

Cell transplantation for myocardial regeneration: myoblast approach

Tomasz Siminiak MD, PhD, FESC, FACC



**Poznan University School of Medical Sciences,
Cardiac and Rehabilitation Hospital
Poznan & Kowanowko, Poland**

**Disclosure statement:
nothing to declare**



Problem:

Damaged tissue or organ

Solution:

Replacement by artificial organ



Helpful, but not ideal...

Problem:

Damaged tissue or organ

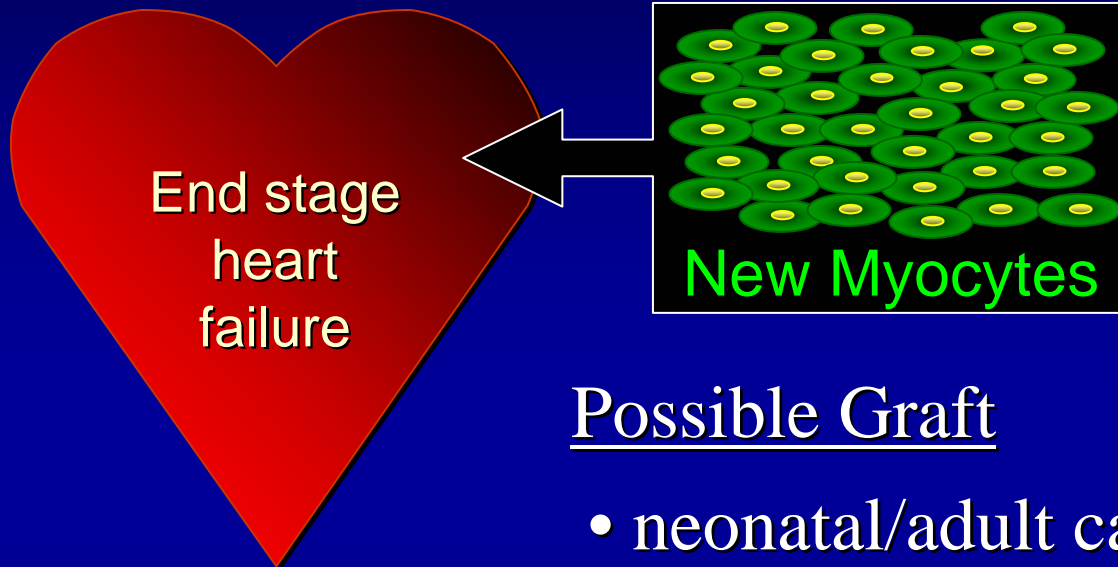
Solution:

Replacement by new tissue



Post MI myocardial injury

Possible sources of cells

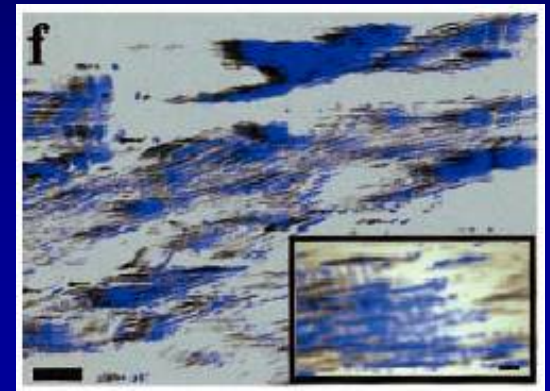
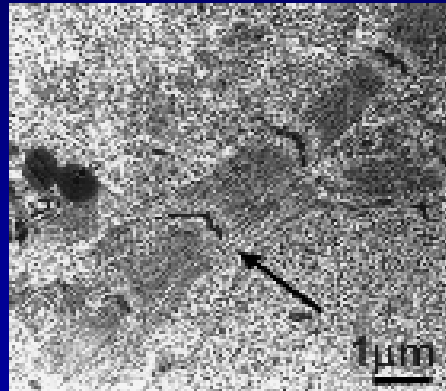
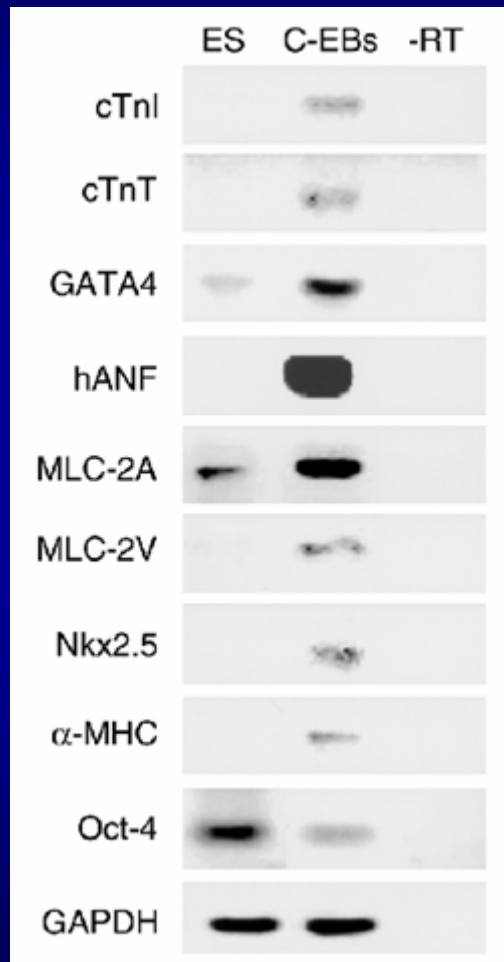


Possible Graft

- neonatal/adult cardiomyocyte
- embryonic stem cell
- bone marrow stem cell
- skeletal myoblast
- other

fibroblast \pm genetic engineering
cardiac stem cells

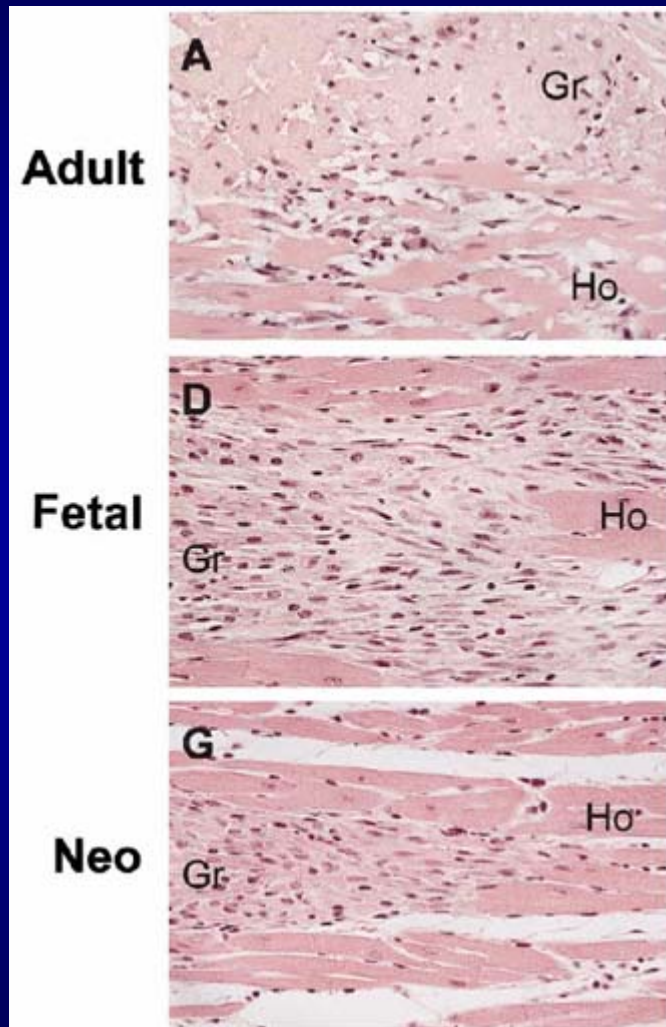
Cell sources: **Embryonic stem cell**



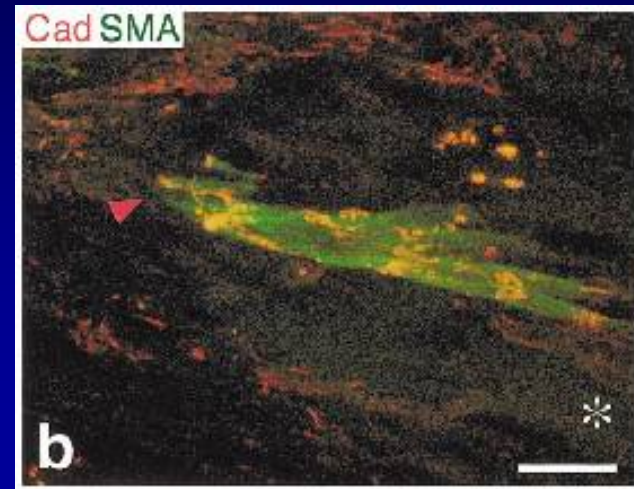
Klug MG / Field LJ et al. J Clin Invest 1996

- Possible differentiation in vitro
- **Ethical issue**
- Tumor induction??
- Control of differentiation??

Cell sources: **cardiomyocytes**



*Reinecke H/Murry CE et al.
Circulation 1999*

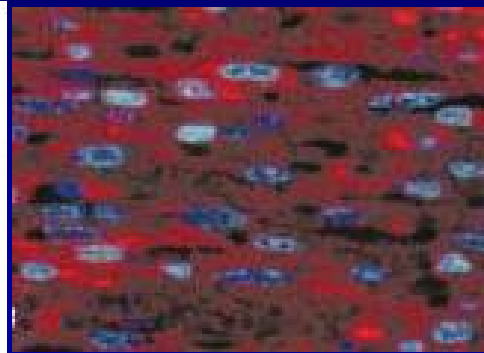
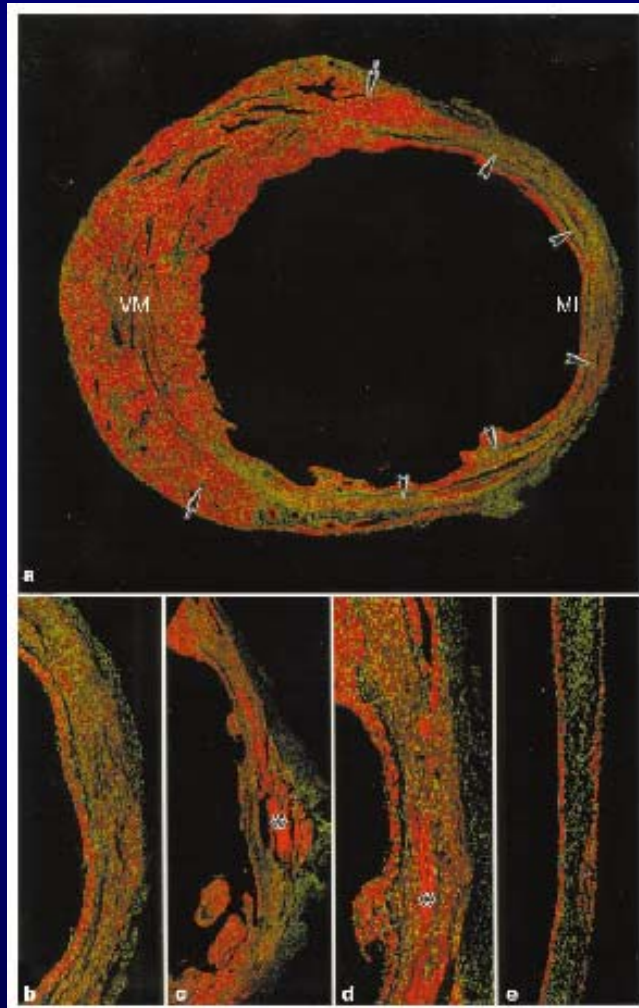


Matsushita T et al. Circulation 1999

- Possible integration
- Cardiac function↑
- Difficult availability
(ethical issues)
- Poor resistance

Cell sources: **Bone marrow stem cells**

Selection of Lin⁻, c-kit⁺ bone marrow cells

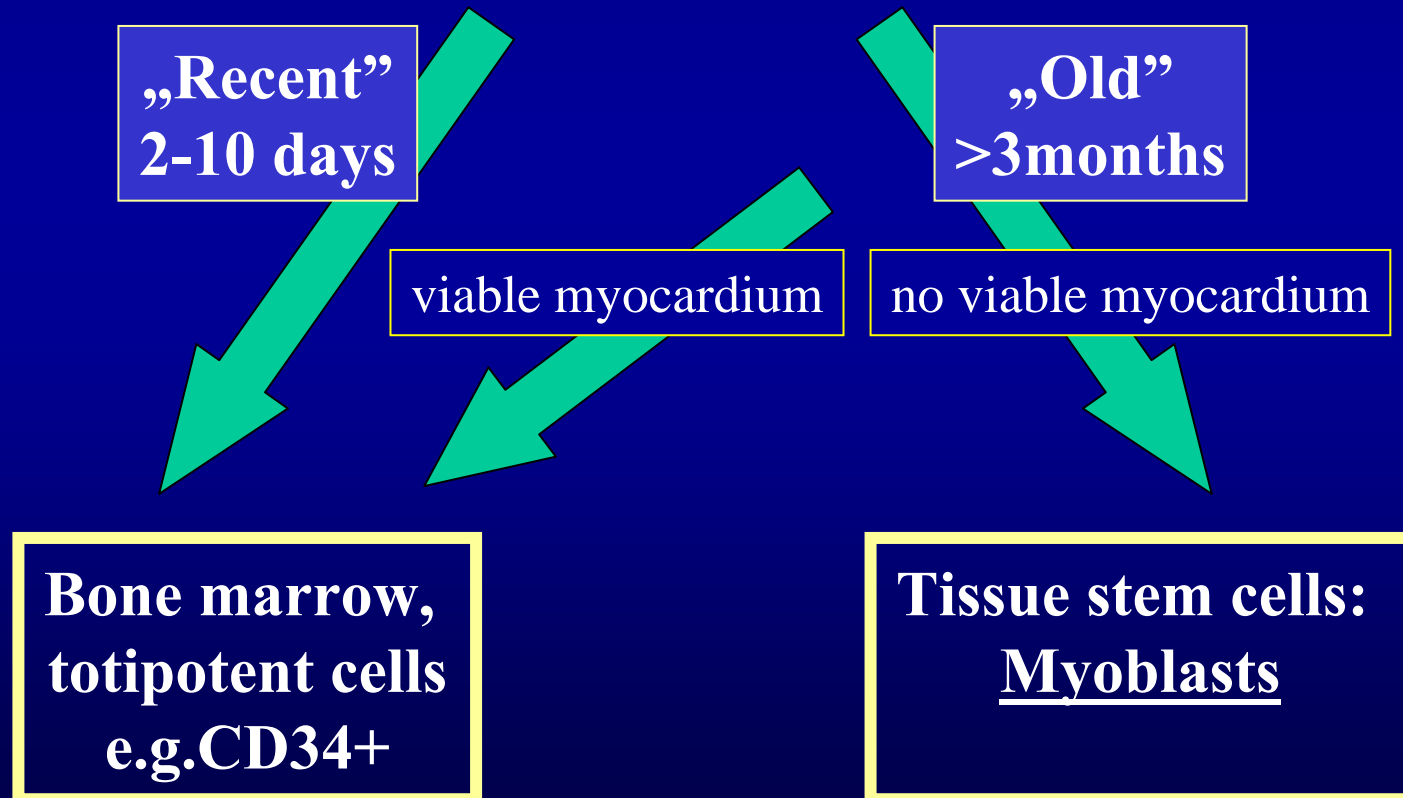


Orlic/Anversa et al. Nature 2001

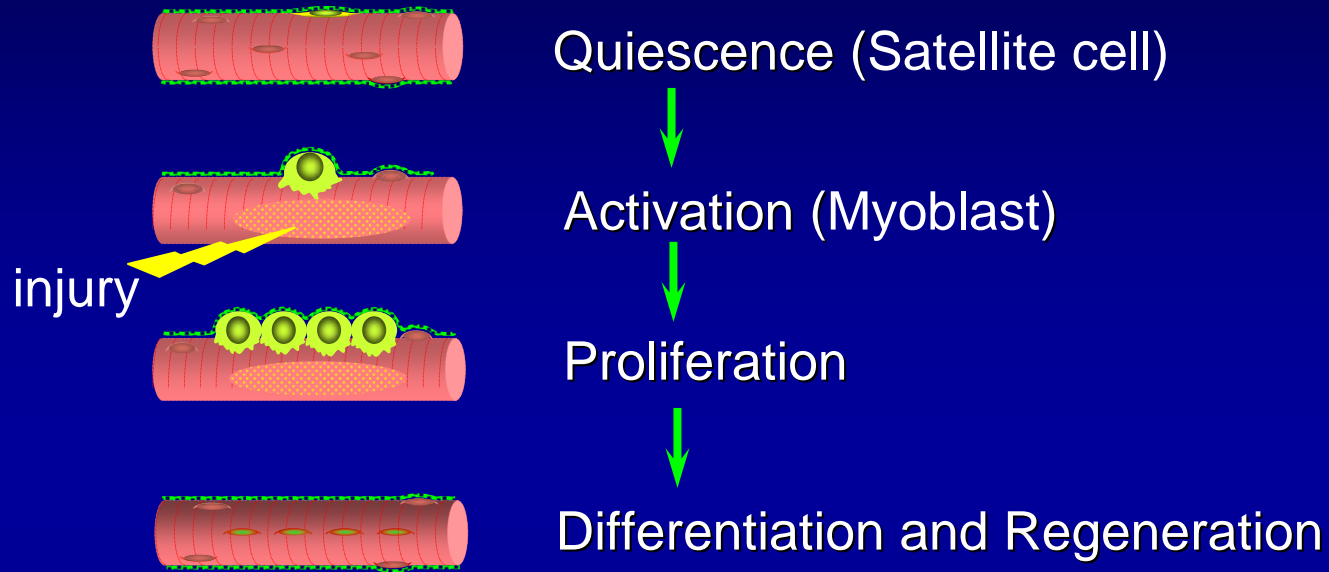
- Cardiac function ↑
- autograft → **human trials**
- Selection method?
- **Differentiation within fibrotic scar**

Current clinical trials on cell transplantation for myocardial regeneration

Postmyocardial injury



Cell sources: **Skeletal myoblasts**

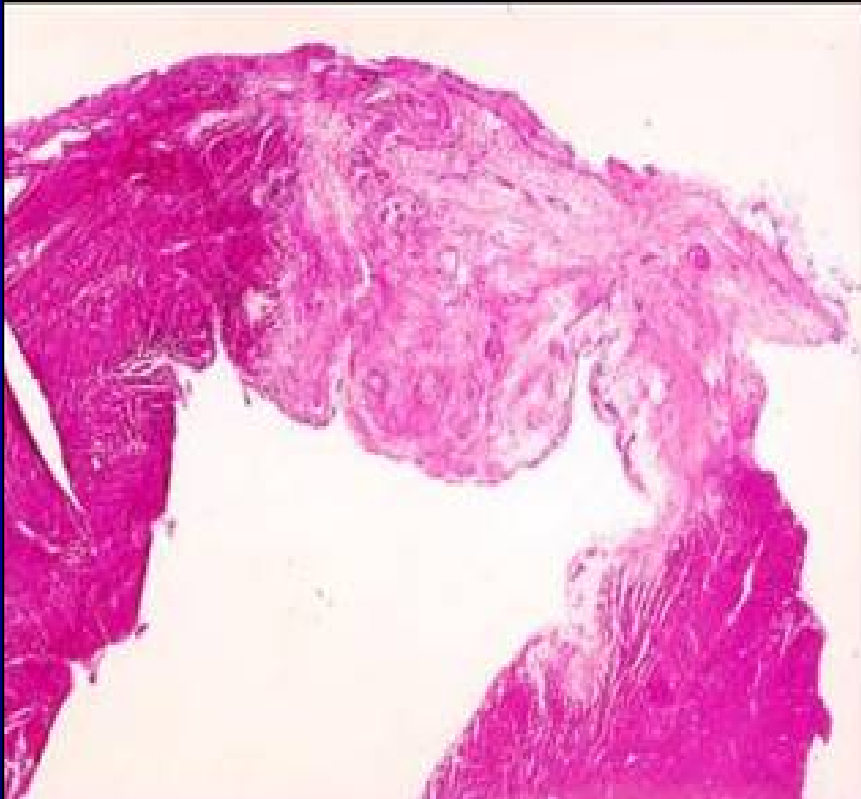


survive, proliferate and differentiate to form myotubes within the heart

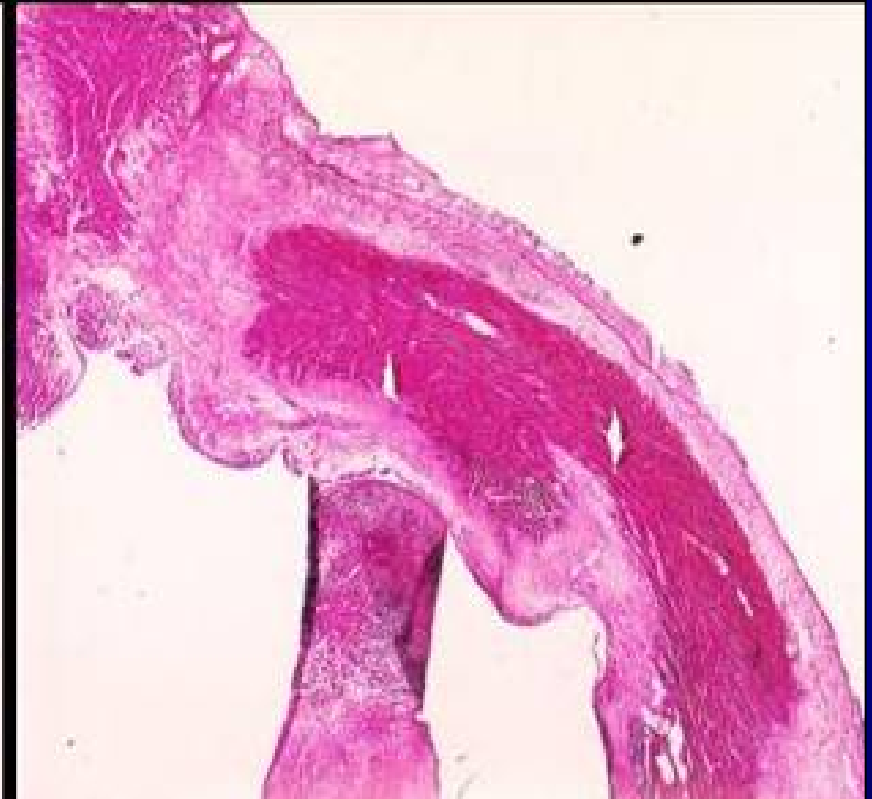


● **improve cardiac function** in experimental heart failure

Skeletal myoblasts transplantation in animal model - rats

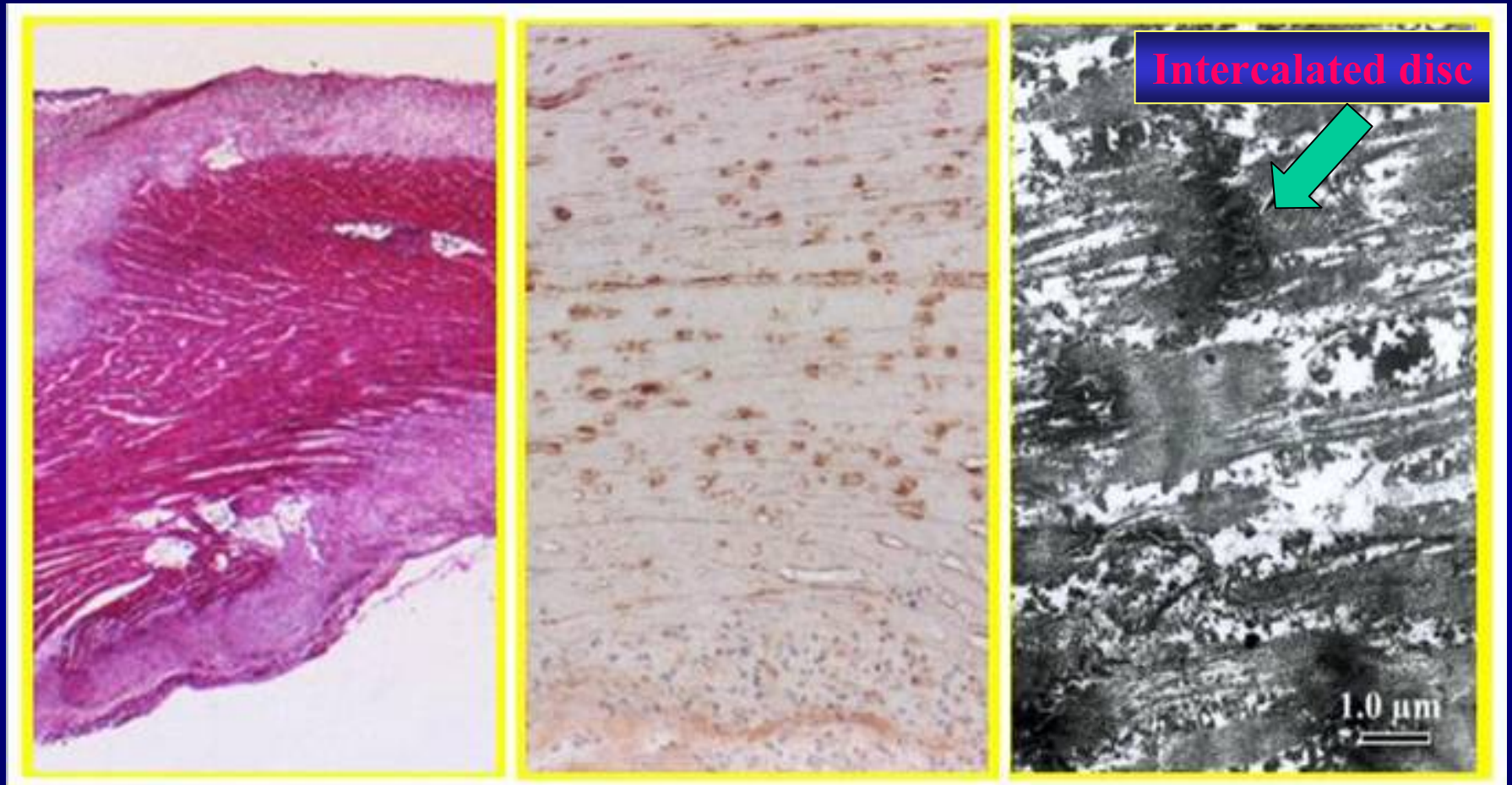


Infarction



After transplantation

Histology of transplanted myoblasts

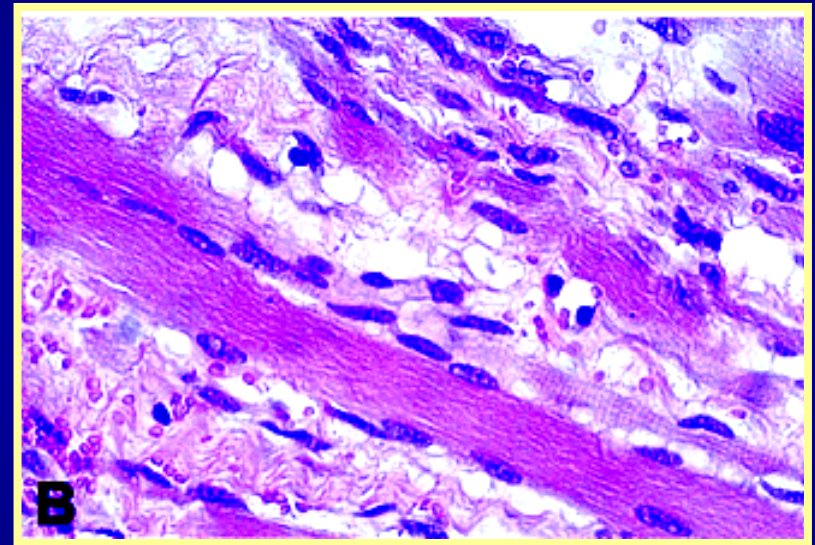
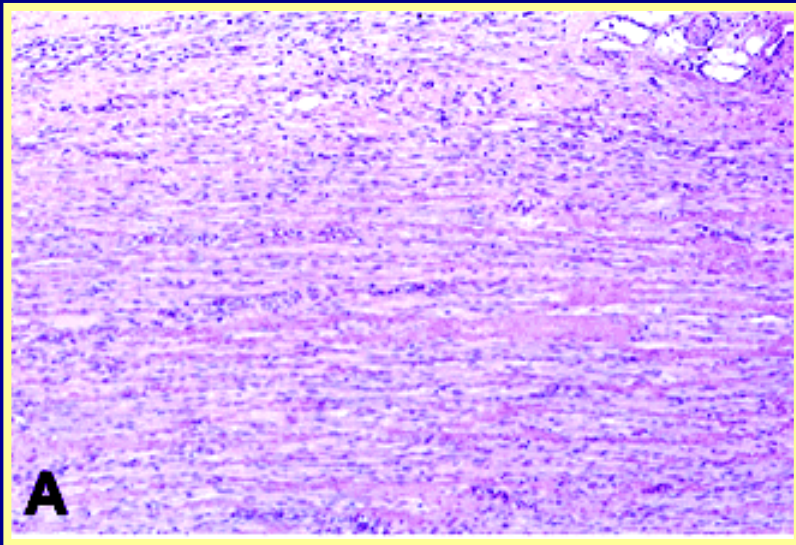


**H+E
staining**

**Myogenin
staining**

**Electron
microscope**

Autologous skeletal myoblasts transplantation in animal model - sheep



H+E staining 4 months after transplantation

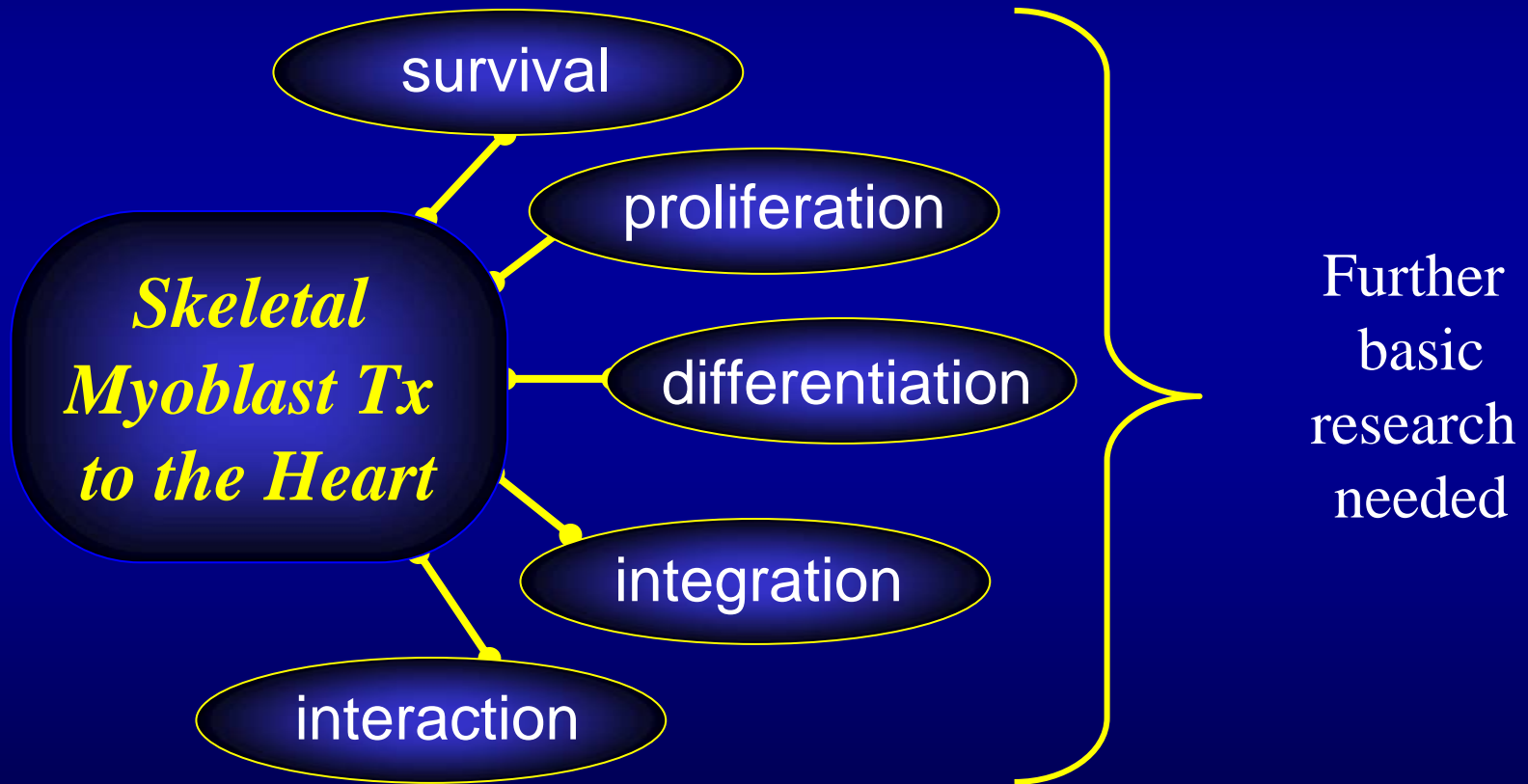
A. Magnification 100x

B. Magnification 800x

Albert A. Hagège, Jean-Thomas Vilquin, Patrick Bruneval, and Philippe Menasché
Hypertension 2001 38: 1413-1415

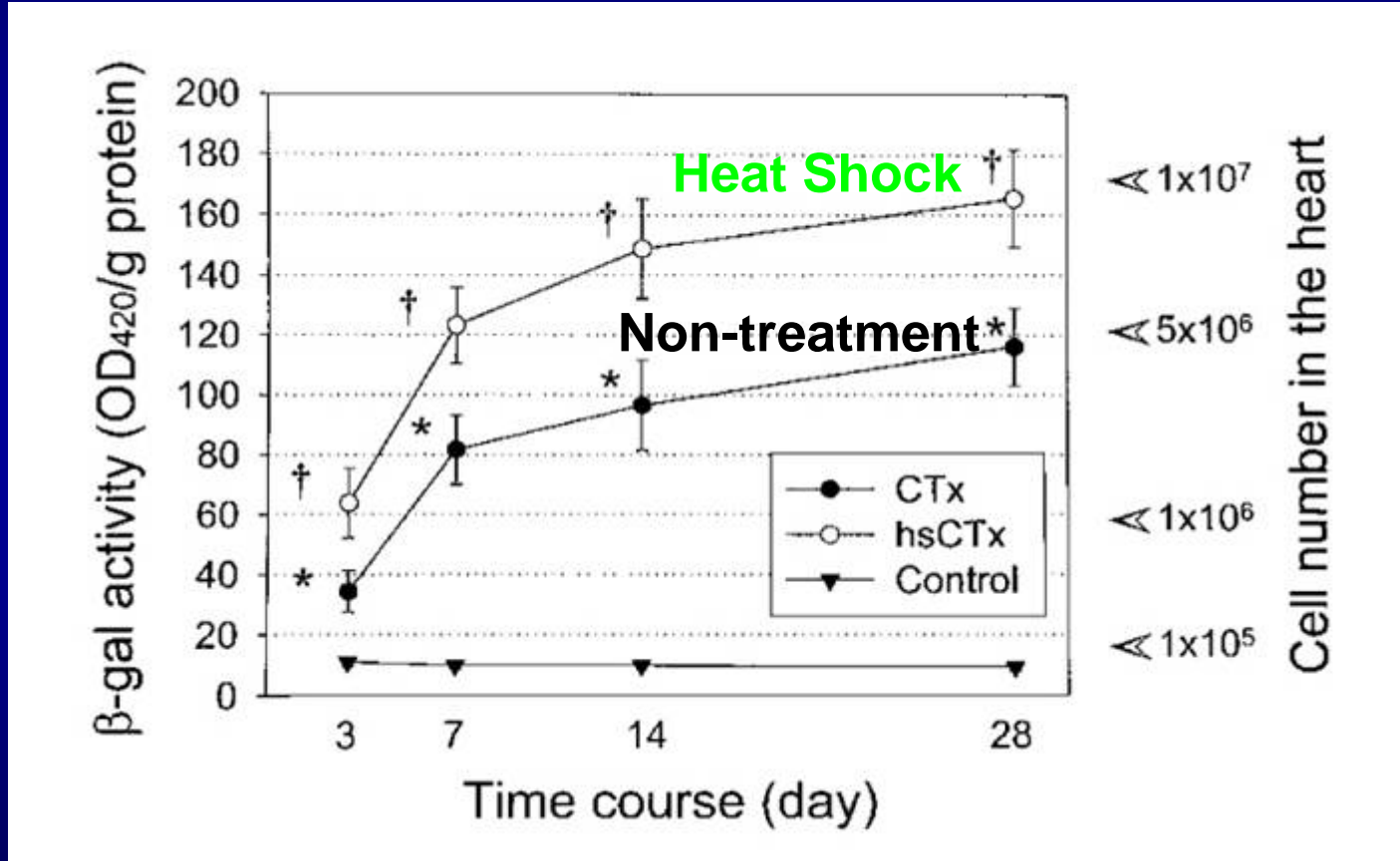
Issues of cell transplantation

Unknown mechanisms & uncertain graft quality



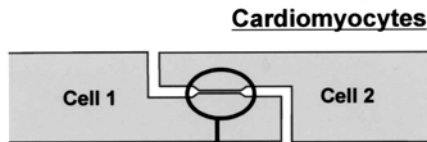
Issues of SMb Transplantation; **Graft survival**

Heat shock improves myoblast survival *in vivo*

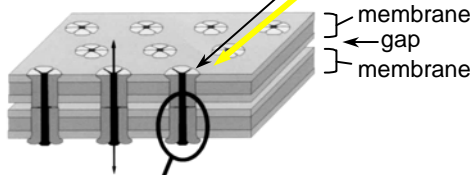


Issues of SMb Transplantation; Graft integration

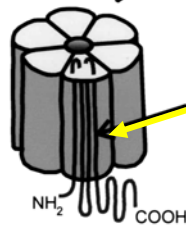
Intercellular communication



Gap junction



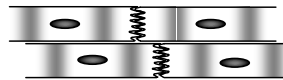
Connexin



Connexon

Cx43

Cardiomyocyte



- single nuclei (central)
- gap junction (+)
- Cx43 expression (+)

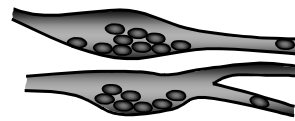
Skeletal muscle



- multinucleated (peripheral)
- gap junction (-)
- Cx43 expression (-)

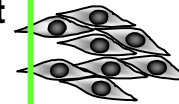
??

Myotube



- Multinucleated
- gap junction (-)
- Cx43 expression (-)

Myoblast (satellite cell)



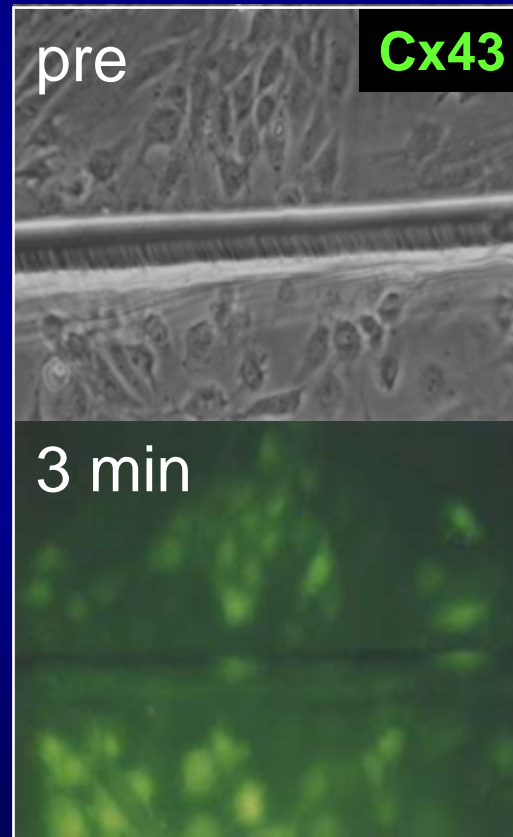
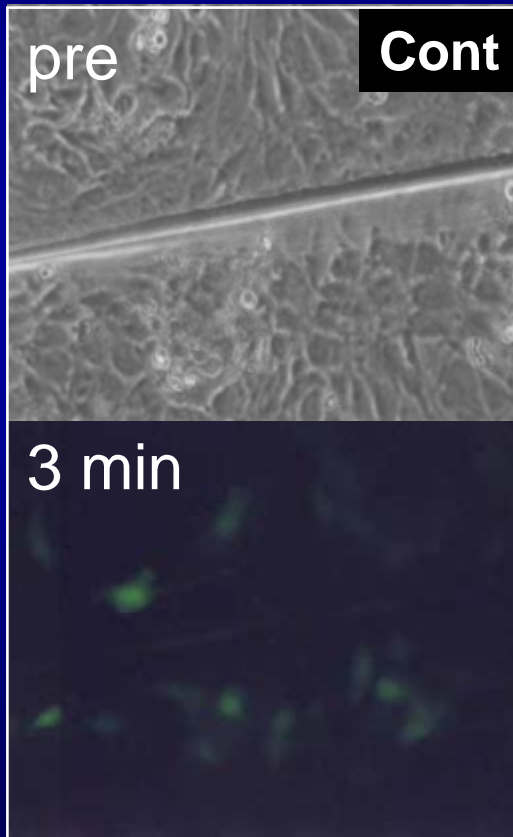
- Single nucleus
- gap junction (+)
- Cx43 expression (+)
- proliferation (+)

fusion & differentiation

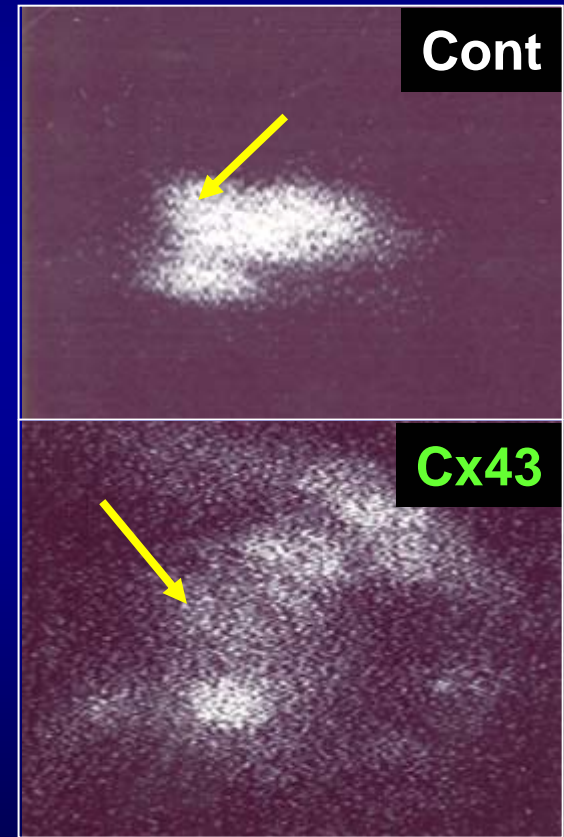
Issues of SMb Transplantation; **Graft integration**

Enhanced GJIC by Cx43-overexpression

Scrape loading



Micro-dye injection



Issues of SMb Transplantation; Cell-based gene therapy

Skeletal myoblast Tx

Functional benefit by
regenerating myofibers

Heart
failure



Gene Therapy

Effects by protein overexpression/suppression
to grafted cell

Cytoprotection ↑, Gap junction ↑, Differentiation ↑

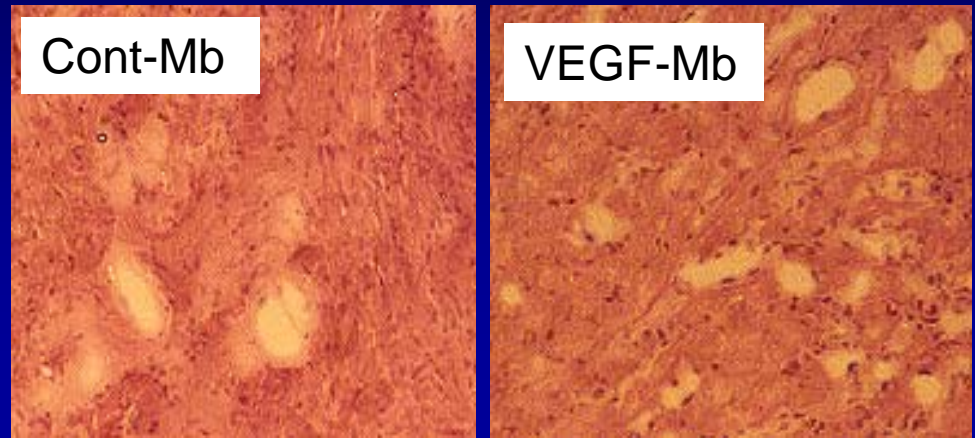
to myocardium

Angiogenesis ↑, remodelling ↓, Contraction ↑

Additional and
enhanced effects

Issues of SMb Transplantation; Cell-based gene therapy

Transplantation
of VEGF-
overexpressing
myoblast



	Mortality (%)	Infarct (%)	Heart rate (bpm)	Max dP/dt (mmHg/s)	Min dP/dt (mmHg/s)	CF (ml/min)
Medium	25.5	43.7±1.6	252.4±12.5	3482.5±90.6	-2124.0±57.9	10.1±0.3
Control-Cell	21.2	38.1±1.4*	242.8±13.0	3772.5±101.1*	-2311.3±57.0*	11.2±0.4*
VEGF-Cell	10.4 ^Δ	33.3±1.4 ^Δ	257.8±11.8	4072.0±113.6 ^Δ	-2504.2±68.5 ^Δ	12.3±0.4 ^Δ

Issues of cell Transplantation: **Cell-delivery method**

Direct intra-myocardial

- measurable efficacy
- selective delivery into intact or infarcted area

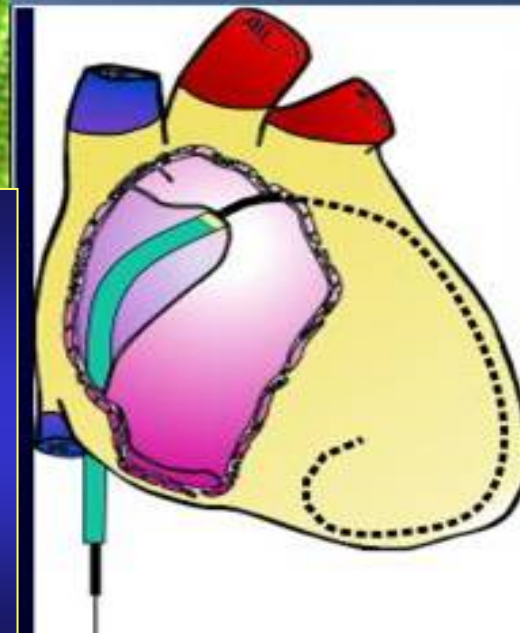
Intra-coronary

- 'natural' cell-dissemination
- little myocardial damage

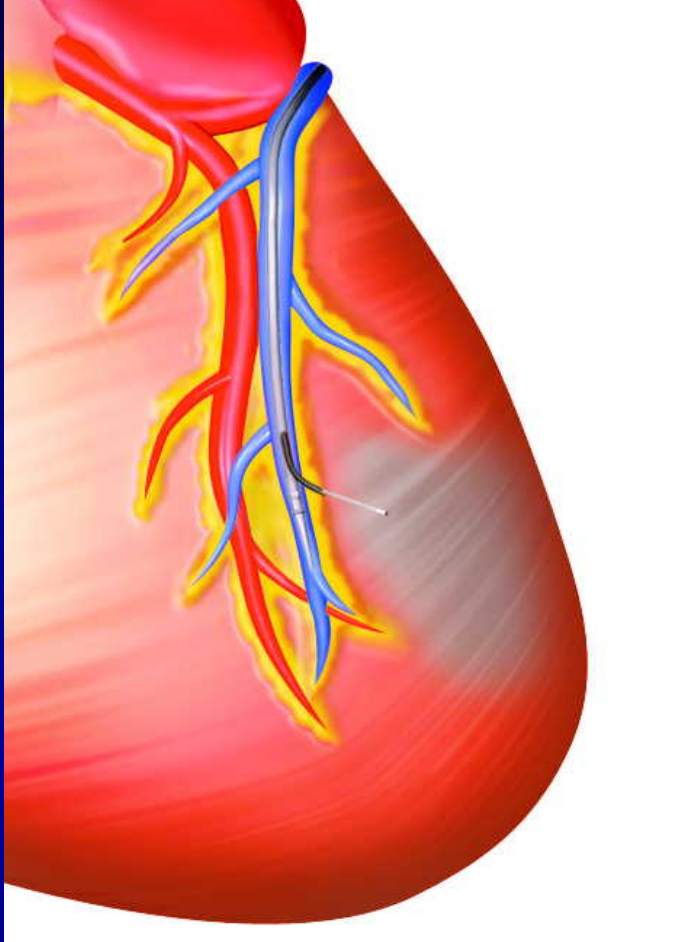
Disadvantages

- localised islet formation
- mechanical injury and inflammation resulting in myocardial damage

- coronary embolism (limited cell number grafted)
- limited delivery to avascular area



Cell injection devices

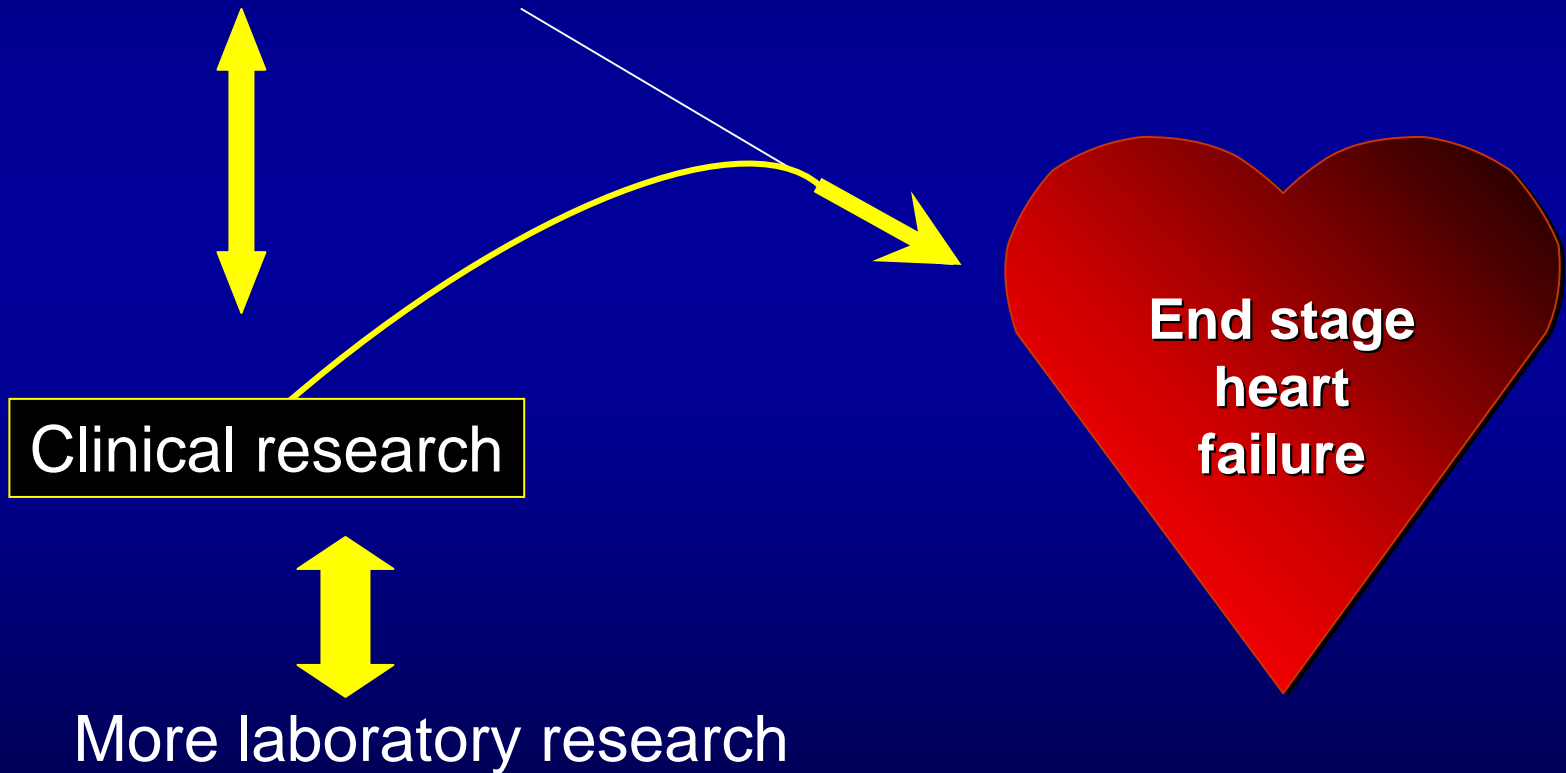


TransAccess[®]
MicroLume[™]
Intramyocardial
Injection System



Myoblast transplantation for heart regeneration

- promising strategy – positive laboratory data



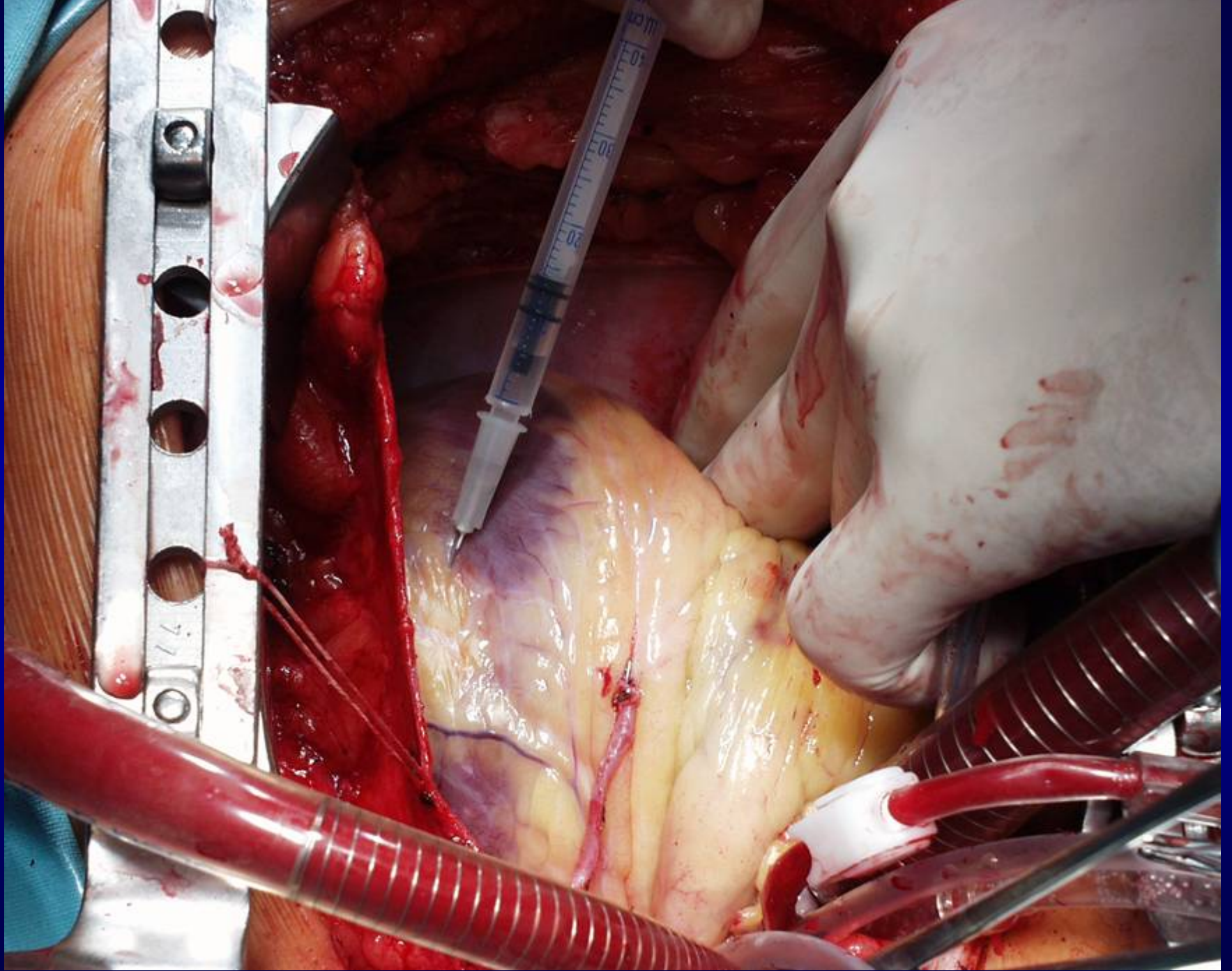
Late Breaking Clinical Trial



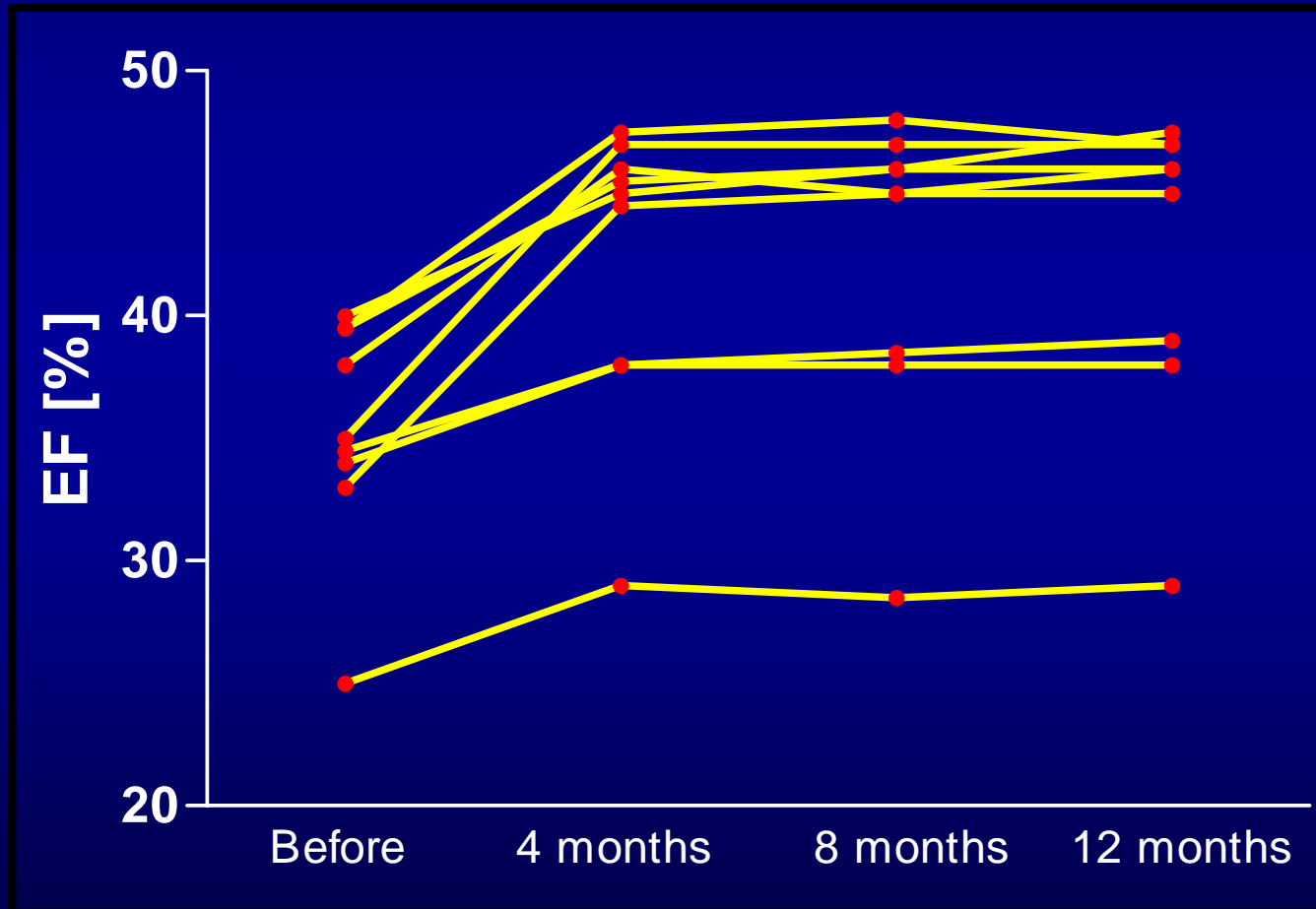
**Transplantation of Autologous Skeletal Myoblasts
in the Treatment of Patients With Postinfarction
Heart Failure – **One Year Experience****

**T. Siminiak, R. Kalawski, D. Fiszler, O. Jerzykowska,
P. Kałmucki, M. Kurpisz**

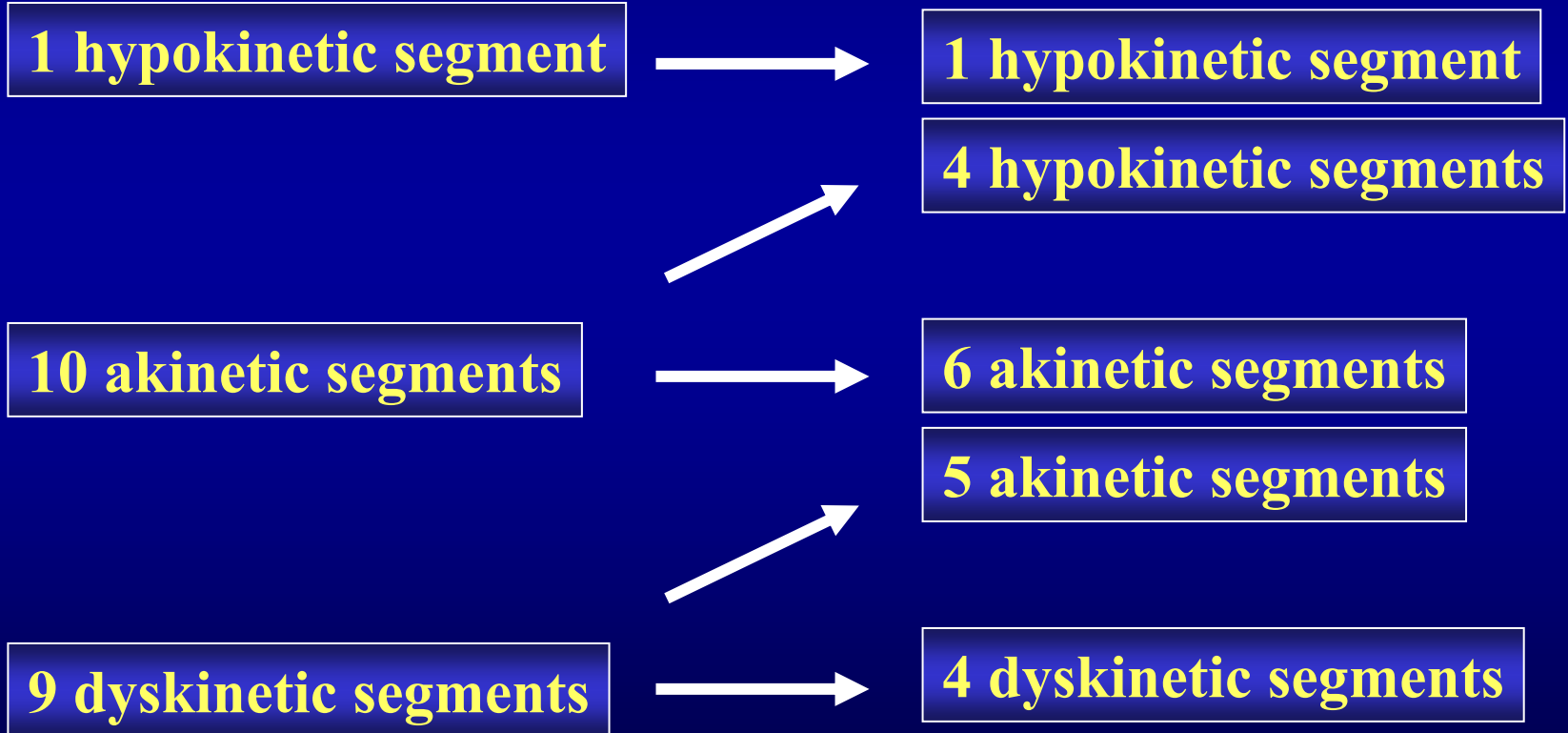
***University School of Medical Sciences,
Institute of Human Genetics, Polish Academy of Sciences,
Poznan, POLAND***



LV ejection fraction after autologous myoblast transplantation – 12 months follow up



Segmental contractility 5 months after autologous myoblast transplantation



Arrhythmia

- **Sustained VT in early postoperative period in first 2 pts**
- **Prophylactic amiodarone infusion in other patients**
 - **no sustained VT episodes 24 postoperatively**
- **2 pts on oral amiodarone at 2 months**
- **No amiodarone treatment at 3-12 months**



Late Breaking Clinical Trial

Percutaneous Transvenous Transplantation of Autologous Myoblasts in the Treatment of Postinfarction Heart Failure

The POZNAN Trial

T. Siminiak, D. Fiszler, O. Jerzykowska, M. Kurpisz

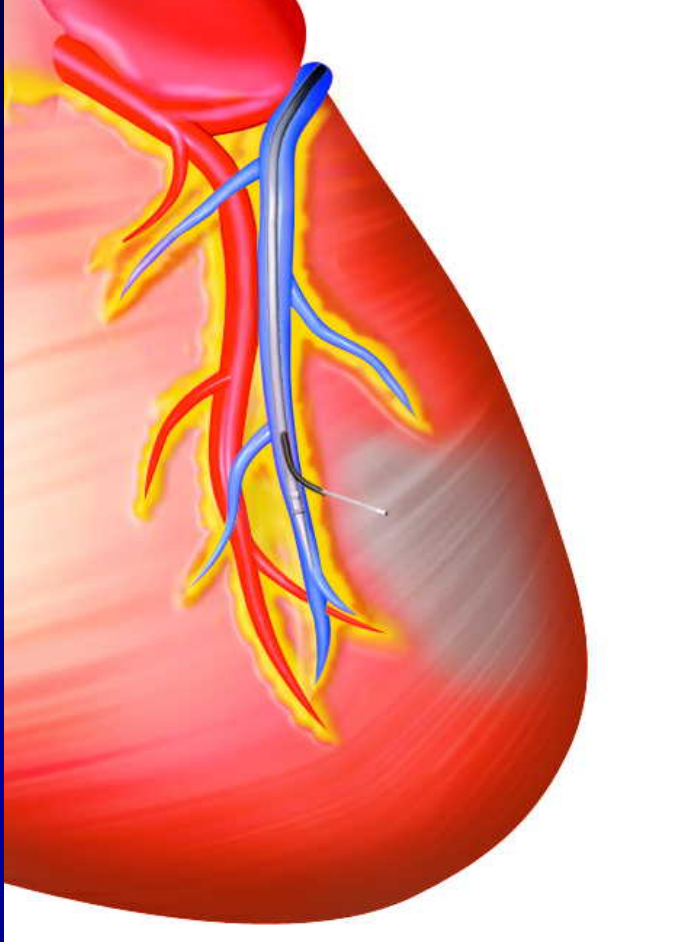
*University School of Medical Sciences,
Institute of Human Genetics, Polish Academy of Sciences,
Poznan, POLAND*

Inclusion criteria

- Documented myocardial infarction
- NYHA class 2 or 3, EF > 25%
- Akinesia or dyskinesia involving 1-3 segments of LV
- Lack of viability within akinetic/dyskinetic segments shown at dobutamine stress echocardiography
- Good coronary flow into the injured area
- Extensively informed consent given

Methods

- Myoblasts (satellite cells) were isolated from the skeletal **muscle biopsy** samples in all patients and grown in cell culture.
- Biopsy sample about 1cm³ was obtained from *vastus lateralis* by an incision performed under local anaesthesia. Biopsy specimen was **cleared** from remains of fatty and connective tissue and **minced** in Hanks' (HBSS) solution. The tissue was **digested** with collagenase I and then **filtered** in sterile conditions through a nylon net 80µm.
- As a result of three weeks **cell culture** up to **3x10⁸ myoblasts** were obtained. **Cell identification** was done by morphological and immunohistochemical assessment.
- **Myoblast injection** into the akinetic area was done via trans-coronary-venous approach using Trans Access® device.
- **Prophylactic amiodarone treatment or ICD**

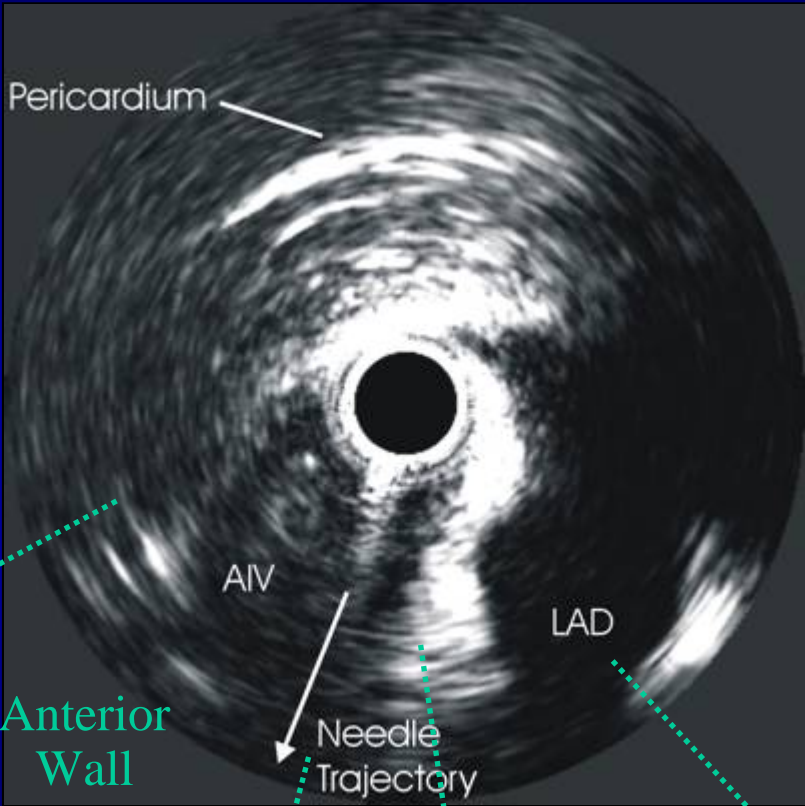
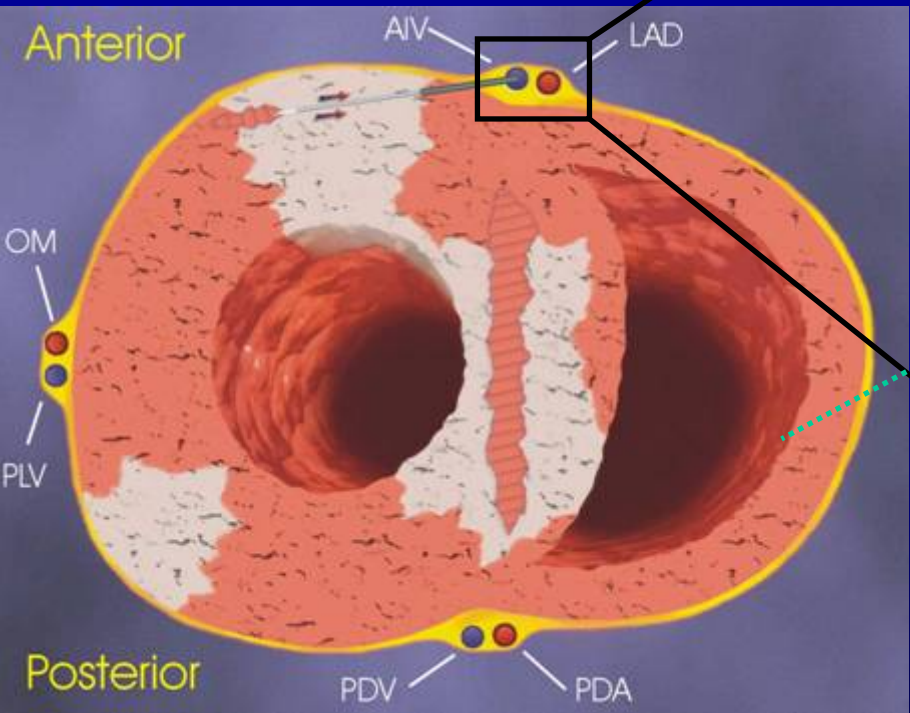


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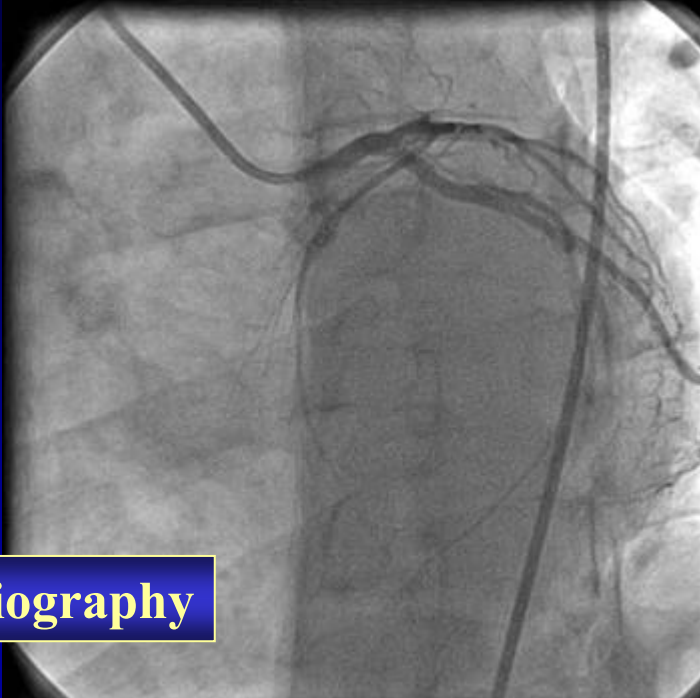
TransVascular Inc.

Intravascular ultrasound and fluoroscopic control

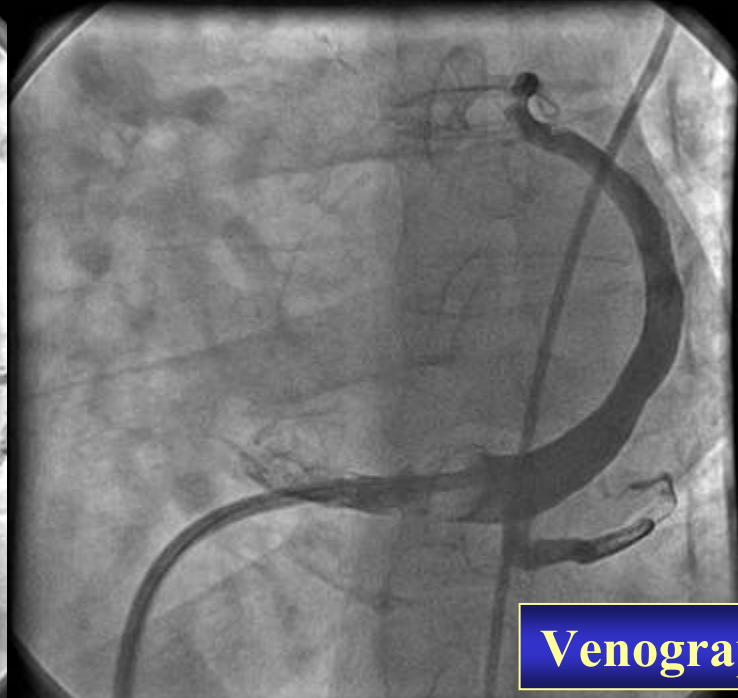


Anterior Wall

Septum



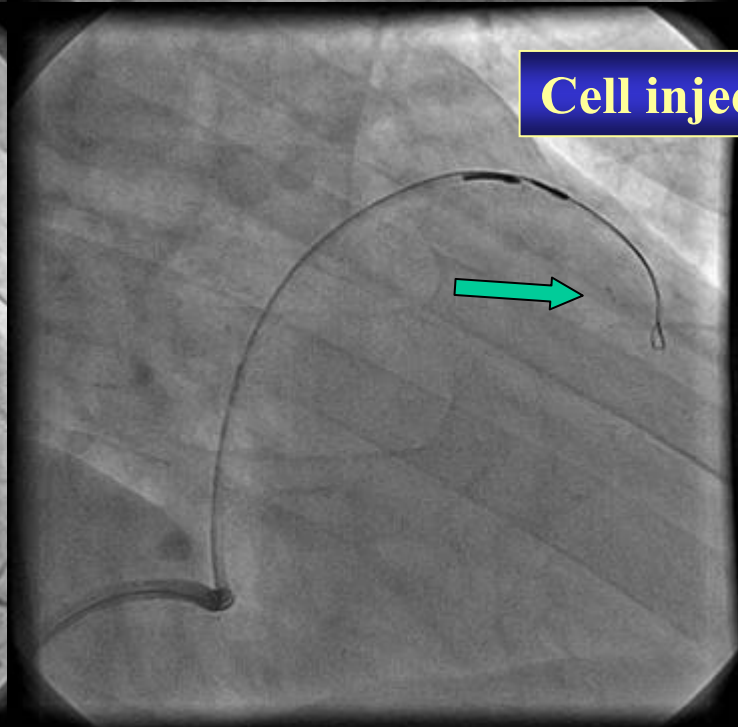
Arteriography



Venography

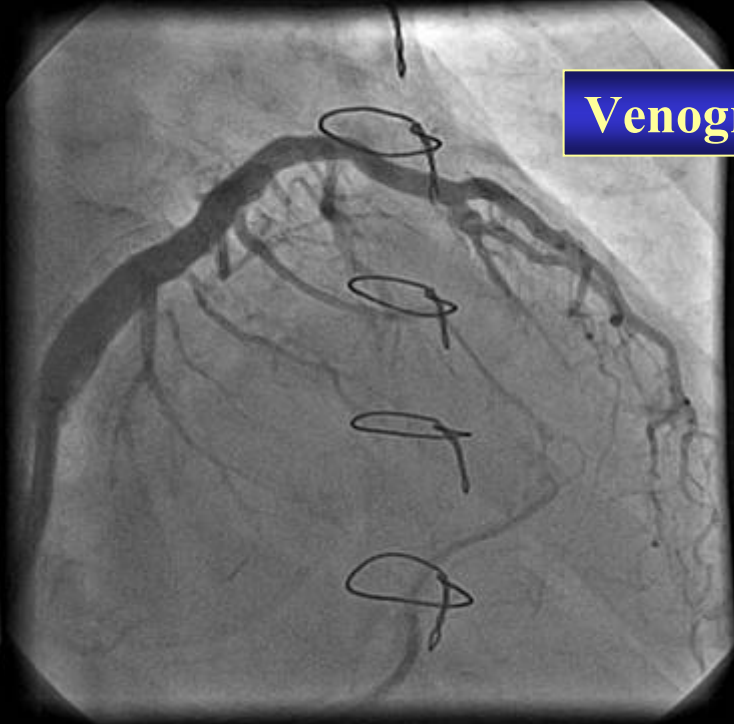


Venography

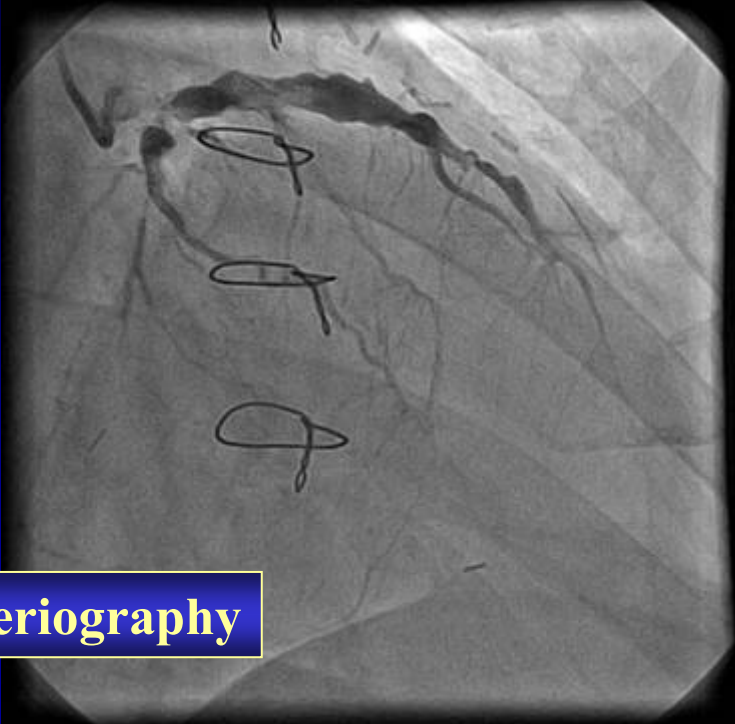


Cell injection

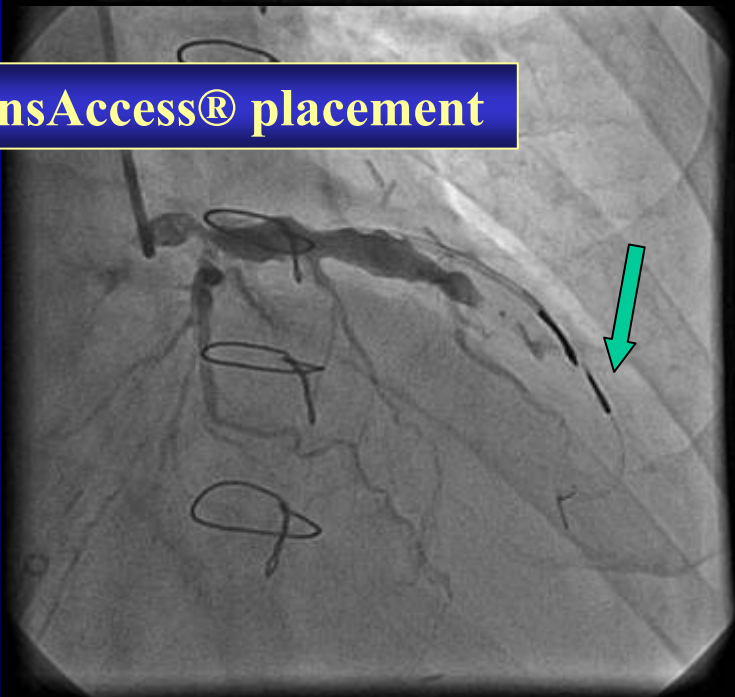
Venography



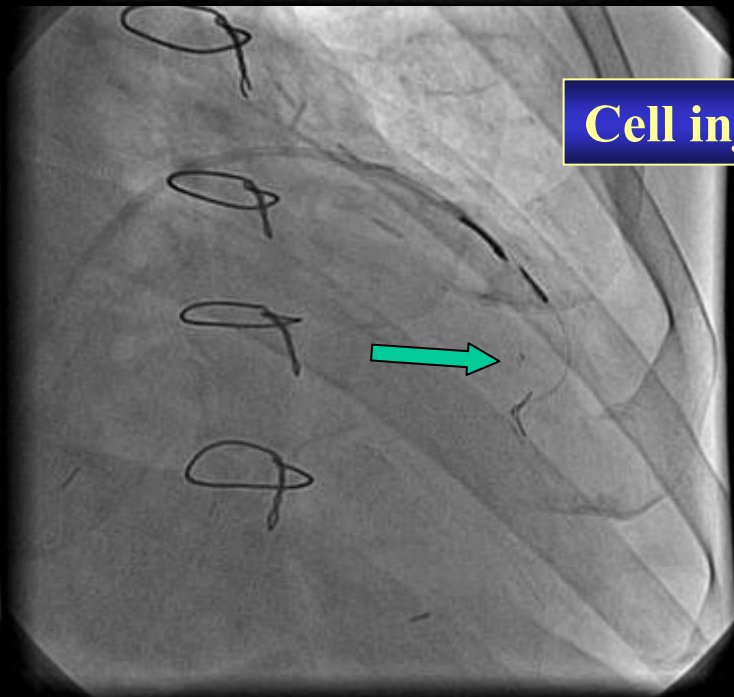
Arteriography



TransAccess® placement



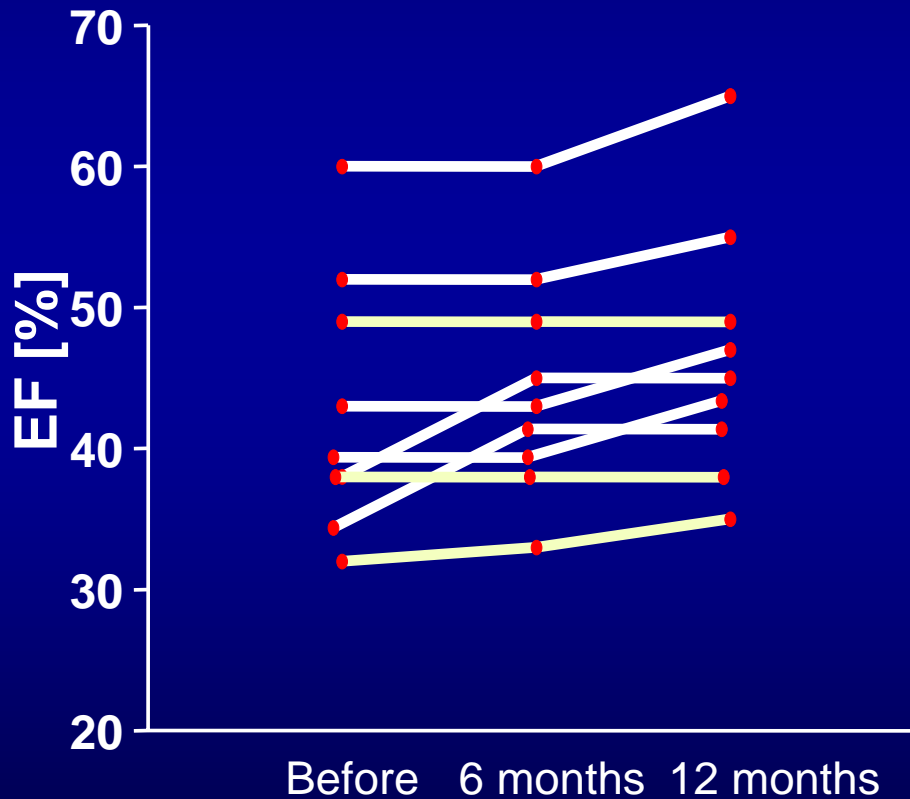
Cell injection



Results

- Procedures safely performed in 9 out of 10 patients.
- In 1 patient procedure was abandoned due to inability of crossing venous valve with the guiding wire
- Anterior interventricular vein in 5 cases and middle vein in 4
- 2-4 intramyocardial channels 1.5-4.5 cm deep
- Up to 2×10^8 myoblasts delivered in 0.8-2.5 suspension volume

Ejection fraction changes



Increase in EF 3-8% in 6 out of 9 cases: Phase II/III trials needed

Phase I clinical trials evaluating myoblast transplantation: 90 pts

Surgical

Menasche et al.	10 pts	safety/feasibility	<i>J.Am.Coll.Cardiol.</i> 2003; 41:1078-1083
Siminiak et al.	10 pts	safety/feasibility	<i>Am. Heart J.</i> 2004; 148:531-537
Dib et al.	30 pts	safety/feasibility	<i>Circulation</i> 2005; 112: 1748-1755
Herreros et al.	12 pts	safety/feasibility	<i>Eur. Heart J.</i> 2003; 24: 2012-2020
Pagani et al.	5 pts	histology	<i>J. Am. Coll.Cardiol.</i> 2003; 42: 2063-2069

Percutaneous

Smits et al.	5 pts	safety/feasibility	<i>J.Am.Coll.Cardiol.</i> 2003;42: 2063-2069
Siminiak et al.	10 pts	safety/feasibility	<i>Eur. Heart J.</i> 2005; 26:1188-1195
Ince et al.	6 pts	safety/feasibility	<i>J.Endovasc.Ther.</i> 2004; 11: 695-704

MAGIC trial: myoblast transplantation during CABG

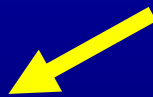
The trial's Data Monitoring Committee (DMC), following a planned review of preliminary safety and efficacy data, recommended this step (termination of the trial) after concluding that there is a low likelihood that the trial would result in the hypothesized improvements in heart function, and that the study hypothesis has been adequately tested.

Cell transplantation during CABG: limitation in efficacy trials

- high background effect of revascularization
- procedural differences between centers
- evaluation of the cells effect independently
of revascularization not possible



**Bioheart supported trials:
percutaneous myoblast transplantation**



MYOHEART trial (US)

SEISMIC trial (Europe)

Conclusions

- ✓ **Autologous skeletal myoblast transplantation in the treatment of postinfarction myocardial injury is feasible and safe**
- ✓ **Initial observations justify further research to validate the method and to establish its role in the clinical practice**
- ✓ **Percutaneous studies are more likely to show any effect in clinical situation**

Remaining questions

- ✓ **Long term and large scale safety observations**
- ✓ **Efficacy in LV performance improvement?**
- ✓ **Number of cells to be implanted?**
- ✓ **Single or multiple transplantations?**

POZNAN Trial Contributors

(all from Poznan, Poland)

University School of Medical Sciences, Poznan

Tomasz Siminiak

Olga Jerzykowska

Piotr Kalmucki

Institute of Human Genetics, Polish Academy of Sciences

Maciej Kurpisz

Dorota Fiszler

Natalia Rozwadowska

**Special thanks to: Theodore Lamson, PhD, Ludwik Firek, MD
and Josh Makower, MD, all from TransVascular Inc.**