Korean Society of Circulation 50th Annual Scientific Meeting

Seoul, Korea – October 12, 2006

Basic Research Session on

Translational Cardiovascular Research

“Cell and Molecular Therapies for Cardiac Repair”

30’

Origins of cell therapy in congestive heart failure:

- Several groups have had a long-standing interest in the use of donor cells to promote angiogenesis with the notion that this could provide revascularization therapy in ischemic myocardium
- In the 1990s, several groups demonstrated that skeletal myoblasts form stable grafts in hearts; some suggested that the donor cells adopted a “cardiac phenotype”
- Studies in the late 1990s / early 2000s suggested that adult stem cells have a greater capacity for cross-lineage differentiation then previously thought (i.e., “trans-differentiation” from blood to brain, blood to muscle, etc)
- Many preclinical cell transplantation studies indicated that donor cell transplantation improved cardiac function in injured hearts
- Clinical studies with cell transplantation have yielded variable results; the impact on cardiac function observed to date has been somewhat disappointing (particularly given some of the pre-clinical results)

- There are several ways that donor cells can help injured hearts (i.e., vasculogenesis, anti-apoptosis, direct cardiomyogenesis)
- True regeneration of damaged hearts requires the formation of new cardiomyocytes (CMs) – this is the main interest of my laboratory
- Tracking the cardiomyogenic fate & utility of donor cells requires the availability of good markers (either intrinsic or genetic tags) and the ability to demonstrate functional competence at the cellular level, respectively
- Today's presentation will demonstrate:

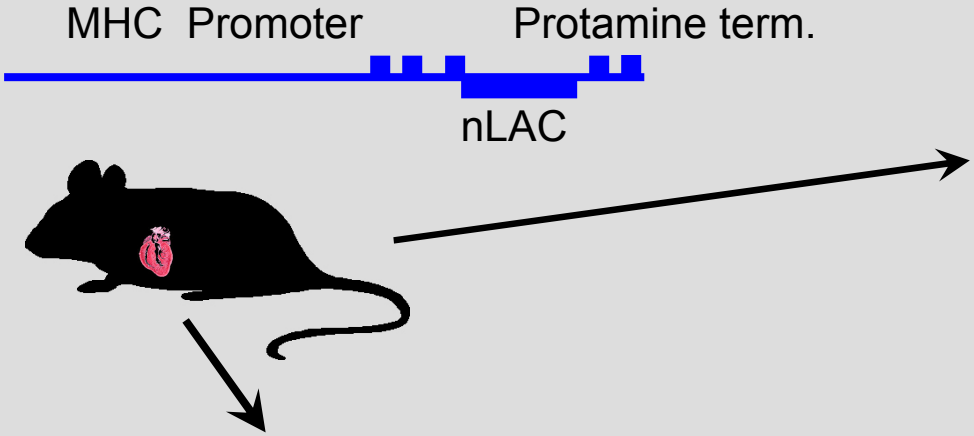
  - that fetal CMs form stable and functionally integrated grafts (we use transgenic reporter mice to demonstrate these traits)

  - that skeletal myoblasts, marrow-derived HSCs, mono-nuclear cells, and Sca-1^{+} cardiac resident cells engraft, but for the most part do not form integrated myocytes (except perhaps via rare cell fusion events)

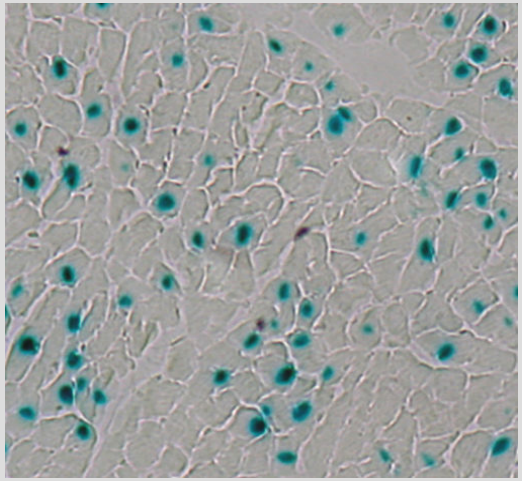
  - that human ES-derived CMs form stable grafts

Tracking differentiation of donor fetal CMs:

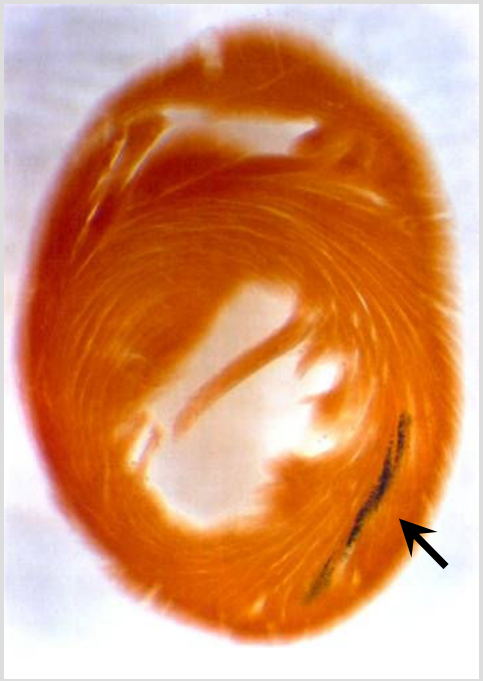
MHC-nLAC mice



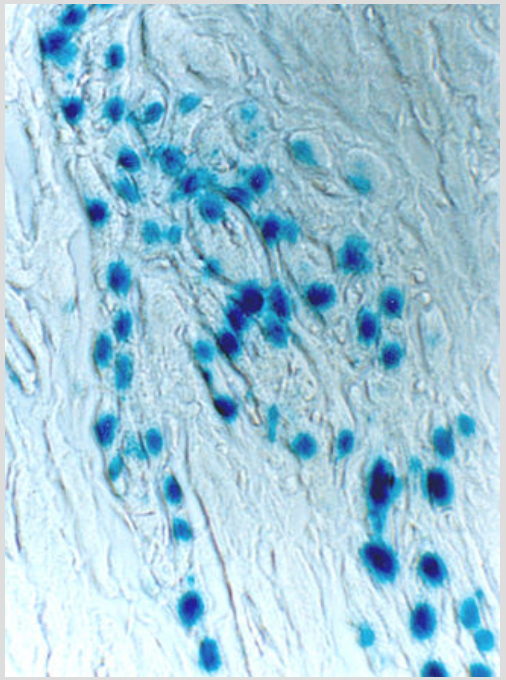
- Isolate E15.5 txg hearts
- Digest with collagenase
- Tsp. CMs into non-txg recipient
- Harvest heart, stain with X-GAL; blue nuclei indicate that donor cells underwent CM differentiation



Adult heart section (X-GAL stained)



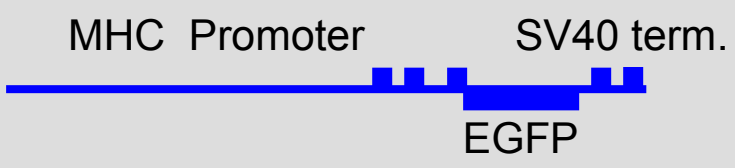
Vibratome Section

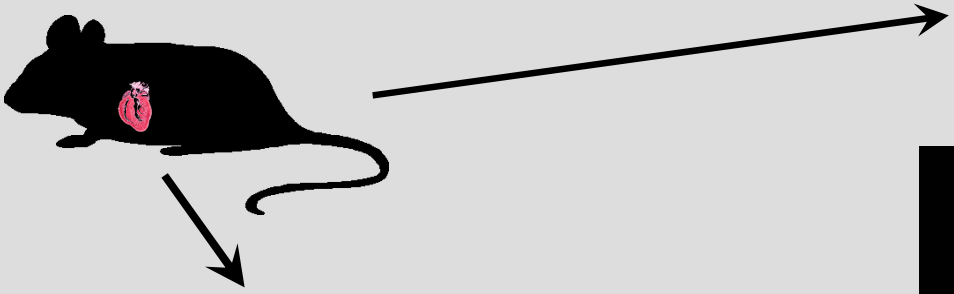


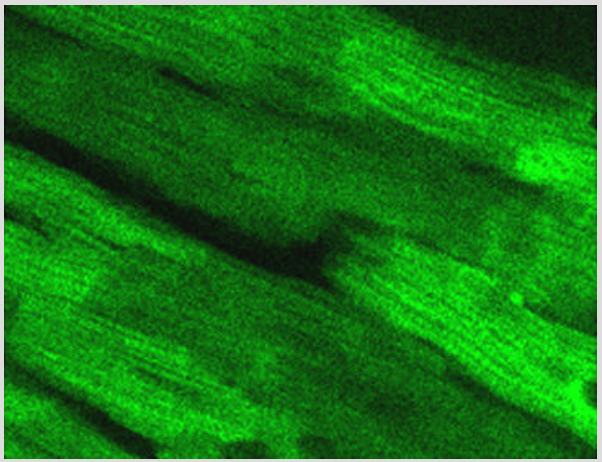
Thin Section

Tracking survival & function of donor fetal CMs:

MHC-EGFP mice

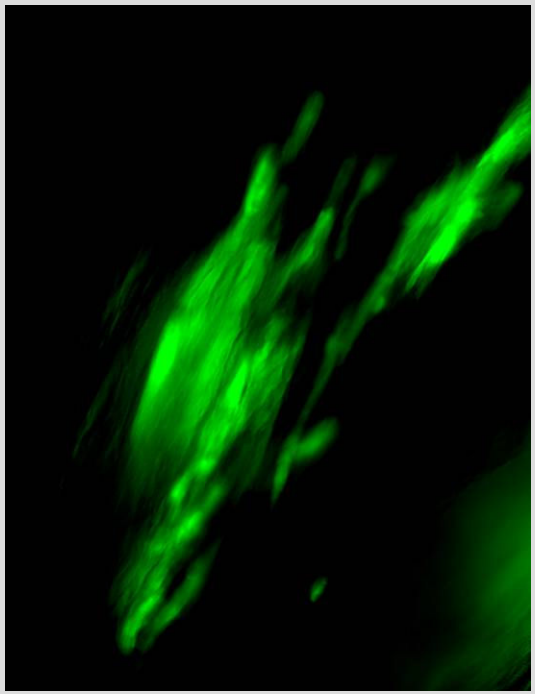




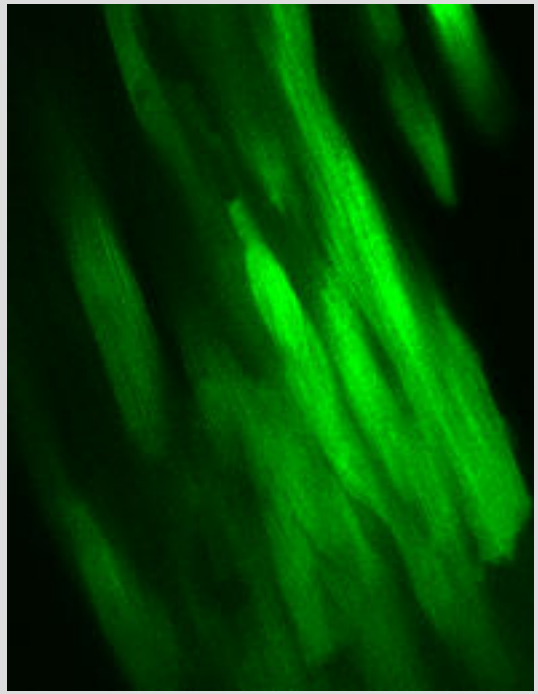


Adult heart section (epi-fluorescence)

- Isolate E15.5 txg hearts
- Digest with collagenase
- Tsp. CMs into non-txg recipient
- Visualize via epi-fluorescence; green signal indicates donor cell survival



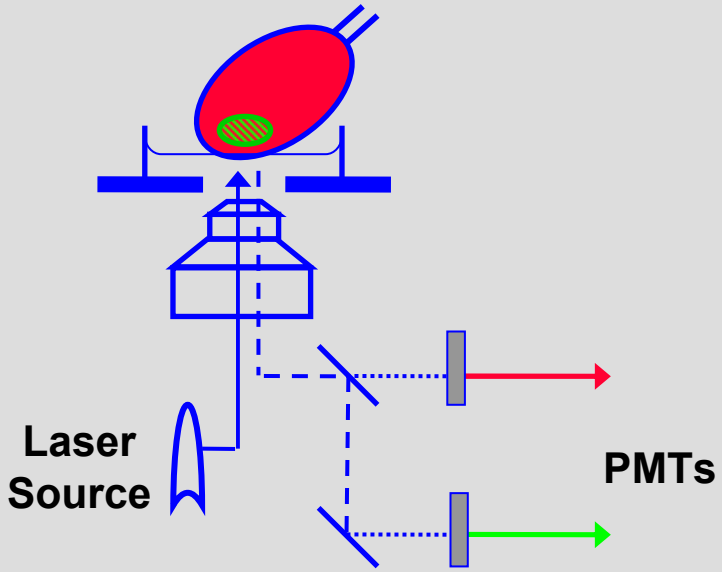
Vibratome Section



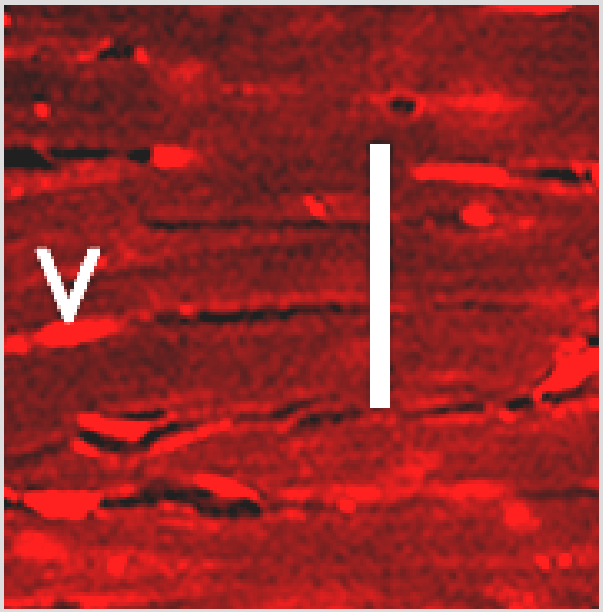
Thin Section

- Image analysis for donor cell function

- Harvest heart with EGFP donor cell graft
- Langendorff-perfuse with cytochalasin D (uncouples excitation/contraction) and Rhod-2 (increases fluorescence with $[Ca^{2+}]_i$)
- Image Rhod-2 and EGFP fluorescence via two photon laser scanning microscopy

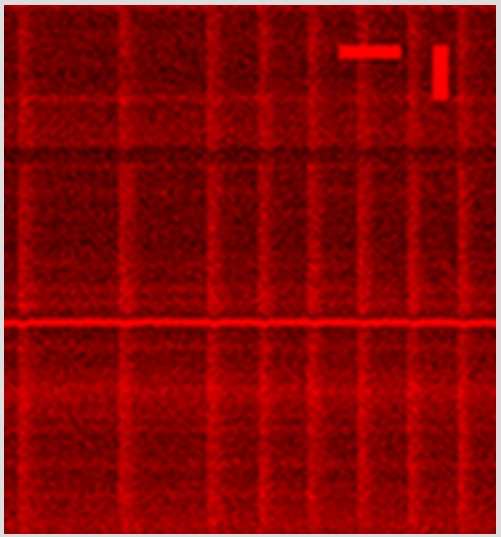


2-D View



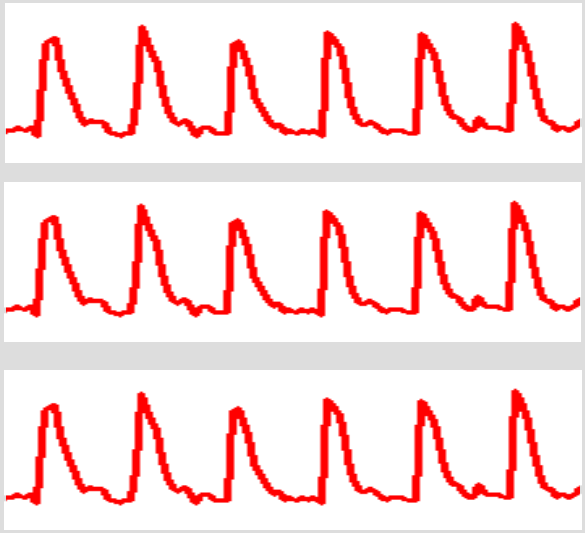
Dist.

Stacked Line Scans



Time

Integrated Traces



- Harvest heart with EGFP donor cell graft
- Langendorff-perfuse with cytochalasin D (uncouples excitation/contraction) and Rhod-2 (increases fluorescence with $[Ca^{2+}]_i$)
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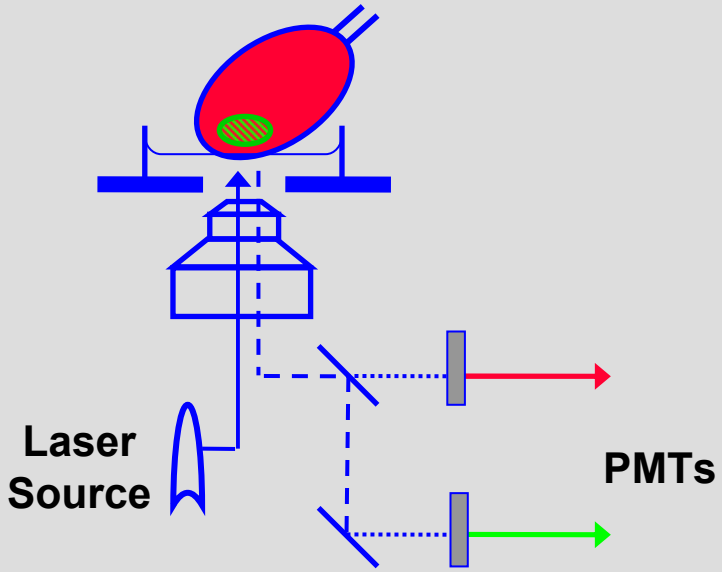
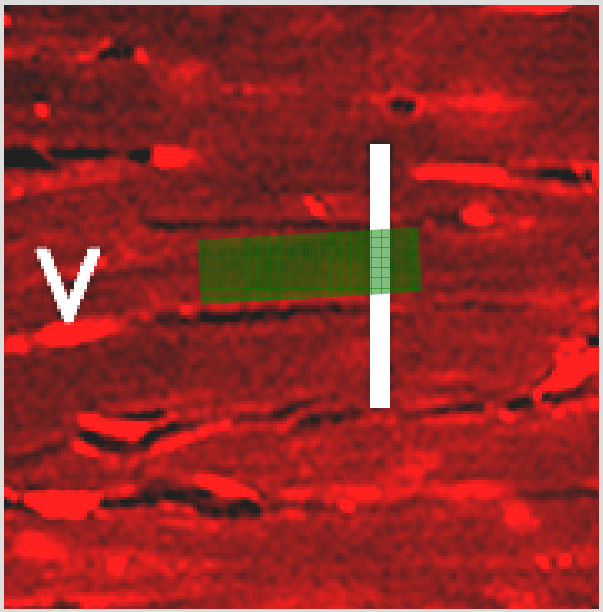
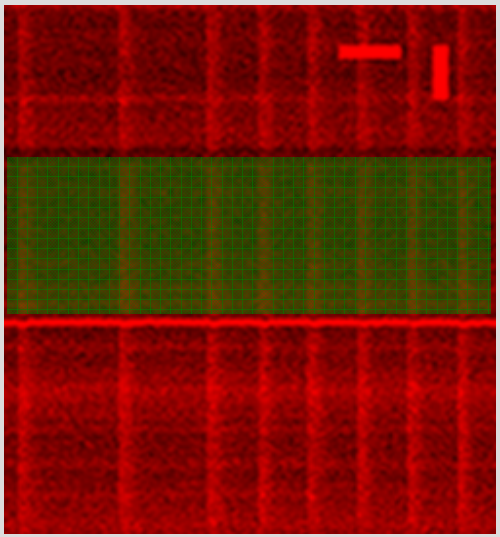


2-D View



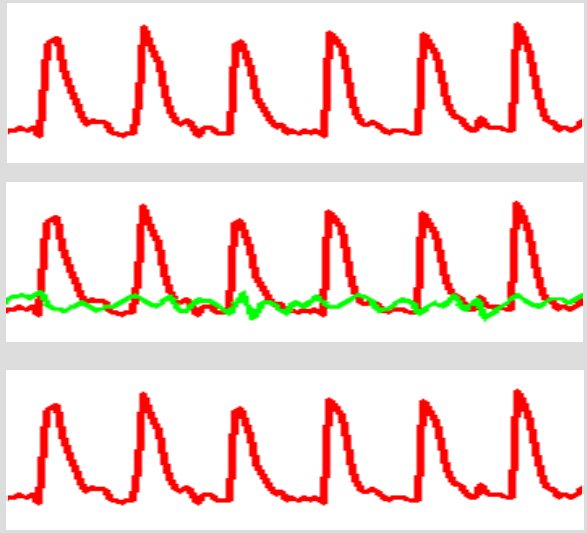
Dist.

Stacked Line Scans

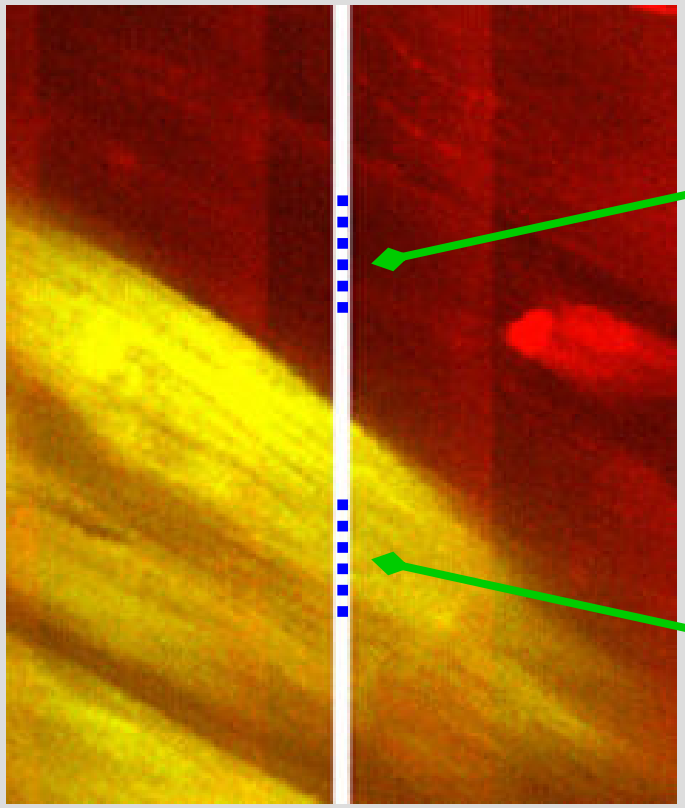


Time

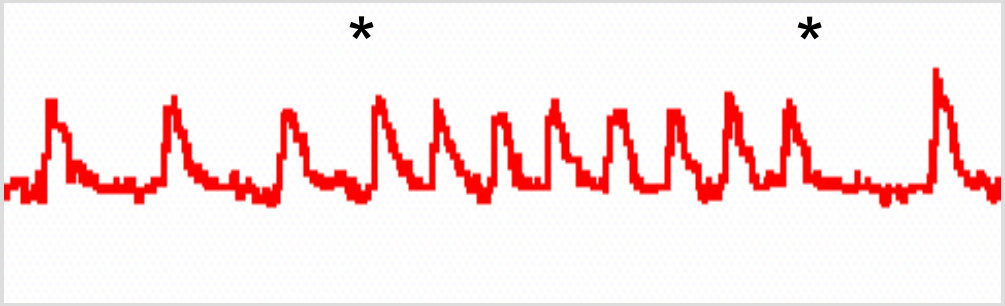
Integrated Traces



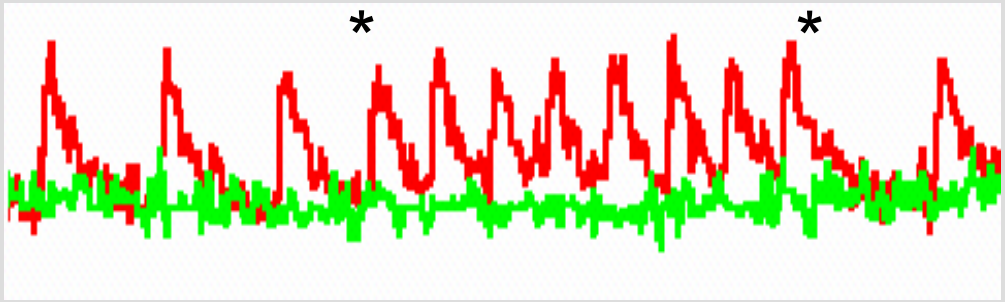
Simultaneous recording of intracellular calcium transients in neighboring donor and host CMs:



Host CM (red due to Rhod2, no EGFP)



Donor CM (red & green due to Rhod2 + EGFP)



All donor CMs transplanted into normal ventricle were observed to be coupled (>400 analyzed; distributed between >18 recipient mice)

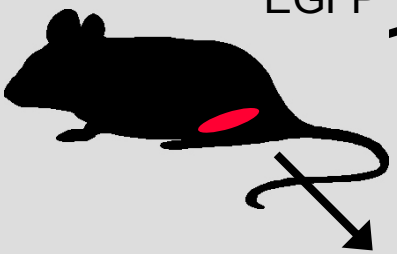
Tracking survival & function of donor SMBs:

Actin-EGFP mice

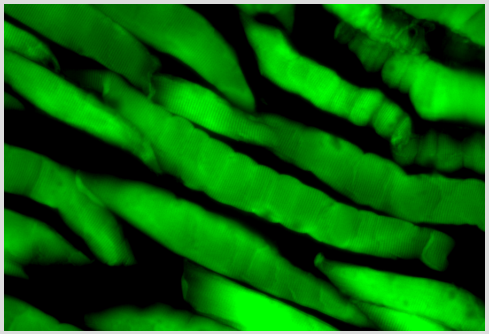
β-ACT Promoter

SV40 term.

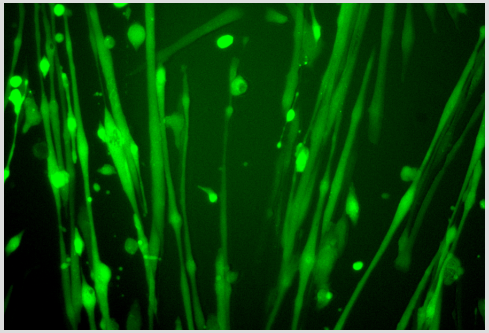
EGFP



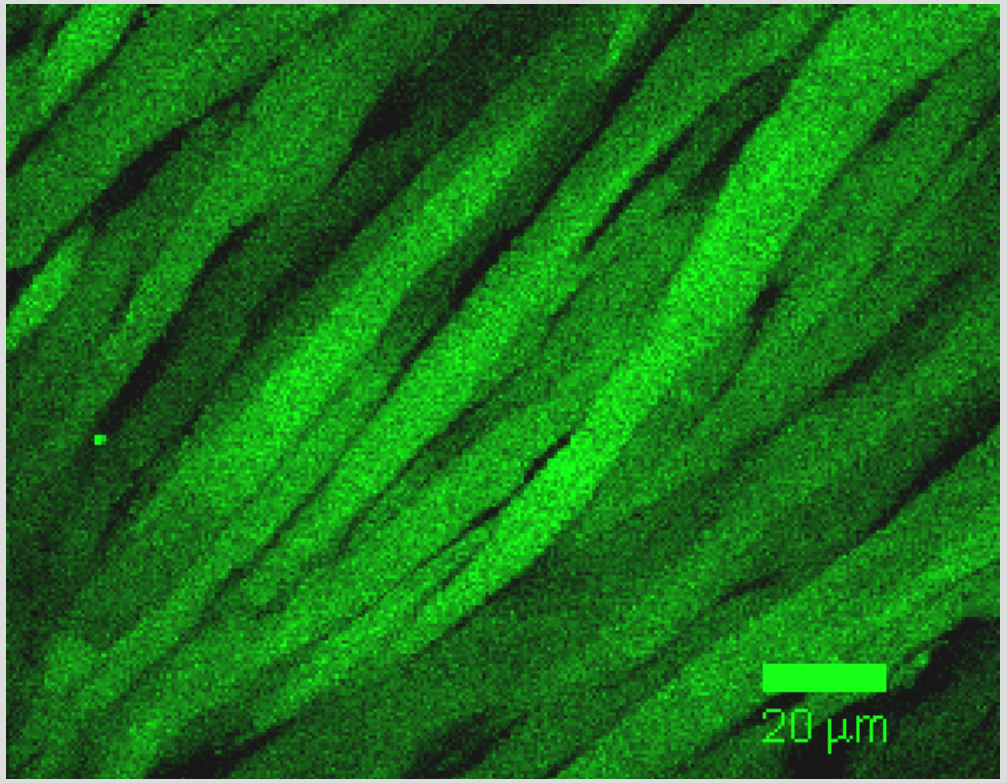
Skeletal muscle section



Cultured myotubes

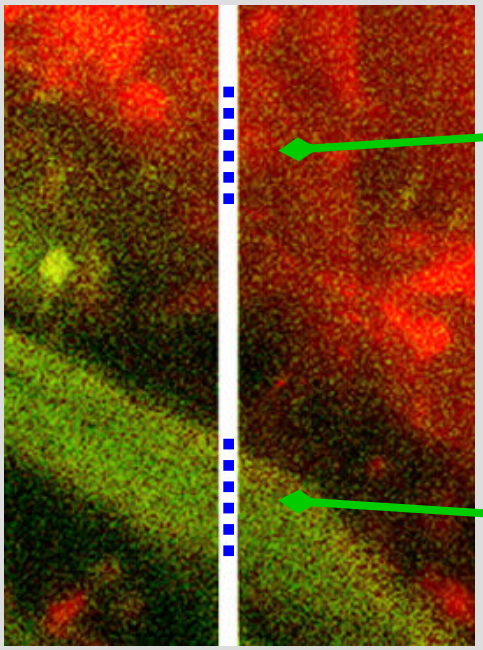


- Isolate and amplify skeletal myoblasts
- Transplant EGFP donor cells into the hearts of non-transgenic mice

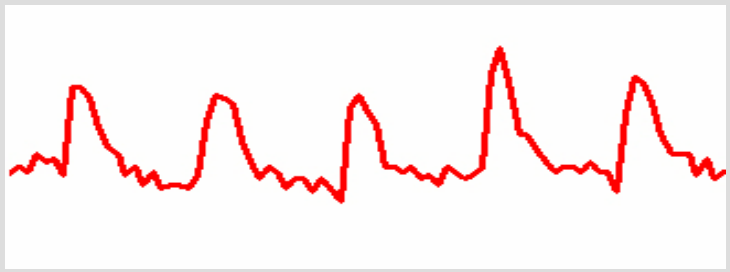


- Image for Rhod-2 and EGFP fluorescence

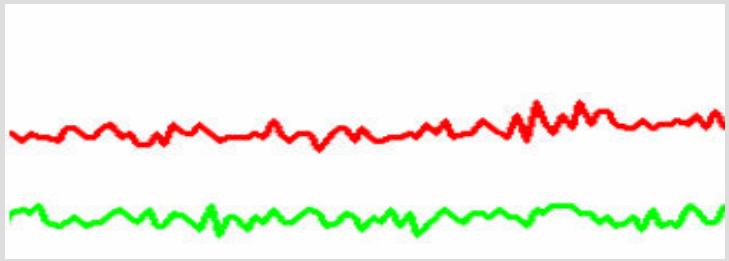
Simultaneous recording of intracellular calcium transients in neighboring host CMS and SMB-derived myocytes:



Host CM (red due to Rhod2, no EGFP)



Donor myocyte (yellow due to Rhod2 + EGFP)



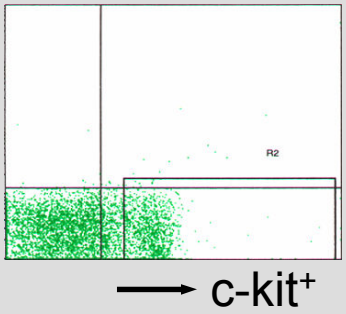
The **vast** majority of donor-derived myocytes do not couple with the host myocardium following skeletal MB transplantation (>99.9%); a few cells at the graft / myocardium border appeared to be coupled, but subsequent analyses indicated that these likely arose from CM-SMB fusion events

Tracking differentiation of donor HSCs:

MHC-nLAC mice

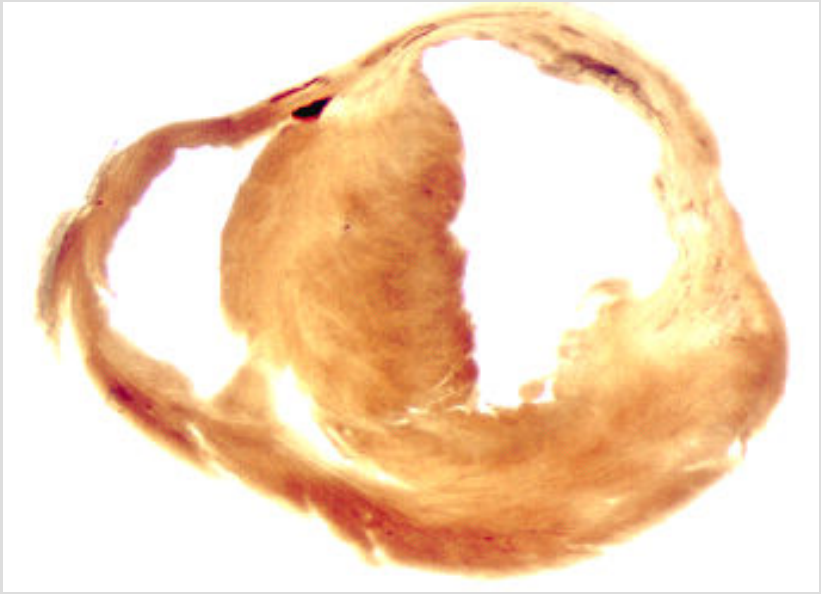
CM nuclei stain blue with X-GAL

- Prepare BM-derived HSCs



- Transplant into infarcted hearts
- Harvest heart, vibratome section and stain with X-GAL

Absence of X-GAL reaction product suggests donor cells do not differentiate into CMs



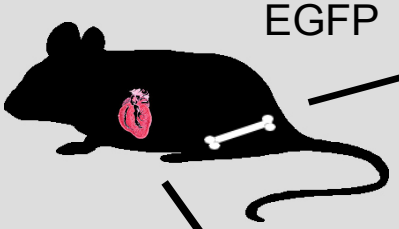
Tracking survival & function of donor HSC:

Actin-EGFP mice

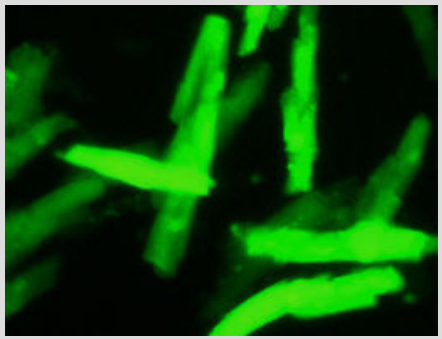
β-ACT Promoter

SV40 term.

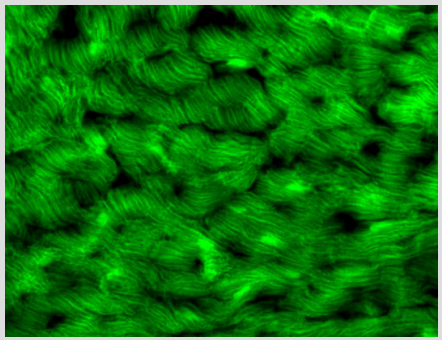
EGFP



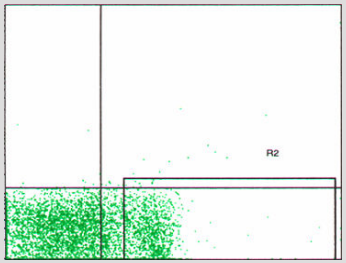
Dispersed cells (epi-fluorescence)



Adult heart section (epi-fluorescence)



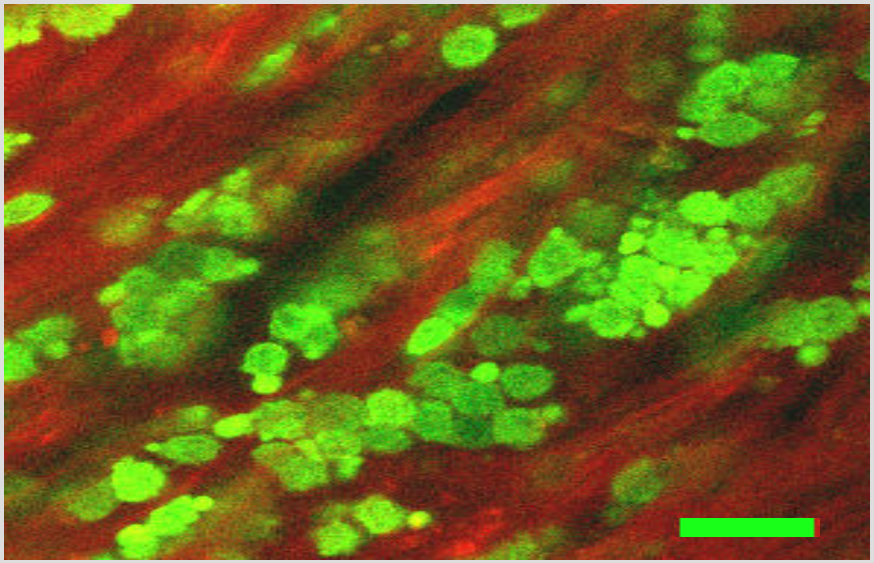
- Prepare BM-derived HSCs



→ c-kit+

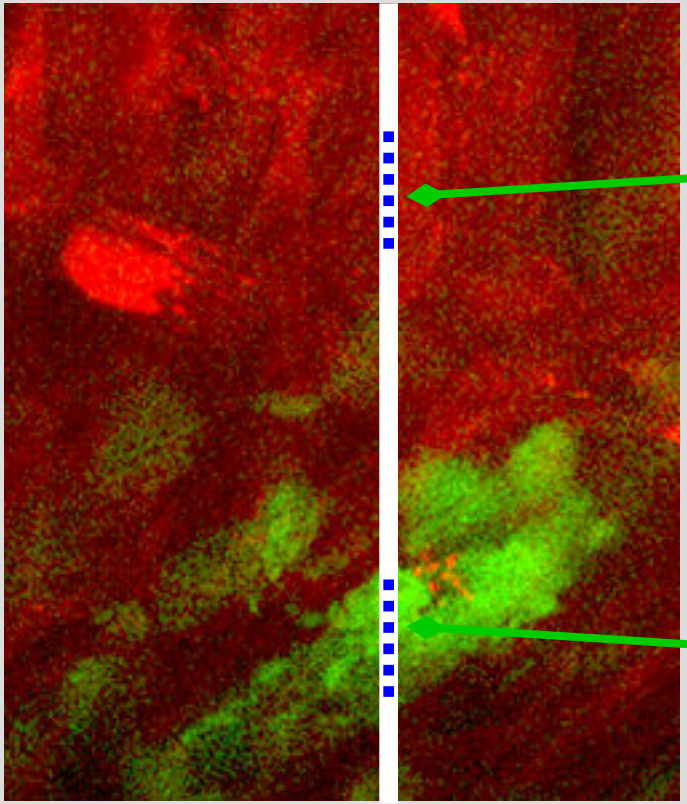
- Transplant into infarcted hearts

Donor HSC-derived cells readily identified based on EGFP epi-fluorescence

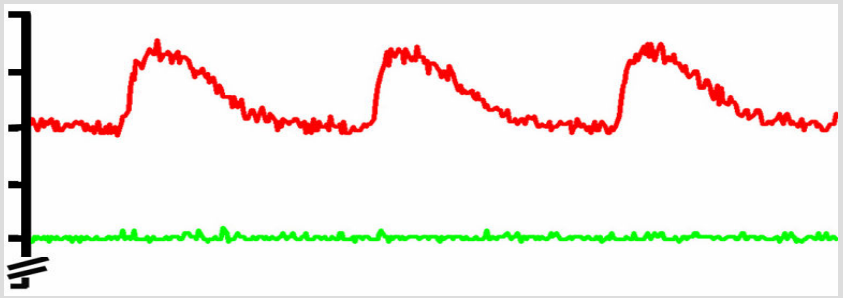


- Image for donor cell function

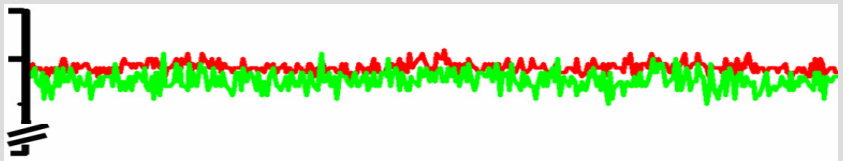
Simultaneous recording of intracellular calcium transients in neighboring host CMs and donor HSC-derived cells:



Host CM (red due to Rhod2, no EGFP)



Donor cell (red & green due to Rhod2 + EGFP)

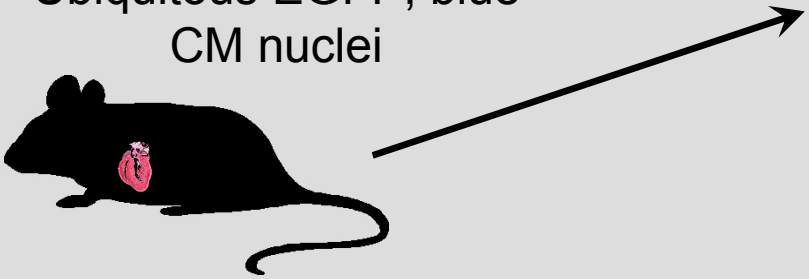


No $[Ca^{2+}]_i$ transients were observed in HSC-derived cells at 9 days post-transplantation with either point or field stimulation (>20 mice, 800 cells analyzed); similar results with crude marrow mononuclear preparations

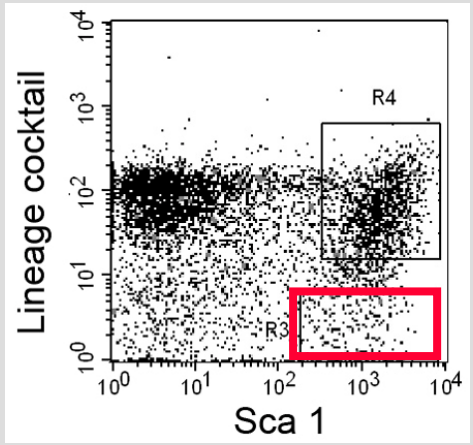
Tracking differentiation & function of donor Sca-1^{+} cells:

Actin-EGFP / MHC-nLAC double txg mice

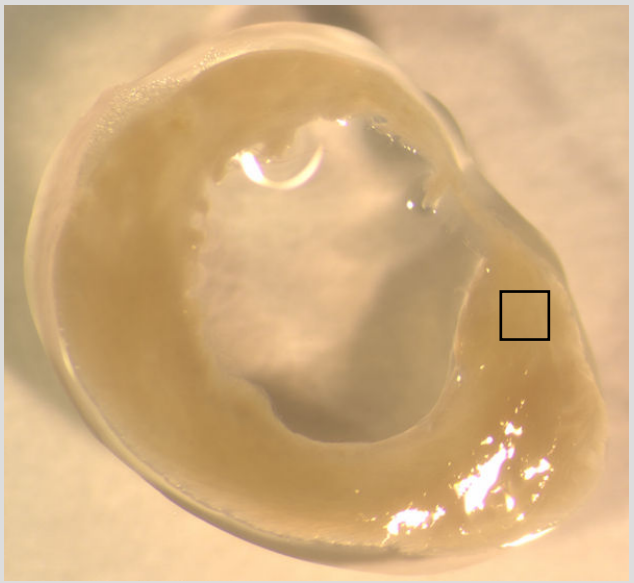
Ubiquitous EGFP, blue CM nuclei



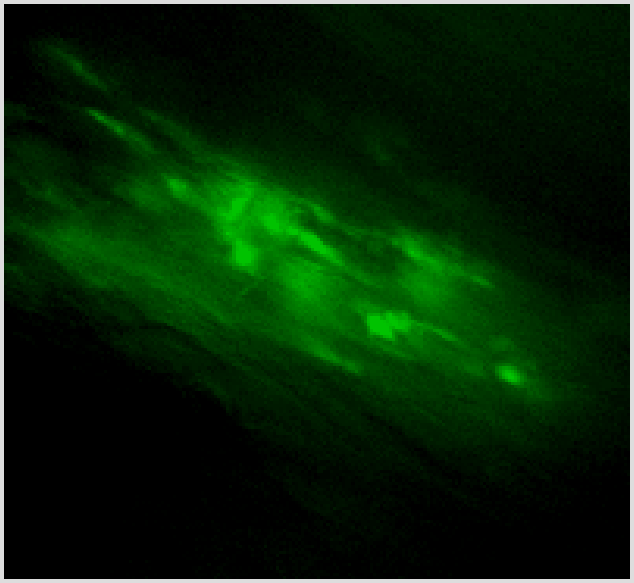
- Mince adult hearts and disperse cells
- Gate on EGFP positive, Sca-1 positive, lineage negative cells
- Transplant into infarcted hearts
- Harvest heart, X-GAL stain, visualize



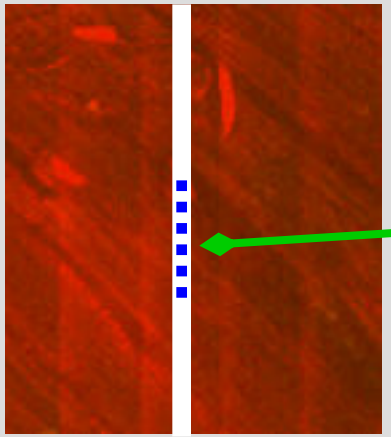
No MHC-nLAC transgene expression (X-GAL stained vibratome section)



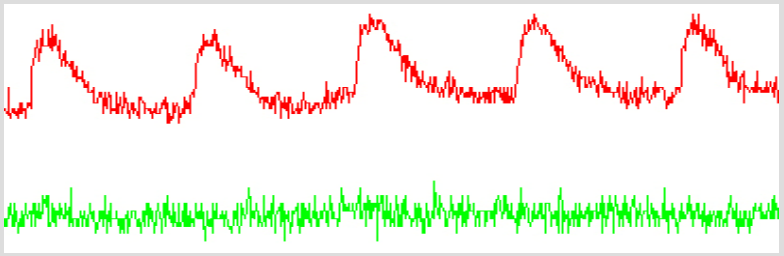
Donor HSCs readily identified via EGFP epi-fluorescence

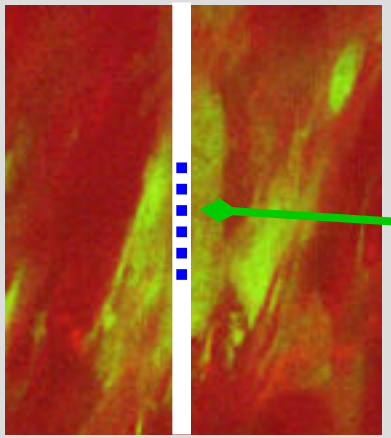


Records of intracellular calcium transients in host CMs and donor Sca-1^{+}-derived cells (field stimulation):

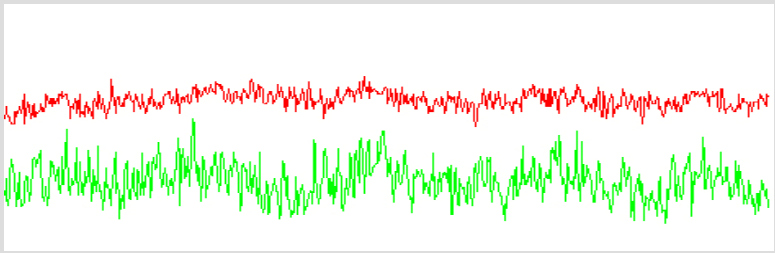


Host CM (red due to Rhod2, no EGFP)





Donor cell (red & green due to Rhod2 + EGFP)

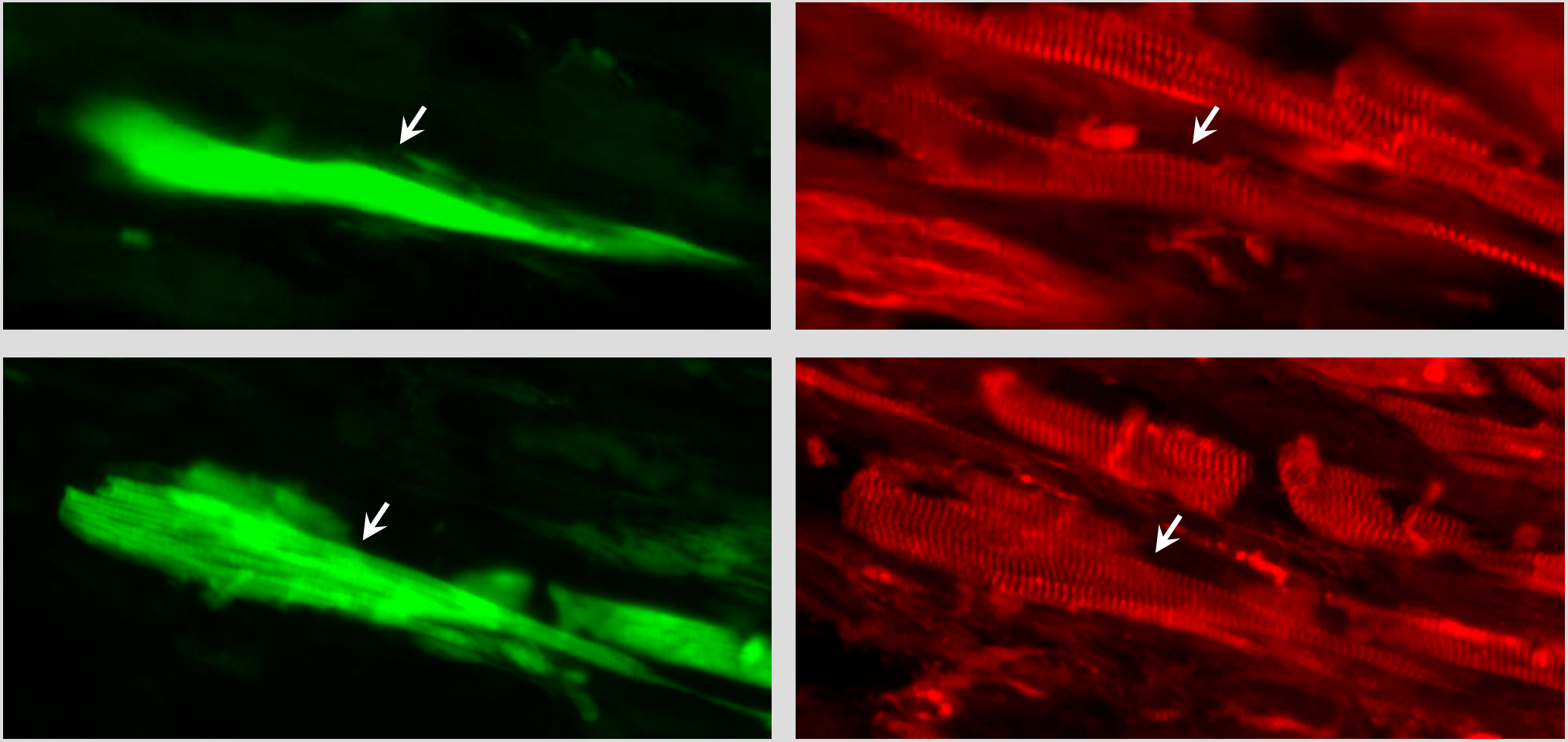


No $[Ca^{2+}]_i$ transients were observed in Sca-1^{+}-derived cells between 9 and 21 days post-transplantation (5 mice, 370 cells imaged), however…

…some EGFP^{+} cells exhibited a “CM-like” morphology:

EGFP epi-fluorescence

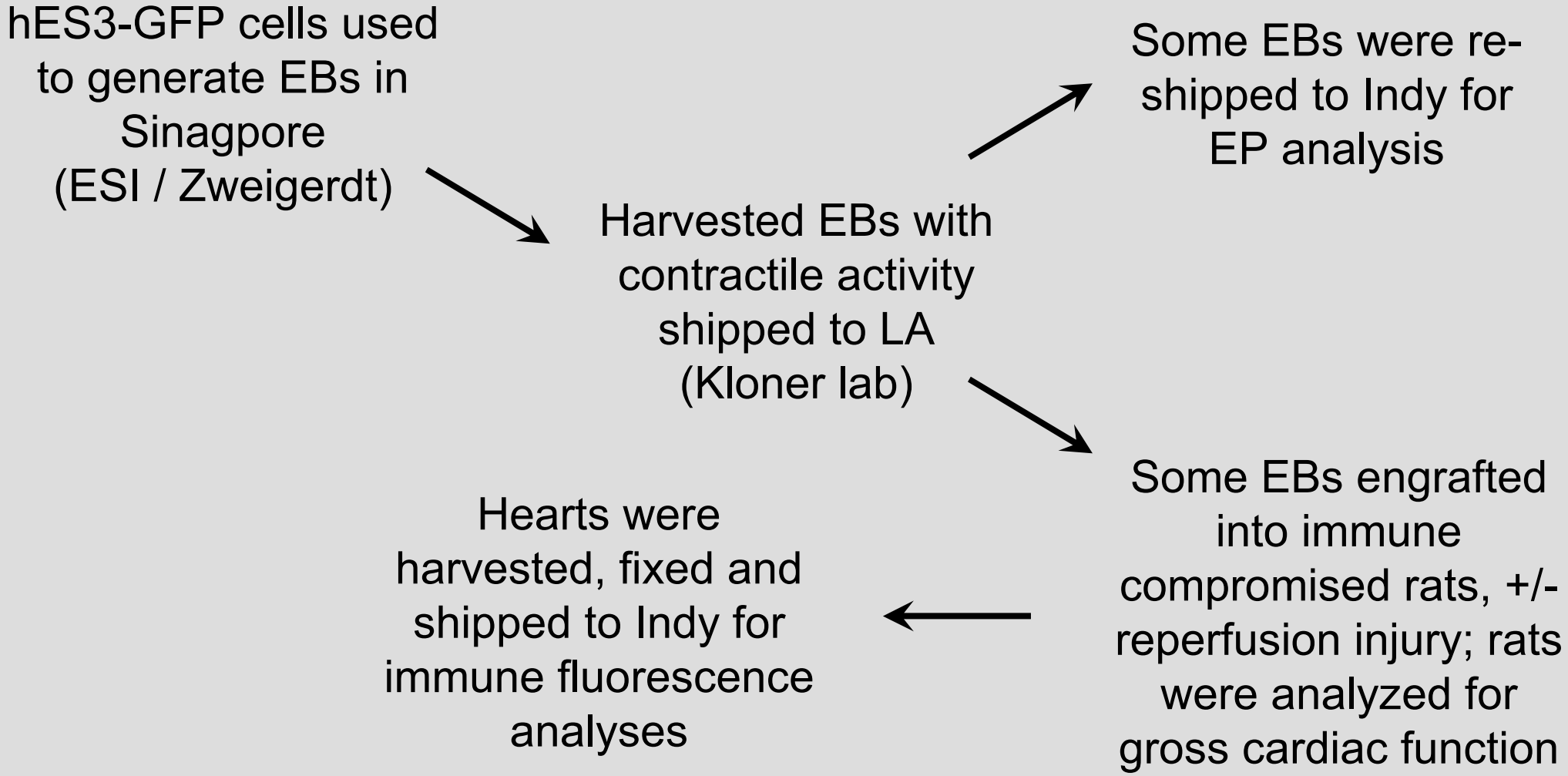
Anti-α-actinin IgG (rhodamine)



The appearance of such cells is very rare (only 3 of ca. 5,000 EGFP^{+} cells screened); absence of X-GAL signal suggests either a failure in terminal differentiation or fusion with subsequent nuclear reprogramming …

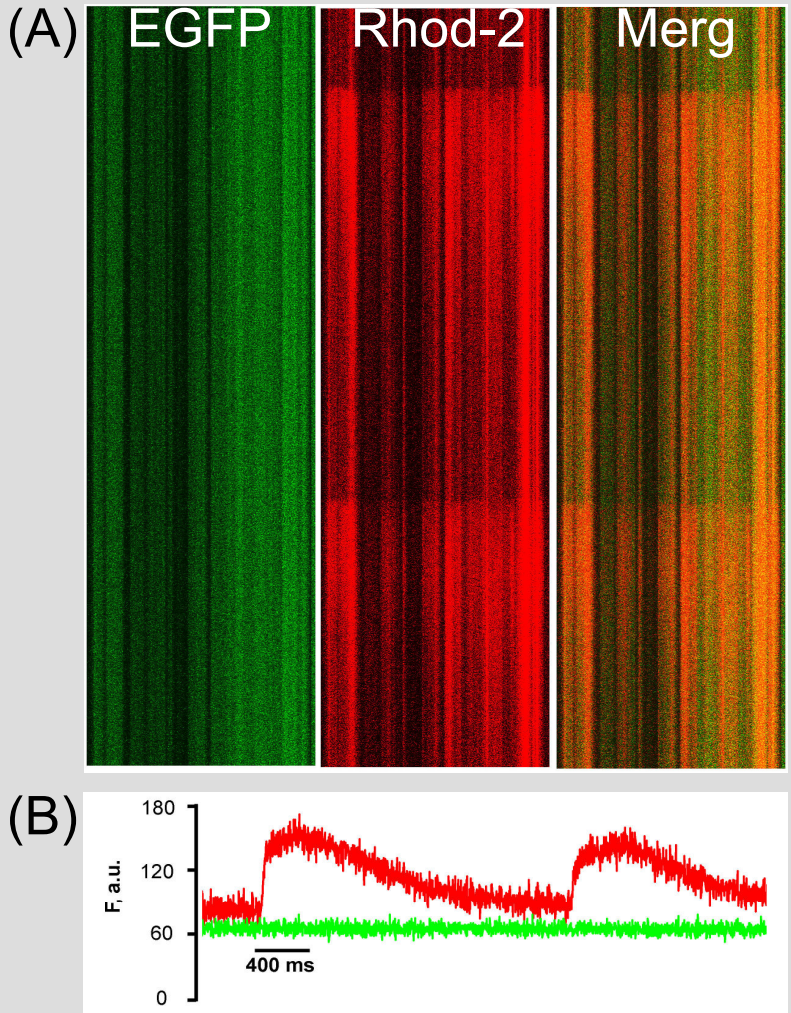
Preliminary experiments with human ES-derived CMs:

Our goal was to see if CMs generated at a remote site could be shipped and successfully engrafted into normal or injured myocardium

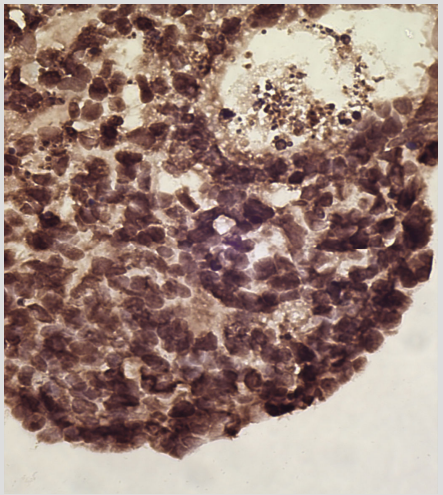


“Shipped” hEBs have spontaneous contractile activity:

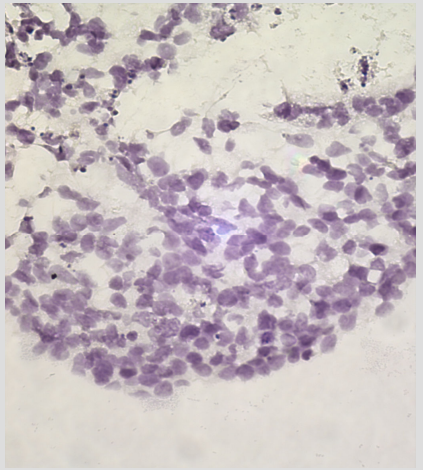
Spontaneous $[Ca^{2+}]_i$ transients (A) and integrated trace (B) in hEBs



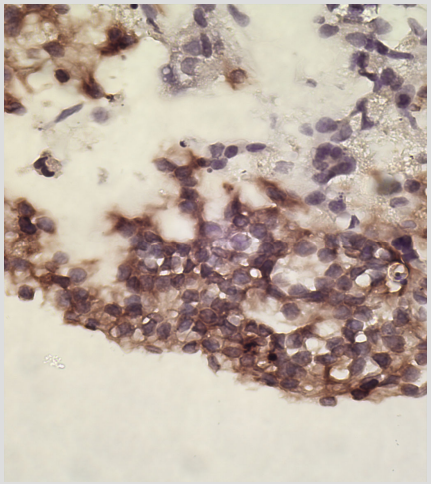
Anti-EGFP IgG



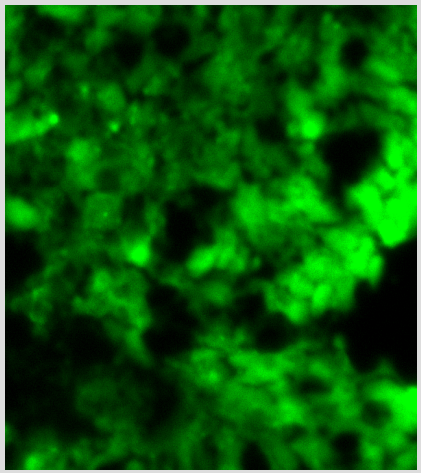
Non-specific IgG



Anti-MHC IgG

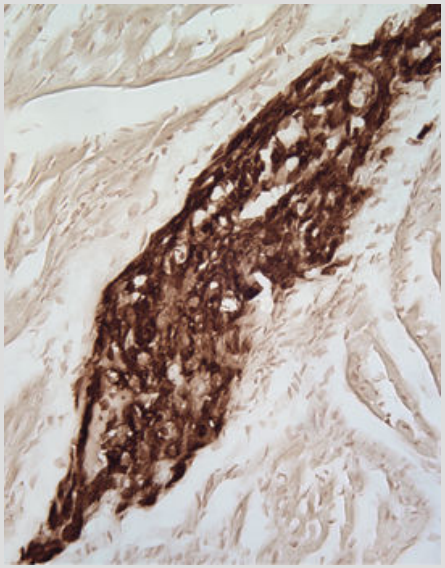


EGFP epi-fluor.

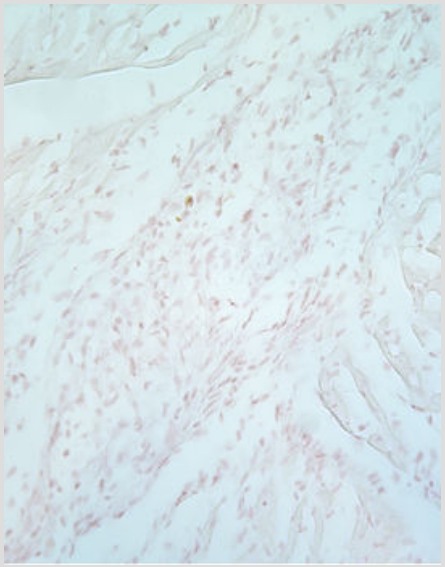


Shipped hES-derived CMs form stable grafts in vivo:

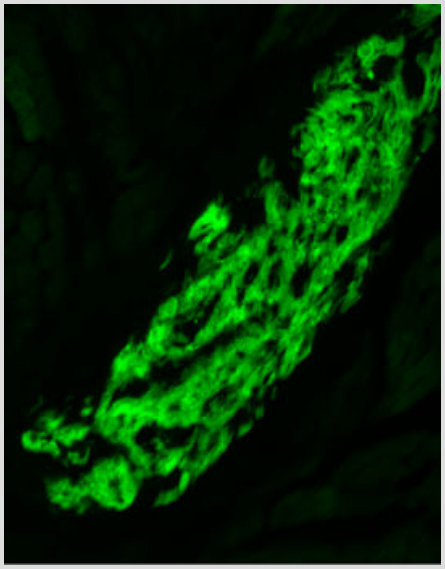
Anti-GFP IgG (HRP secondary)



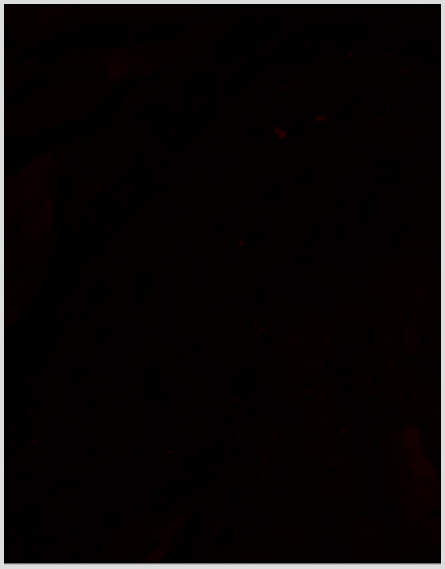
Non-specific IgG (HRP secondary)



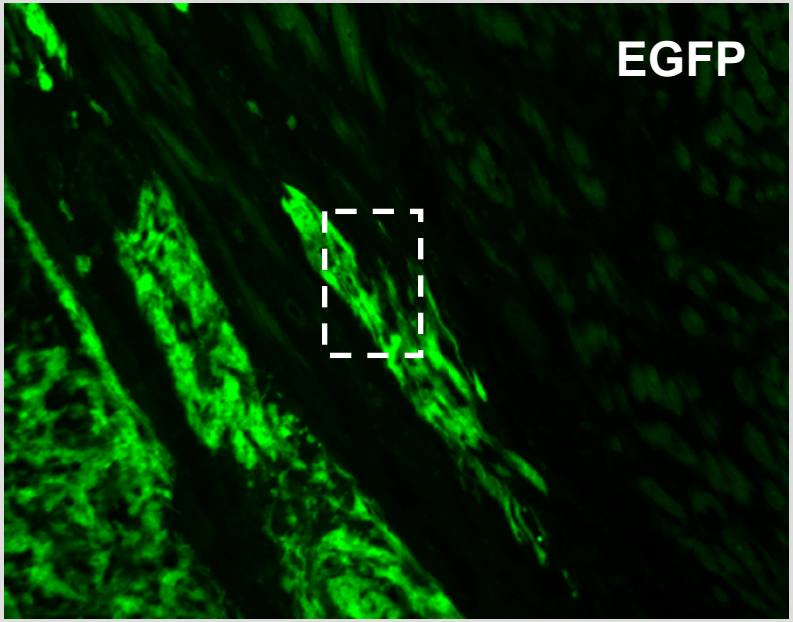
EGFP epi-fluorescence

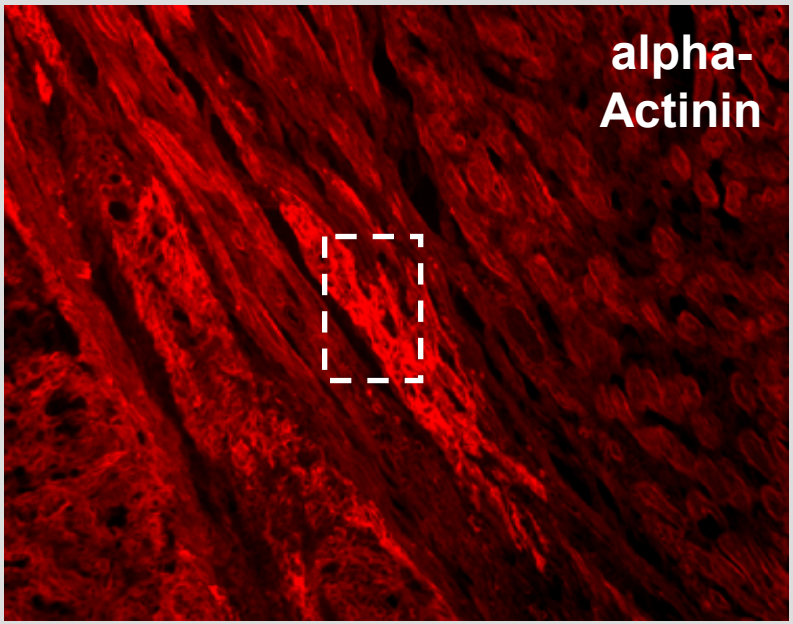


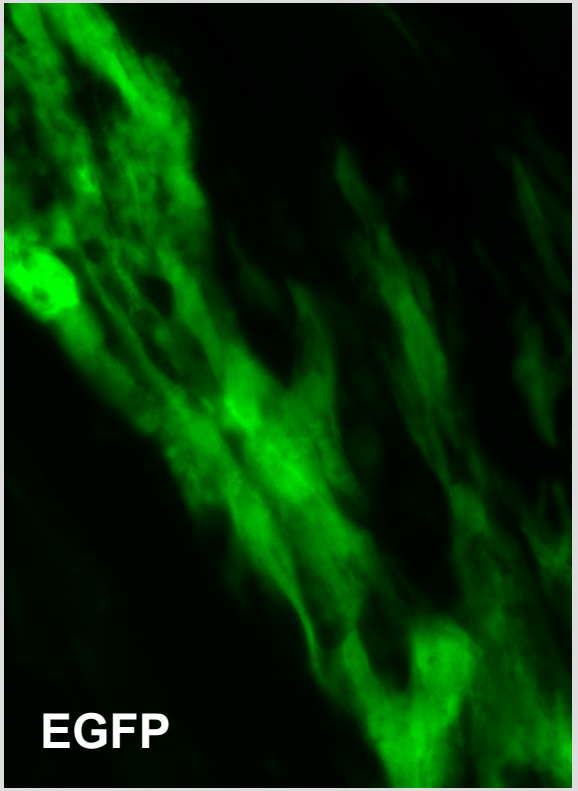
Rhodamine channel Epi-fluorescence

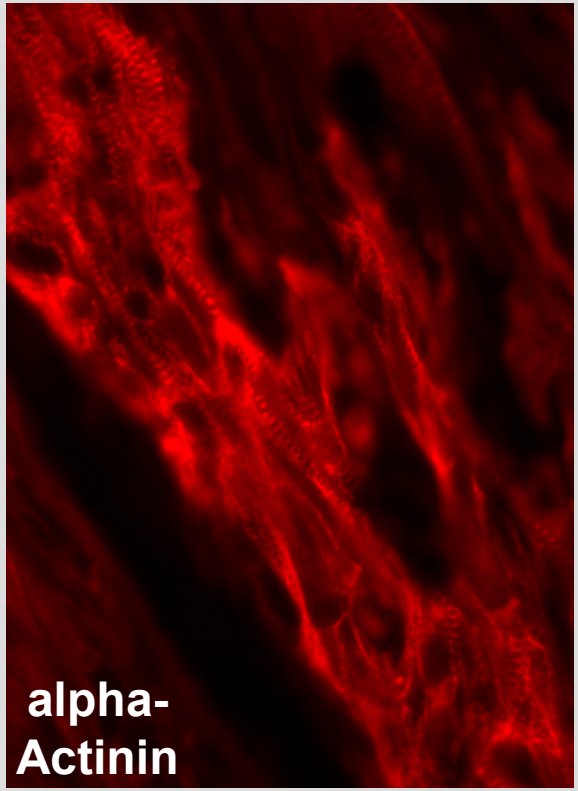


CM differentiation at 4 weeks post-transplantation:









- 6 of 6 non-infarcted hearts had hES cells
- IgG analyses of infarcted hearts is in progress; there appears to be poorer CM survival when injected into scar
- Bob Kloner's data suggest modest functional improvement (direct vs. indirect effect?)

Summary / take home messages:

- Fetal CMs form stable grafts and functionally integrate with the host myocardium
- Skeletal myoblasts form stable grafts but are not electromechanically coupled to the host myocardium; rare fusion events give rise to myocytes with heterogeneous $[Ca^{2+}]_i$ (source of clinical arrhythmias?)
- Lin^- / $cKit^+$ BMSCs or marrow-derived mononuclear cells form stable grafts but to not form CMs; any clinical benefit from these cells is not due to their ability to directly form new muscle
- Cardiac resident Sca-1+ cells form stable grafts, but the majority of cells do not form cardiomyocytes and are not electromechanically coupled; studies are underway to determine if the few CM-like cells observed arise from fusion events or from cardiomyogenic differentiation
- Human ES-derived CMs can form stable grafts following transplantation into normal and infarcted hearts; preliminary coupling analyses look promising

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Pascal LaFontant, Ph.D.

TPME imaging & analyses:

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Ed Srour, Ph.D. (MED, IUSM)

Human ES cell experiments:

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Robert Zweigerdt Ph.D. (ESI)

2007 Weinstein Cardiovascular Development Conference

Indianapolis, Indiana

Union Station Crowne Plaza Hotel
May 10th – 12th 2007

Keynote speakers:
Drs. Oliver Smithies and Peggy Kirby

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& stay an extra night to see 'Indy 500 time-trials'