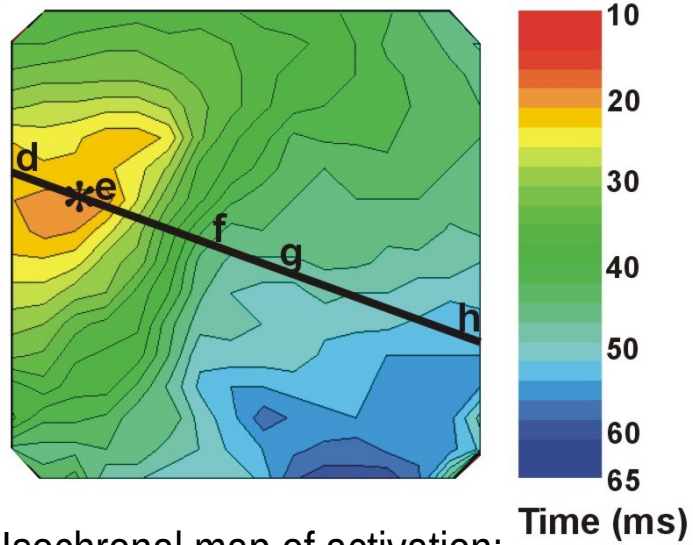
Epicardial activation during epicardial pacing in non-infarcted zone (NZ)

(i)



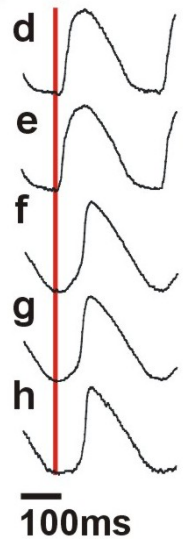
Epicardial area studied
NZ: normal area
PZ: border zone
IZ: infarct

(ii)



Isochronal map of activation: optical mapping of electrical activity

(iii)



Optically recorded AP

Rabbit transmural MI (8 weeks).

Electrical conduction in transmural MI.

Walker *et al*., *JCE* 2007/**18**:862-8.

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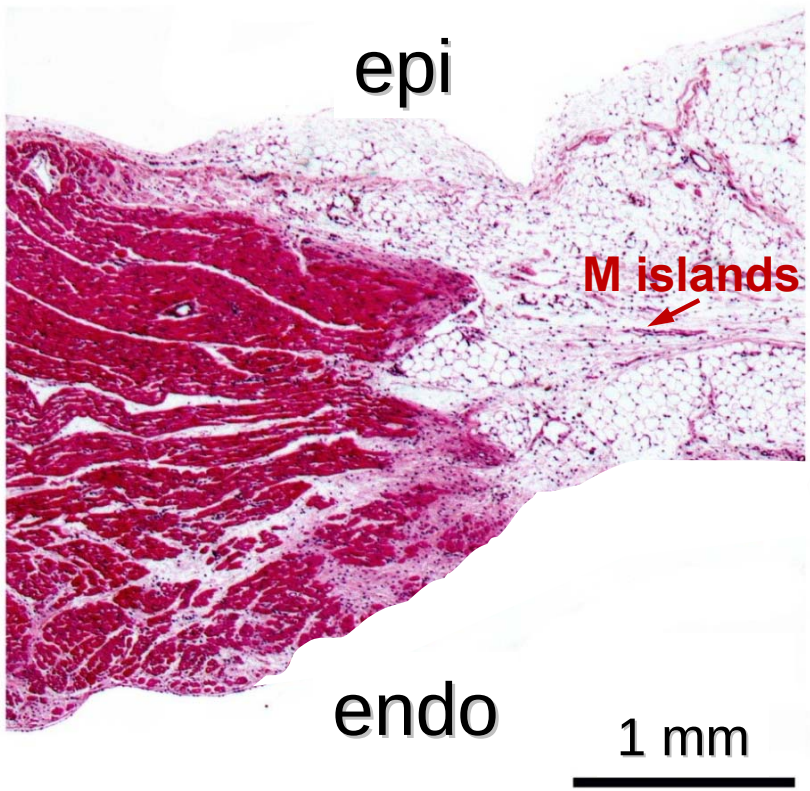
F-M and F-F Coupling *in situ*: Rabbit post-MI model – Conduction Pathways



Electrical propagation across the infarct: conduction between M islands via F

…would require electrical coupling at M-F and F-F contact via GJ.

Walker *et al*. *JCE* 2007/**18**:862-8; Kohl *et al*. *J Electrocardiol* 2005/**38**:45-50.

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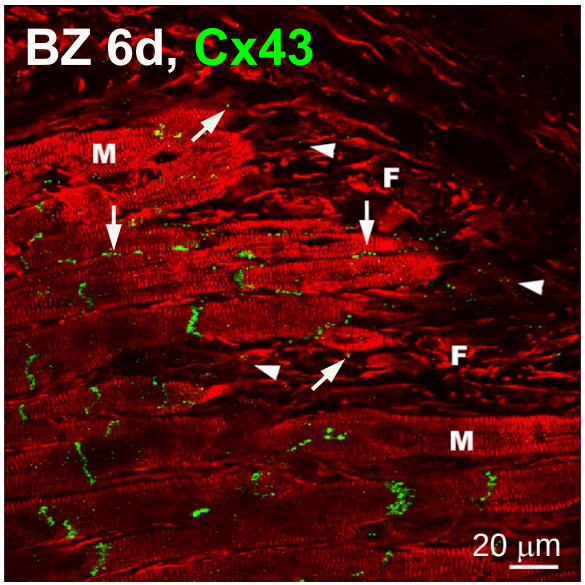
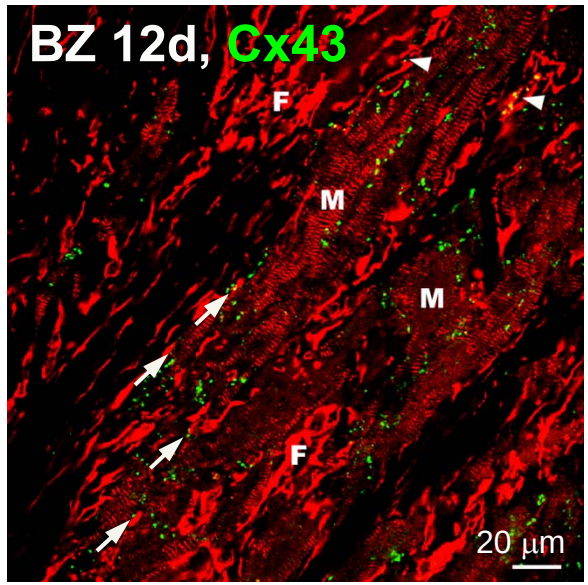
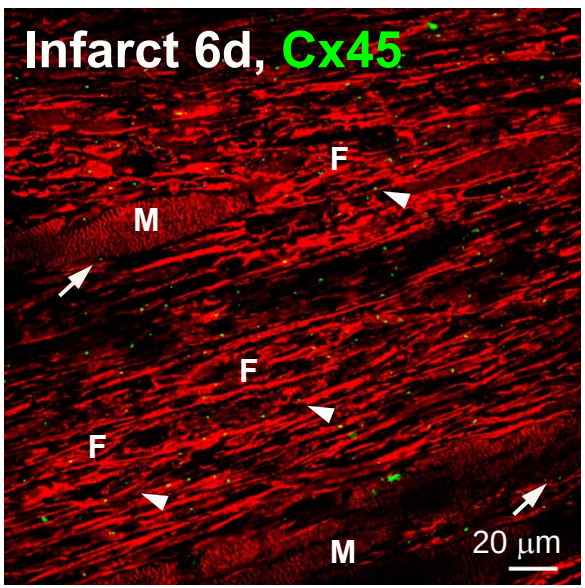
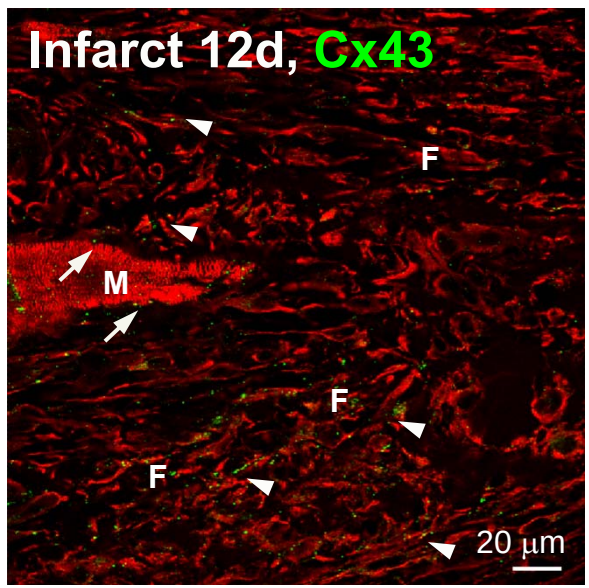
F-M and F-F Coupling *in situ*: Sheep post-MI model – Cx43 & Cx45









MM ↓
MF? ↗
FF ◄

Sheep infarct F express Cx45 and Cx43.

Camelliti P. *et al*. *CVR* 2004/**62**:415-425.

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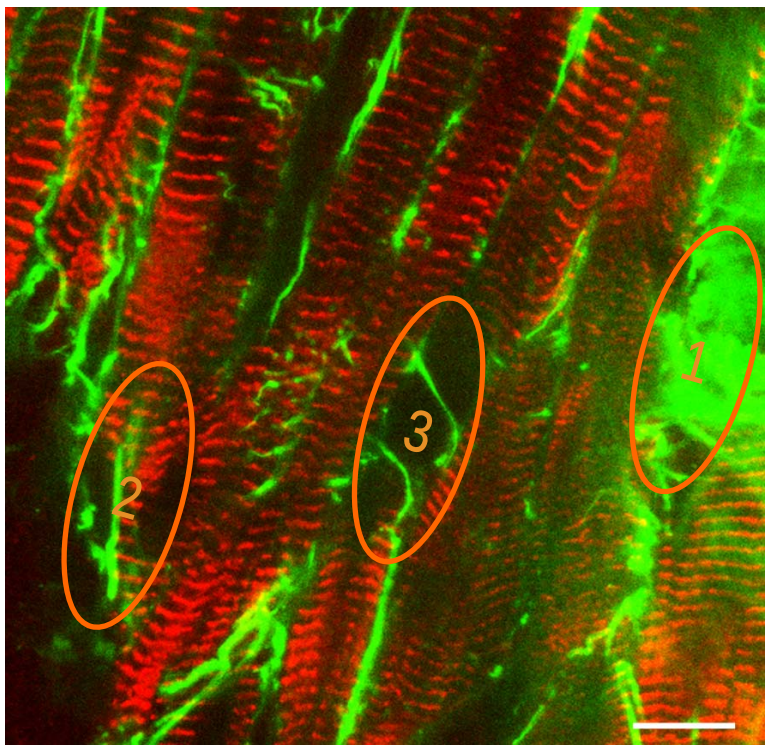
Fibroblasts and Cardiac Electrophysiology

If there is no coupling: 1) F not coupled to M
F = Obstacle (scars)

If there IS coupling: 2) F coupled to a single group of M
F = Current Sink (fibrosis)



3) F interconnecting separated M
F = Conductor

A) short-range
(electrical propagation between groups of SAN cells/myocardial layers)

B) long-range
(electrical conduction across scar, electrical coupling in 10% transplants)

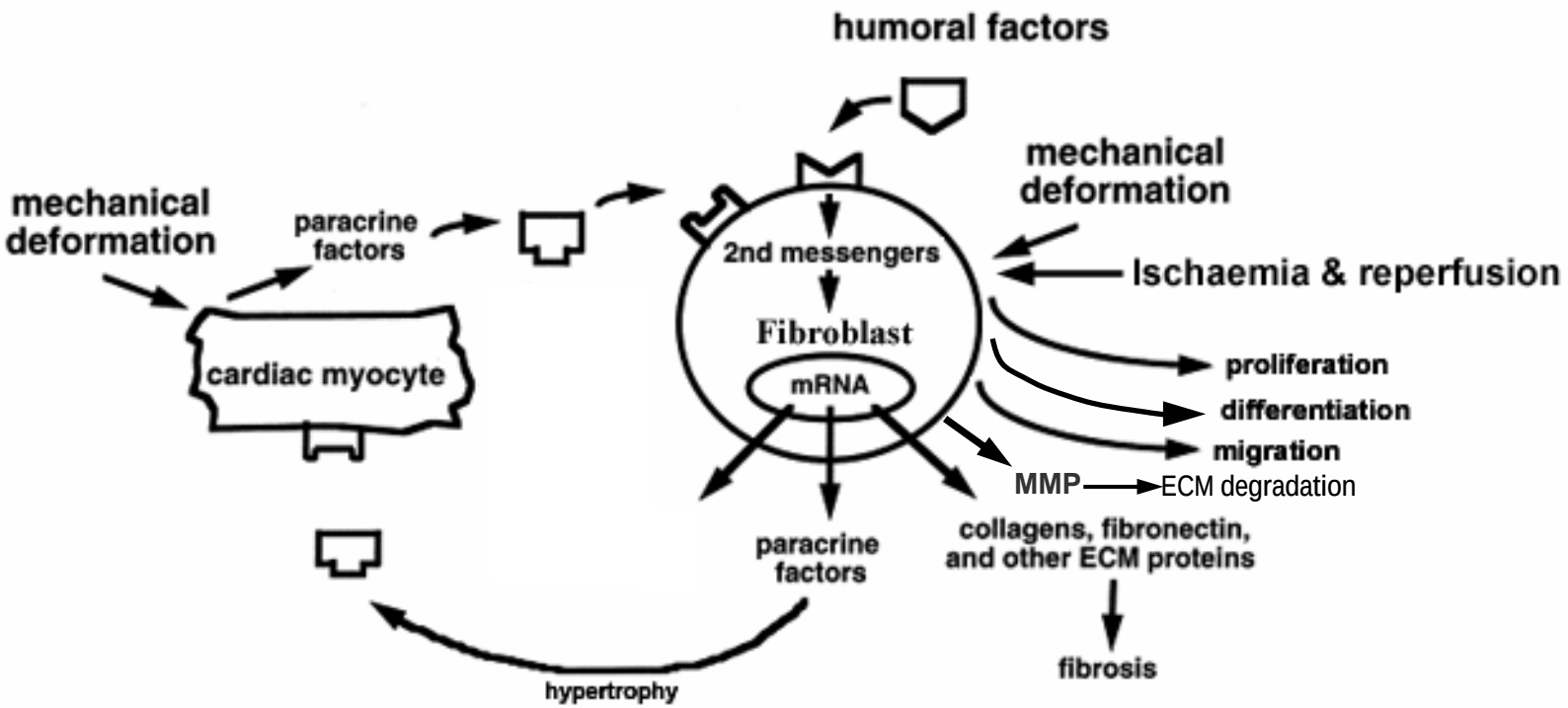
Kohl & Camelliti, Heart Rhythm 2007/**4**: 233-235.

Electrophysiological Function

F can affect cardiac electrophysiology via

- ***Direct gap-junctional coupling with M and other F***
- ***Paracrine signalling***

Paracrine F-M crosstalk



Modified from MacKenna *et al*., *Cardiovasc Res* 2000/**46**:257-63.

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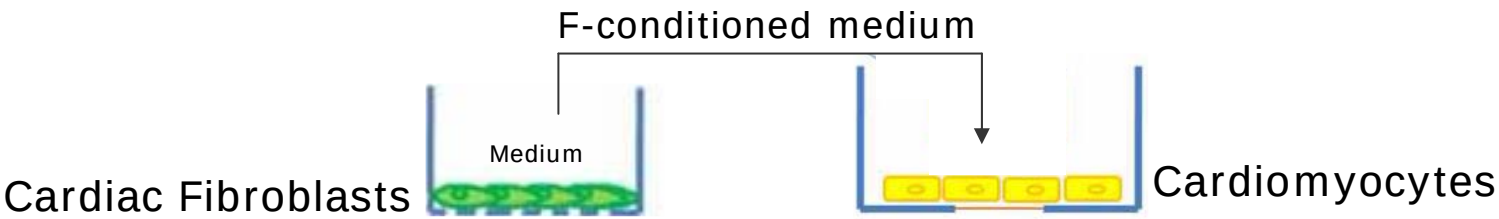
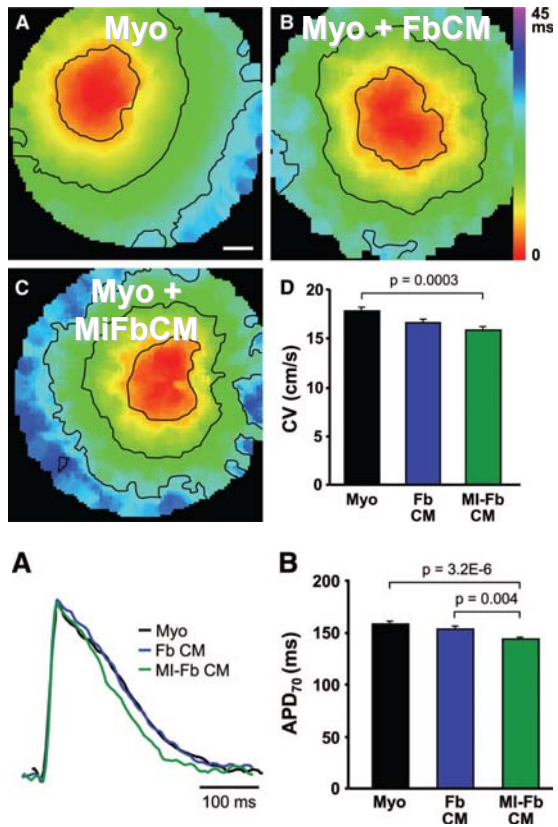
Paracrine Signalling *in culture*



F-conditioned media induce:

- neonatal M hyperthophy
- reduce M spontaneous activity
- affect CV and APD

Conditioned media from infarcted F affect neonatal M CV & APD to a greater degree than conditioned media from normal F.



Vasquez *et al*, *Circ Res* 2010/**107**:1011-20.

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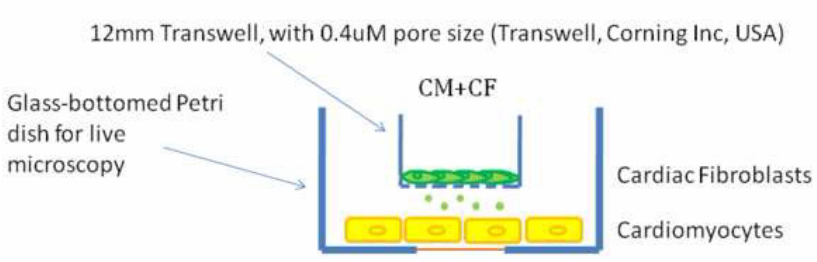
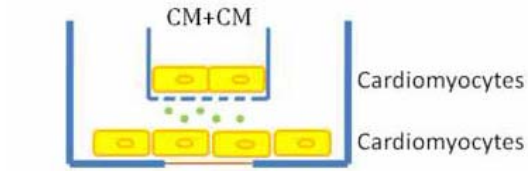
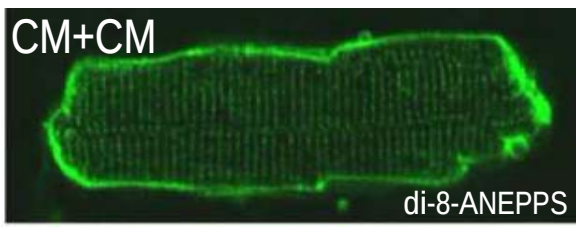
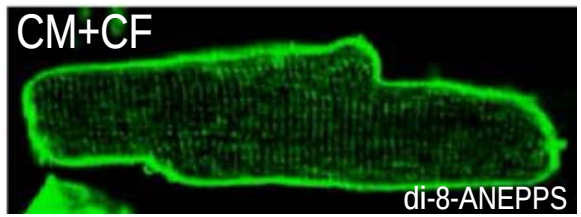
Paracrine Signalling *in culture*:
Effect on Adult M Structure & Excitation Contraction Coupling

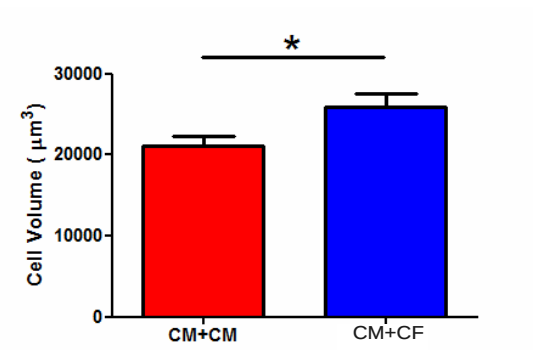




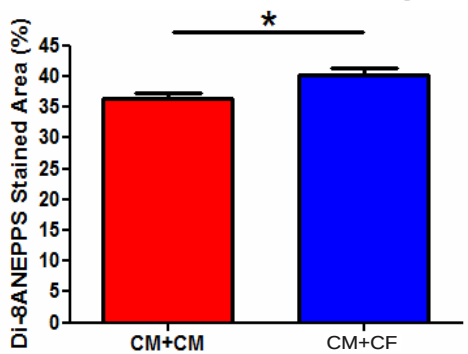




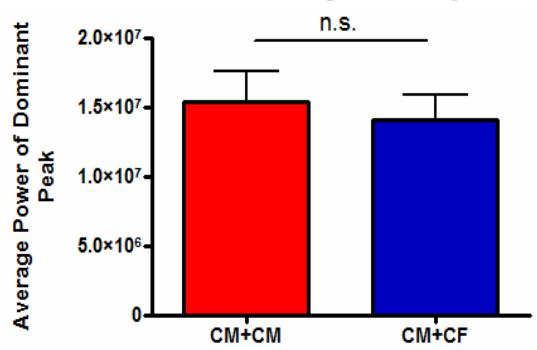
Myocyte Volume



T-tubule Density



T-tubule Regularity



Clark *et al*, under review.

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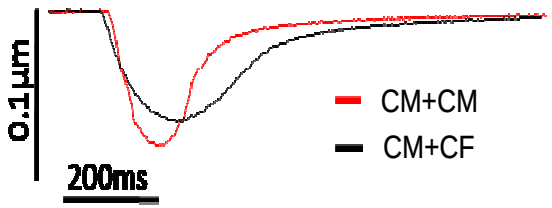
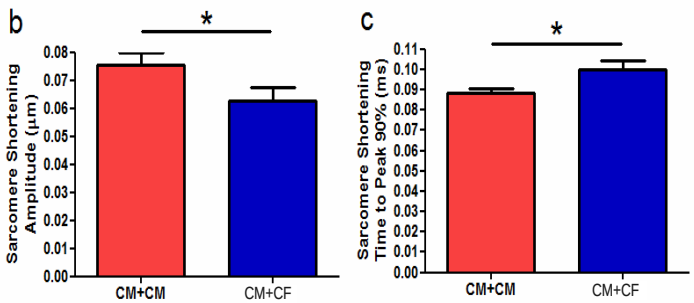
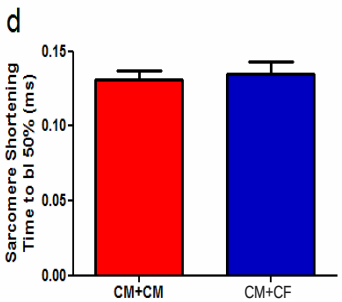
Paracrine Signalling *in culture*:

Effect on Adult M Structure & Excitation Contraction Coupling

Contractility

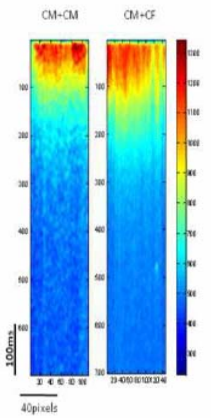




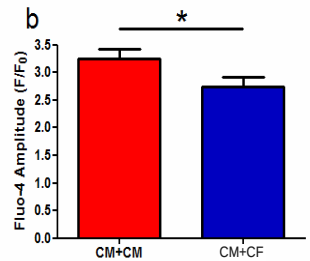


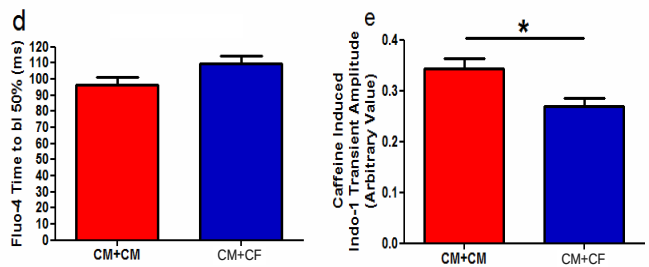
Smaller and slower sarcomere shortening.

Calcium Handling



- Depressed Ca^{2+} transients.
- Reduced SR Ca^{2+} content.





Clark *et al*, under review.

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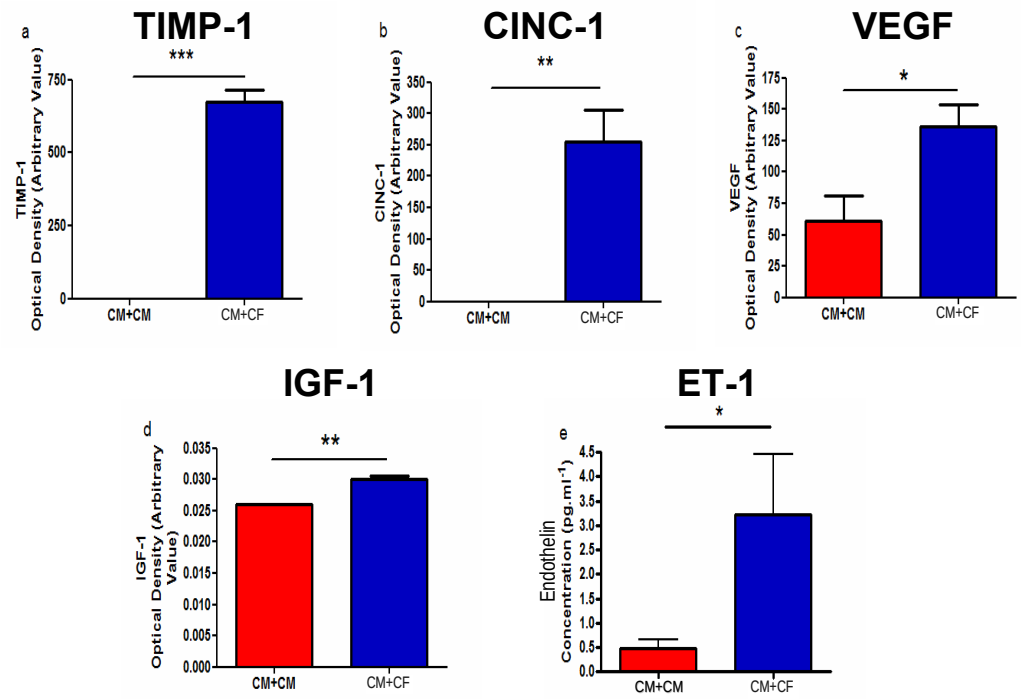
Paracrine Signalling *in culture*:
Effect on Adult M Structure & Excitation Contraction Coupling

Soluble Factors in culture media



Adult F can affect adult M structure and ECC via paracrine signalling.

Clark *et al*, under review.

Therapies directed at Cardiac Fibroblasts

- **Pharmacological agents**
- **Cell therapy**

Therapies directed at Cardiac Fibroblasts

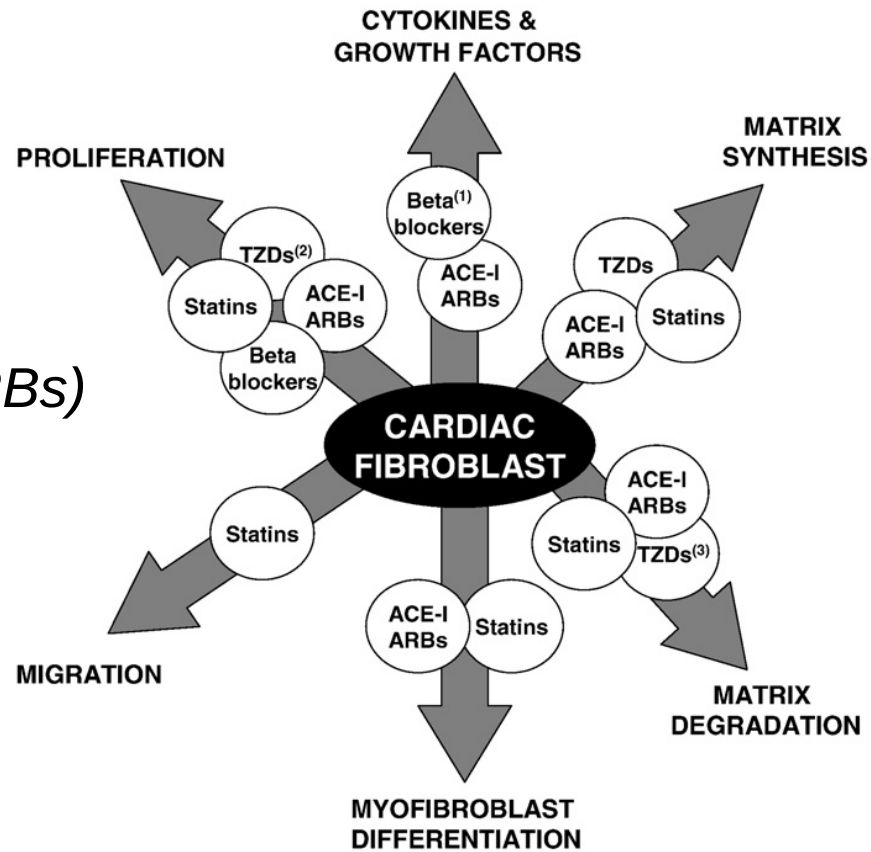
Pharmacological agents:

- anti-hypertensive agents

  ACE inhibitors (ACE-I)
  Angiotensin receptor blockers (ARBs)
  Beta-blockers

- lipid-lowering drugs

  Statins



Porter & Turner, *Pharmacology & Therapeutics* 2009/123:25-78.

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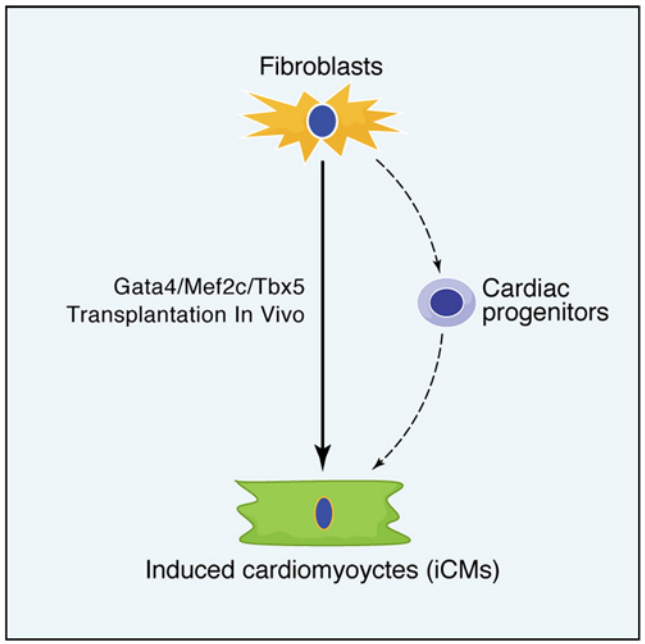
Therapies directed at Cardiac Fibroblasts

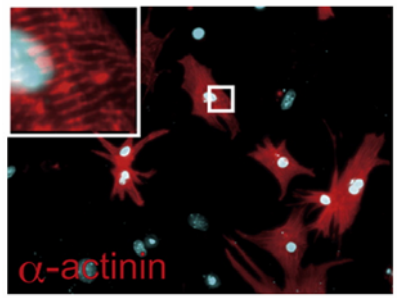
Cell therapy:

Direct reprogramming of cardiac F into functional cardiomyocytes

In vitro…

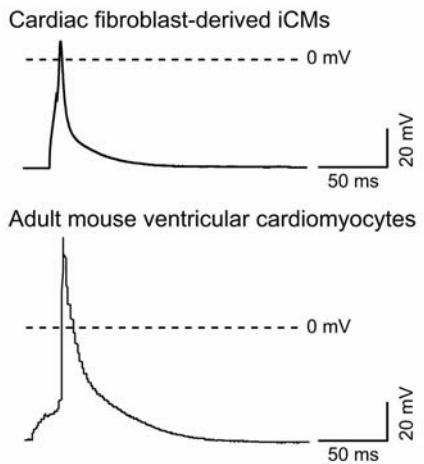


M-like sarcomeric structure…

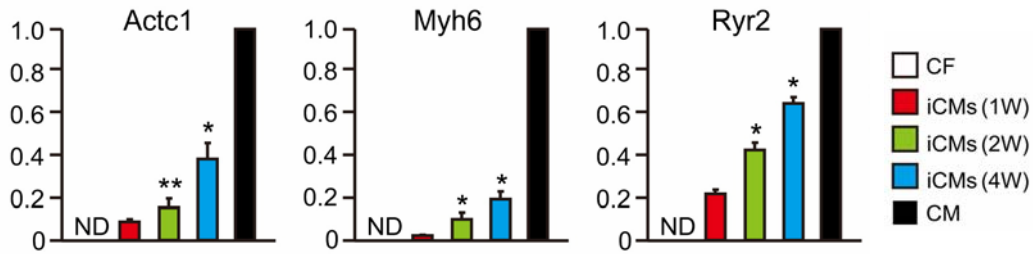


…and spontaneous contraction

Action potentials



Global gene expression profile similar to M



Ieda *et al.*, *Cell* 2010/1142:375-86.

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Conclusions

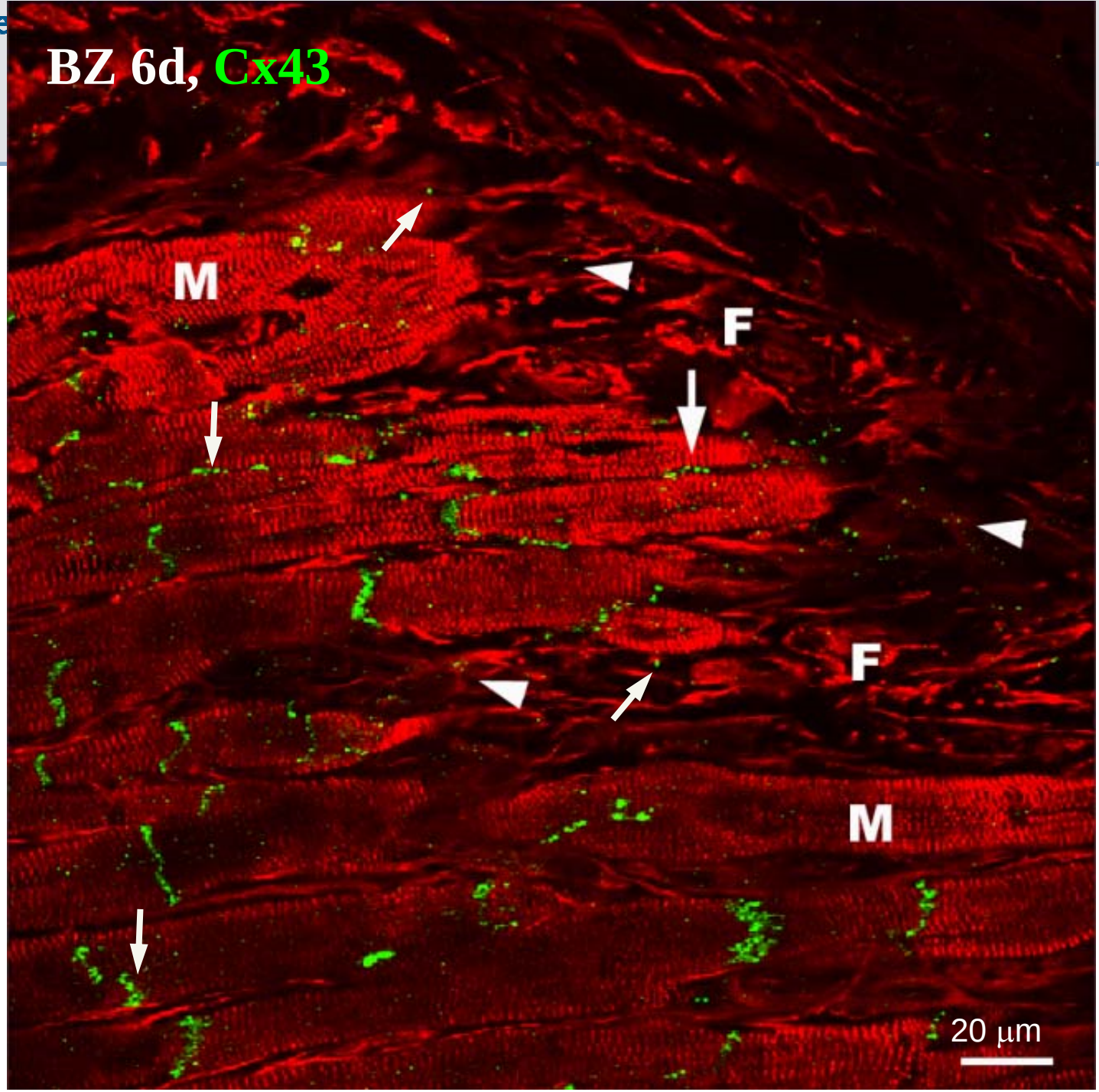
Fibroblasts are the largest cell population in the healthy heart.

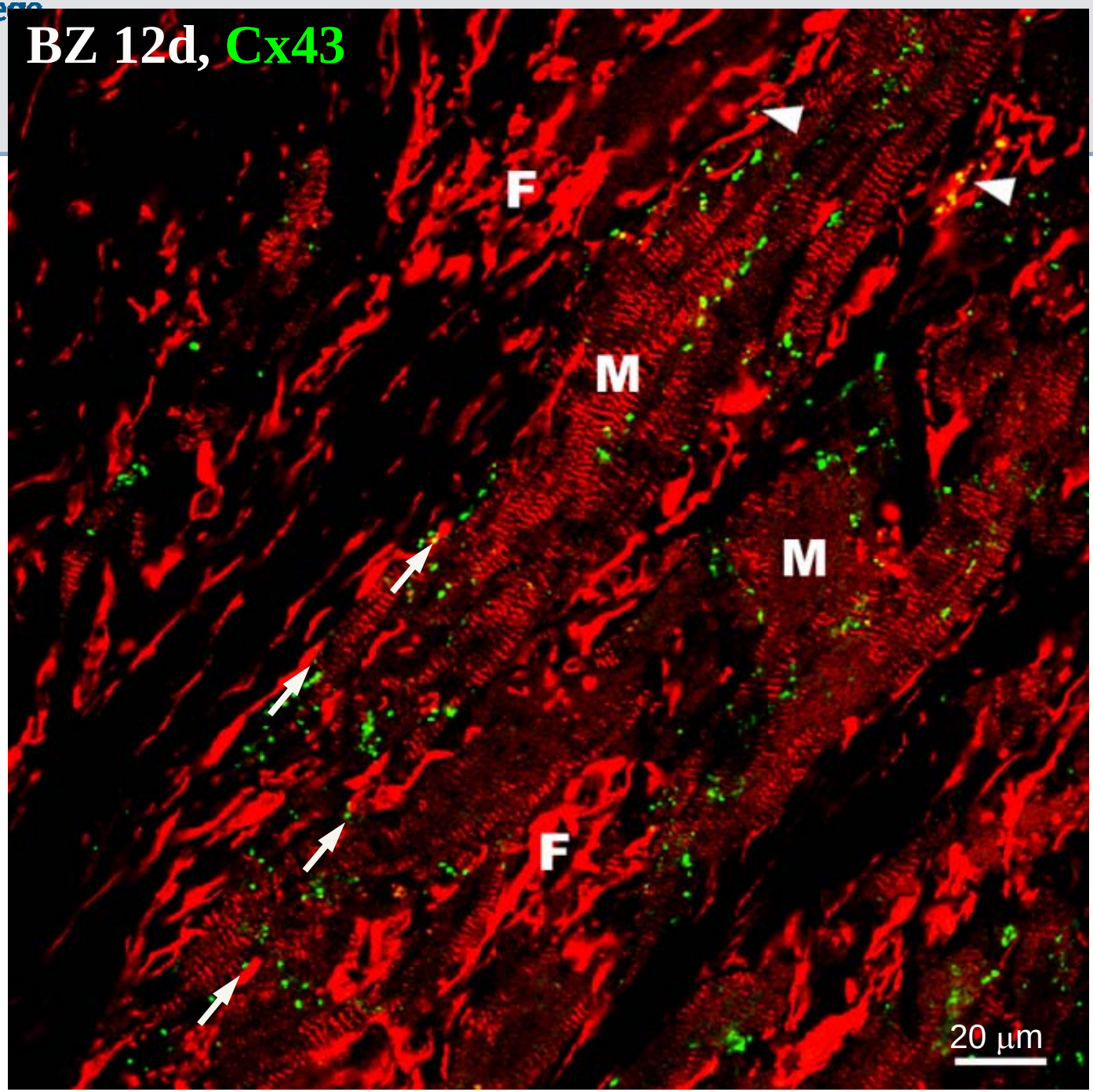
Their number is further increased in pathological conditions.

Fibroblasts are active players in cardiac structure and function.

Fibroblasts are a promising target for novel therapeutic strategies.

Questions?


BZ 6d, Cx43
M
F
F
M
20 μm


BZ 12d, Cx43
F
M
M
F
20 μm