TE for heart

- About 3,000 individuals in the United States are awaiting a donor heart; worldwide, 22 million individuals are living with heart failure
- a bioartificial heart requires engineering of cardiac architecture, appropriate cellular constituents and pump function

- needle injection has some disadvantages
  - loss of transplanted cells by leakage, poor survival of grafted cells, myocardial damage resulting from injury by the needle, subsequent acute inflammation, potential lethal arrhythmia
  - three-layered sheets of cardiomyocytes could be vascularized in subcutaneous tissue without necrosis, but that sheets with four or five layers have areas with disorganized vasculature and primary ischemi
  - increasing the number of layered myoblast sheets to estimate the optimal number of myoblast sheet layers for regeneration of host heart

experiment

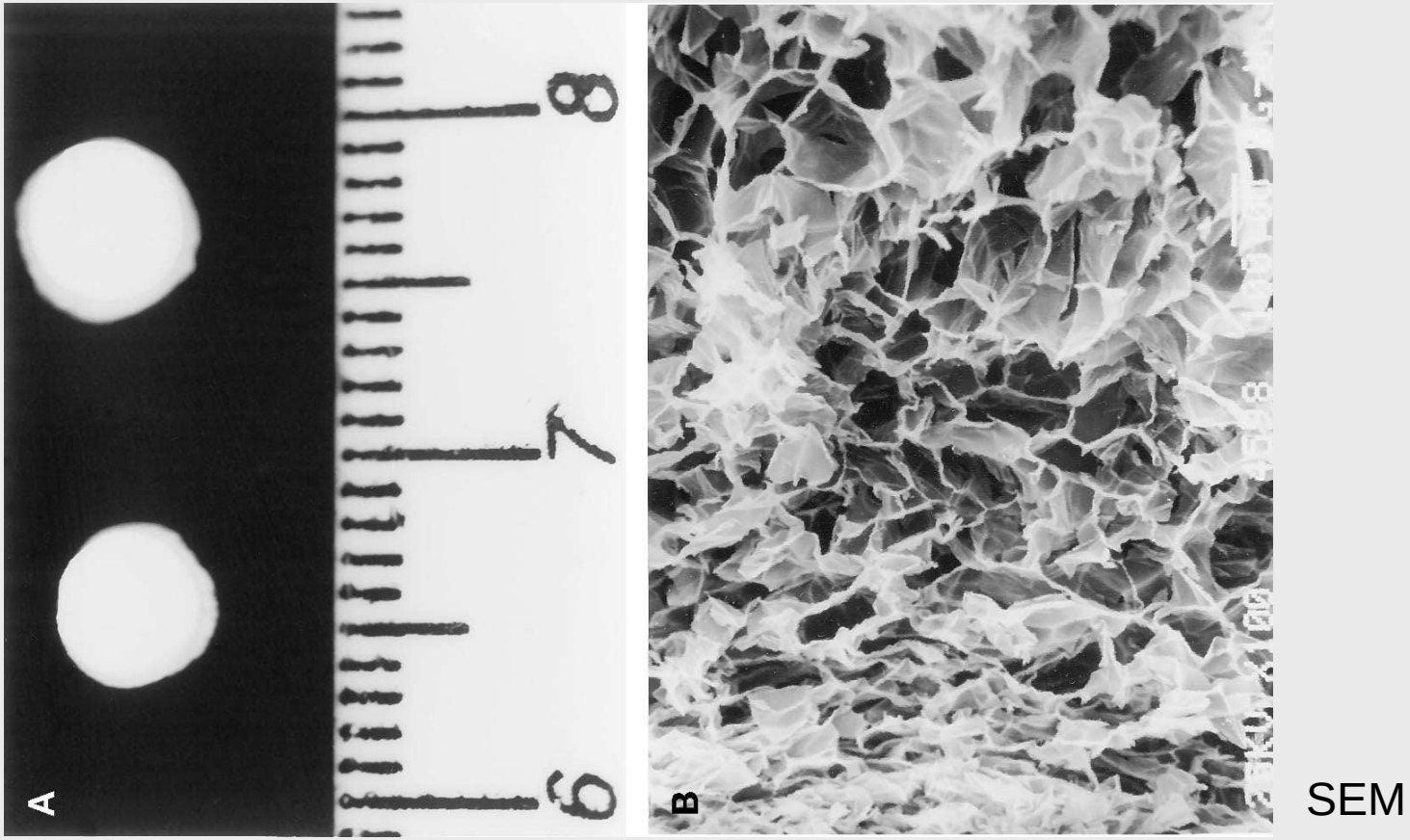
- fetal cardiac cells were grown within 3D porous alginate scaffolds for 4 d.
- After 2-3 d: multicellular contracting aggregates appeared
- Transplanted in rat, 7 d after experimental infarction
- Echography done at d 65
- Hearts harvested after 9 weeks and examined

(Leor et al. *Circulation*. 2000;102[suppl III]III-56-III-61.)

results

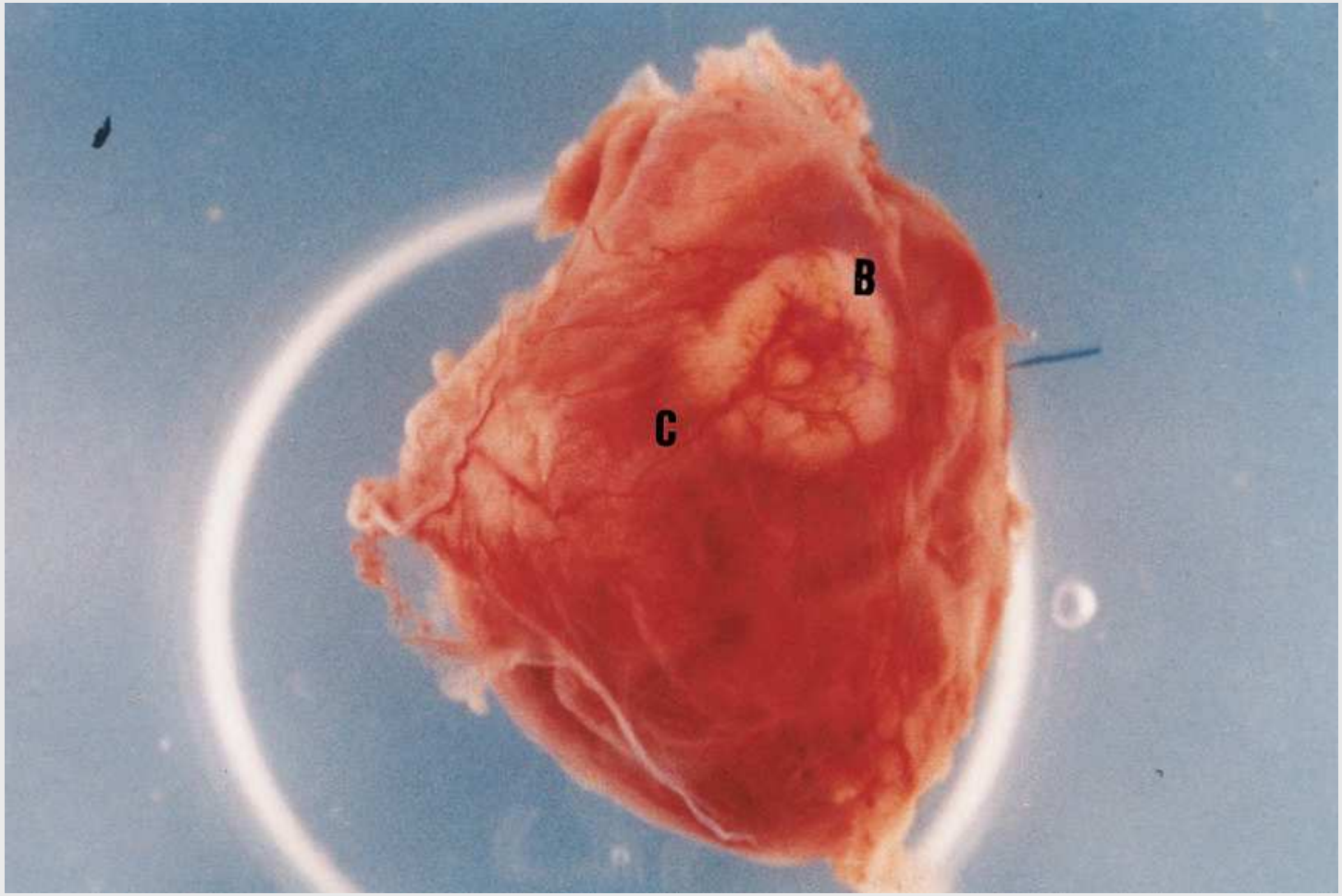
- Extensive neovascularization
- Myofibers embedded in collagen fibers
- Almost complete absence of the scaffold
- good integration into the host
- Attenuation of LV dilatation
- No change in LV contractility

Alginate scaffold

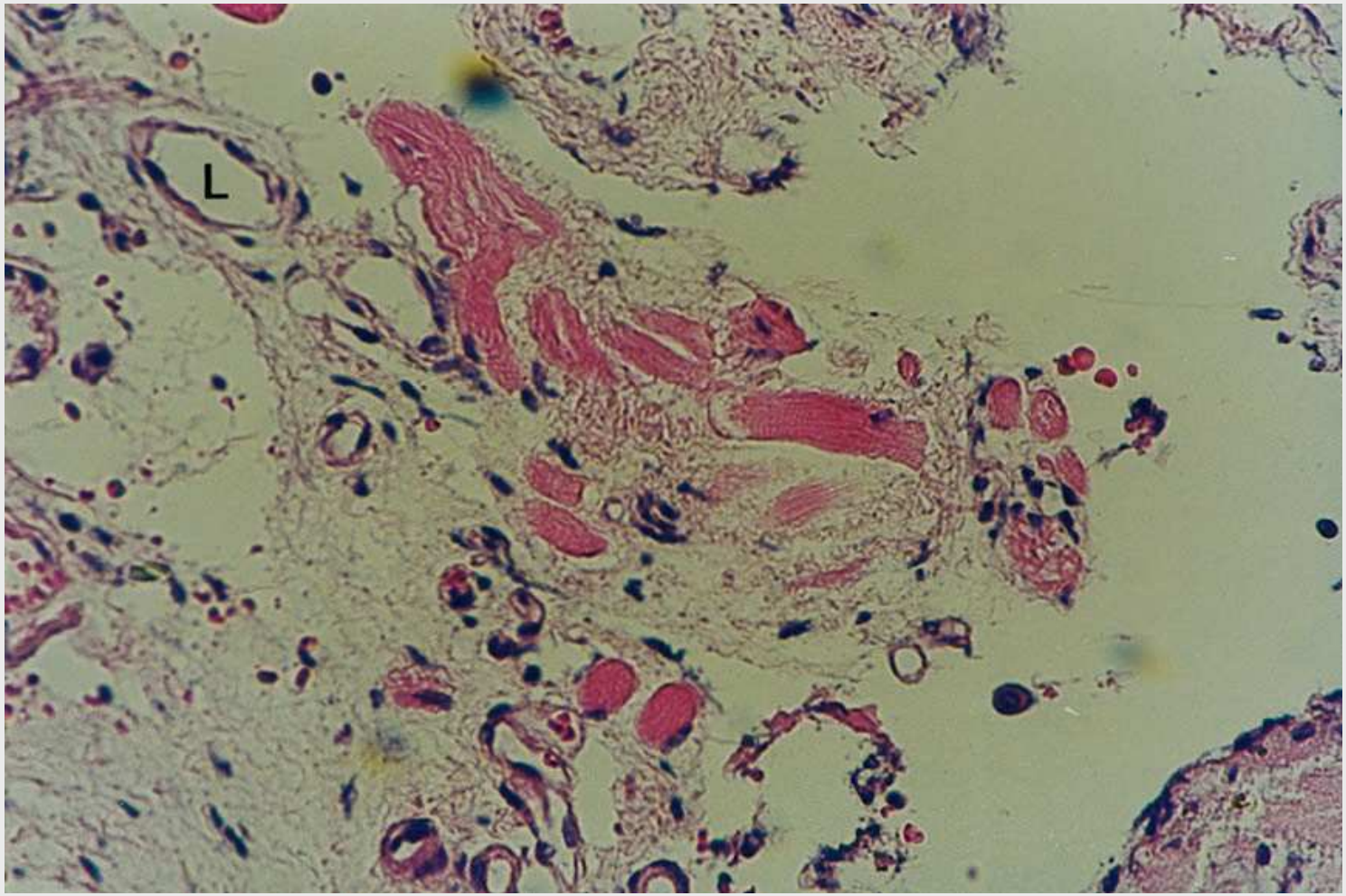


SEM

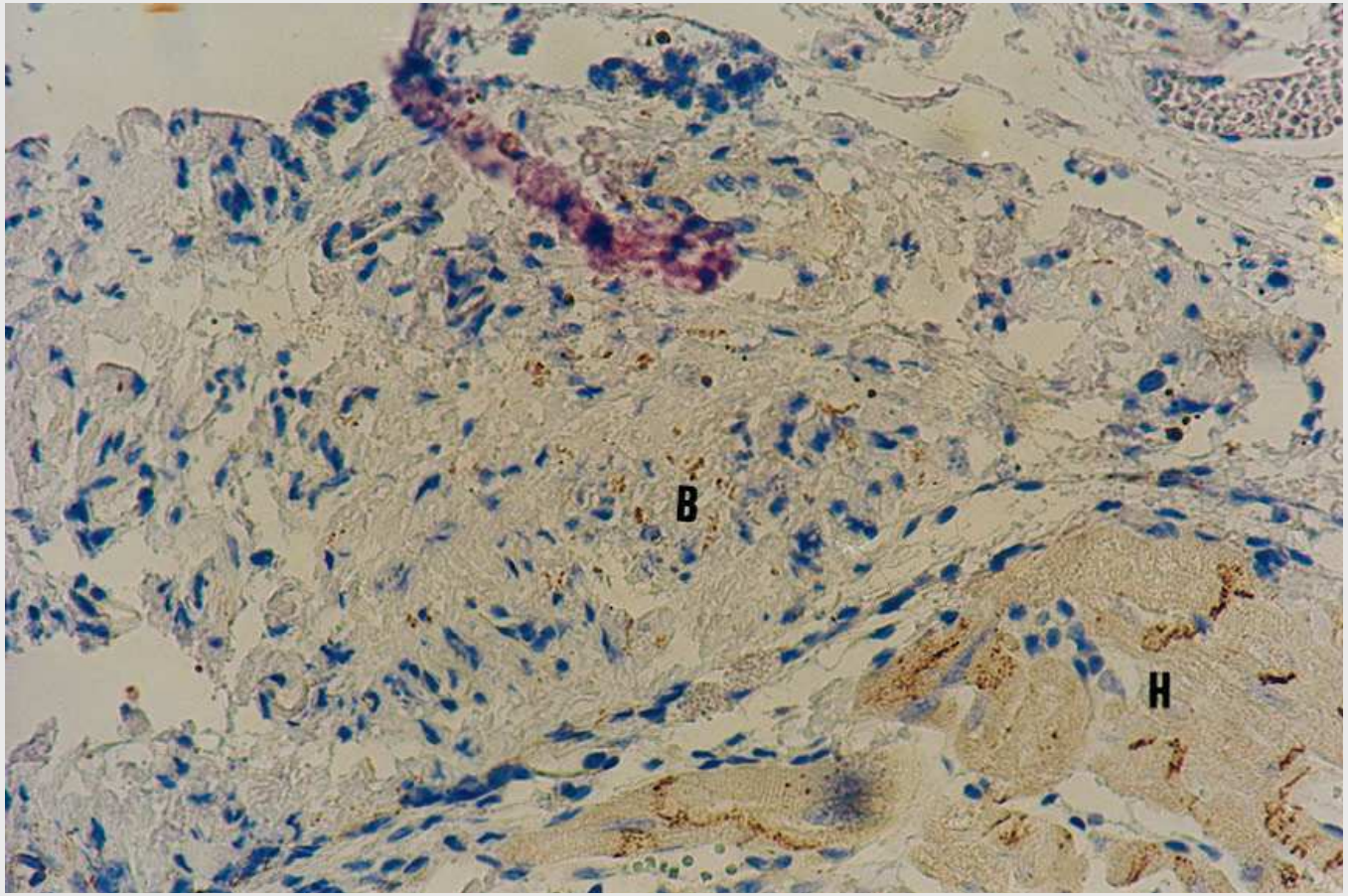
Heart 9 weeks after implantation of cellular construct



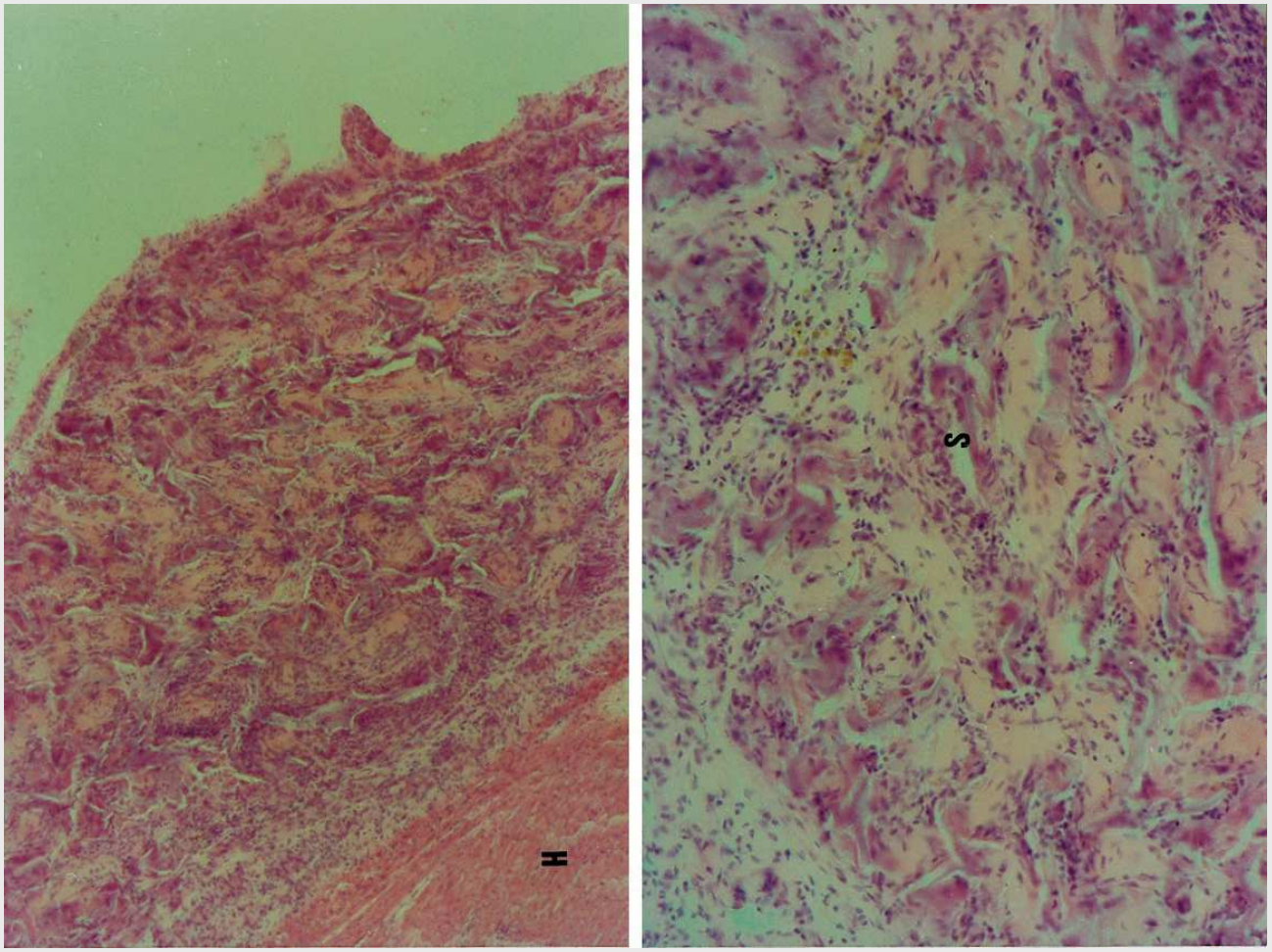
HE stained heart section



Connexin 43 IIC



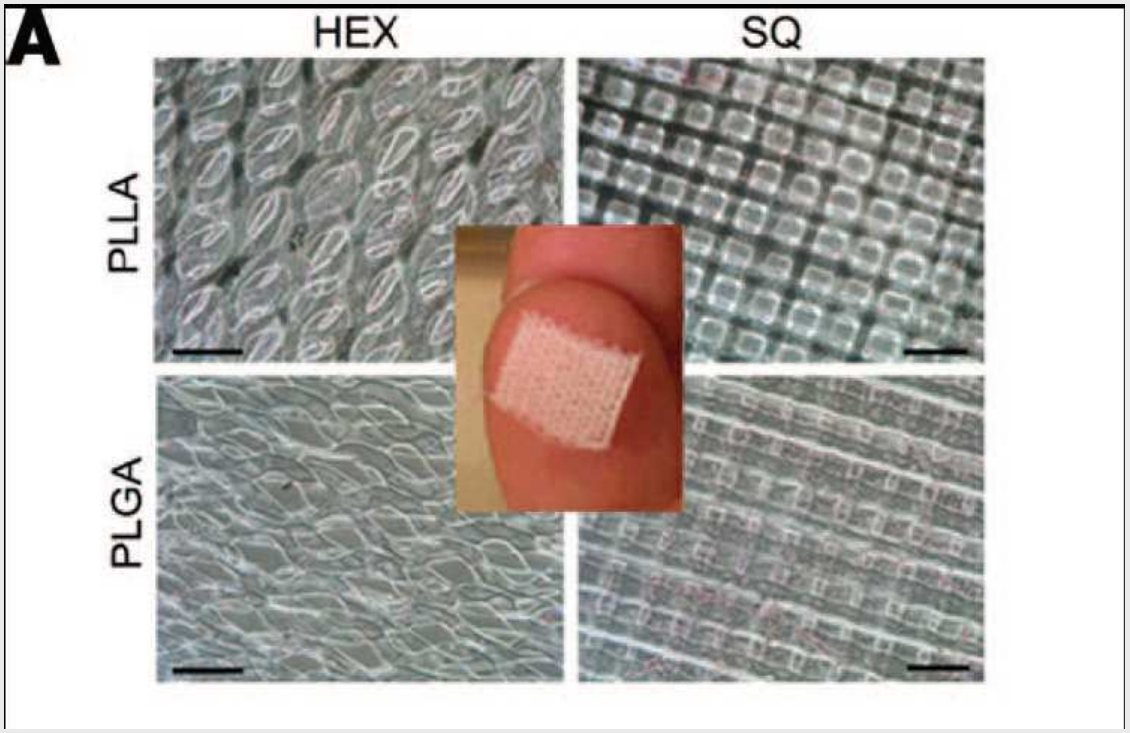
Integration and nearly complete disappearance of scafold. Inflammatory infiltrate



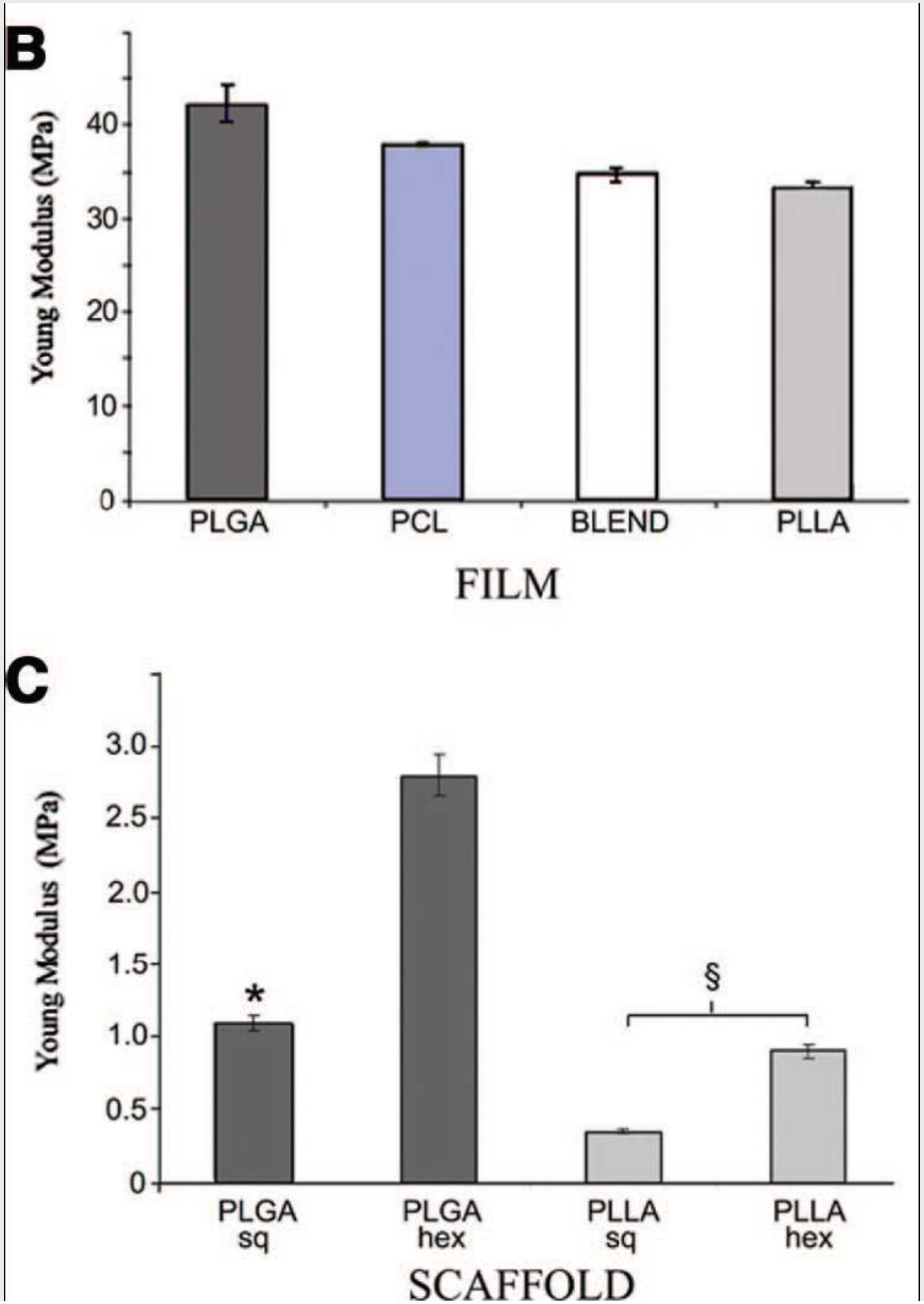
Role of the scaffold geometry and stiffness

Forte et al, Stem cells 2008; 26:2093-2103

PLGA and PLLA scaffold pattern



Elastic modulus

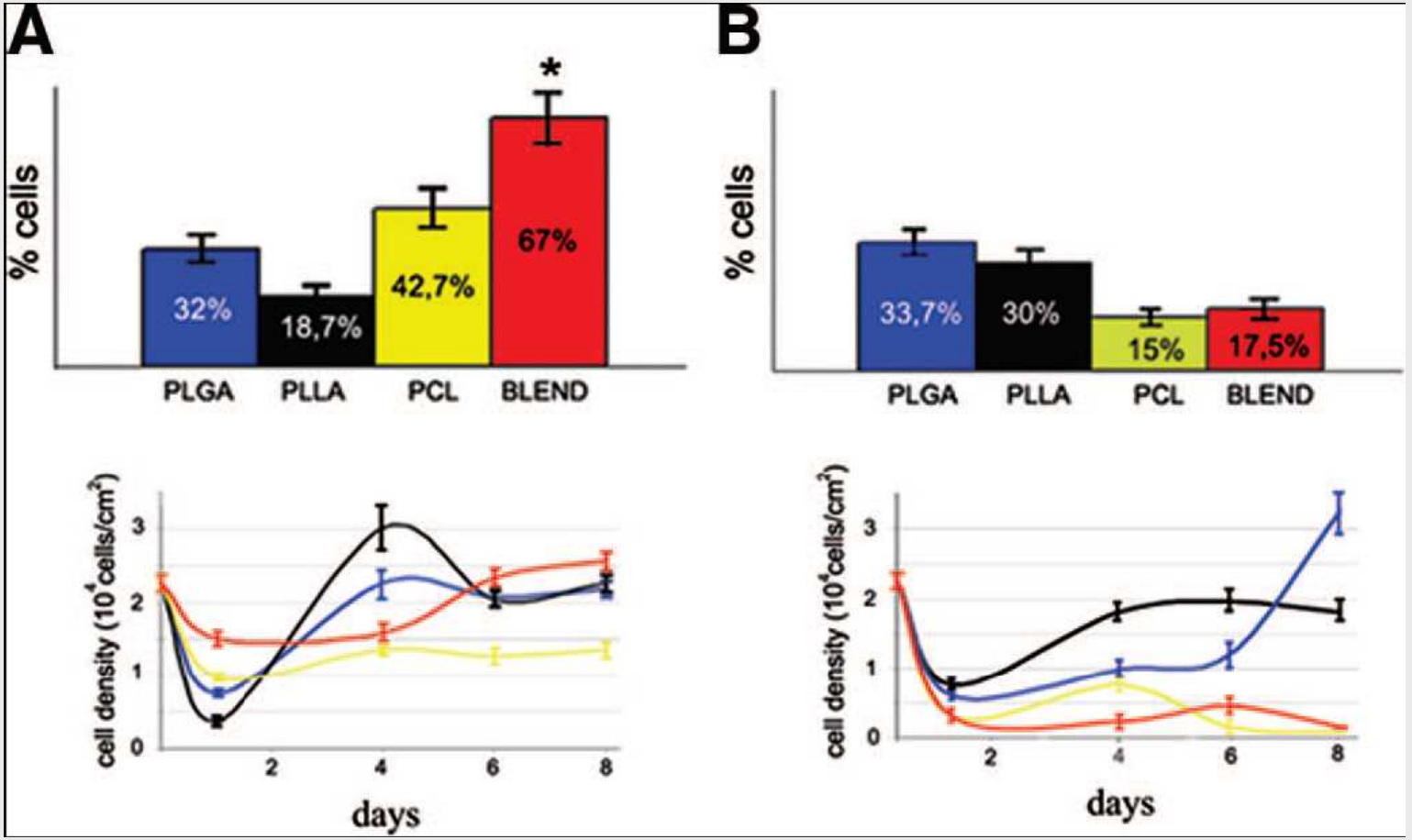


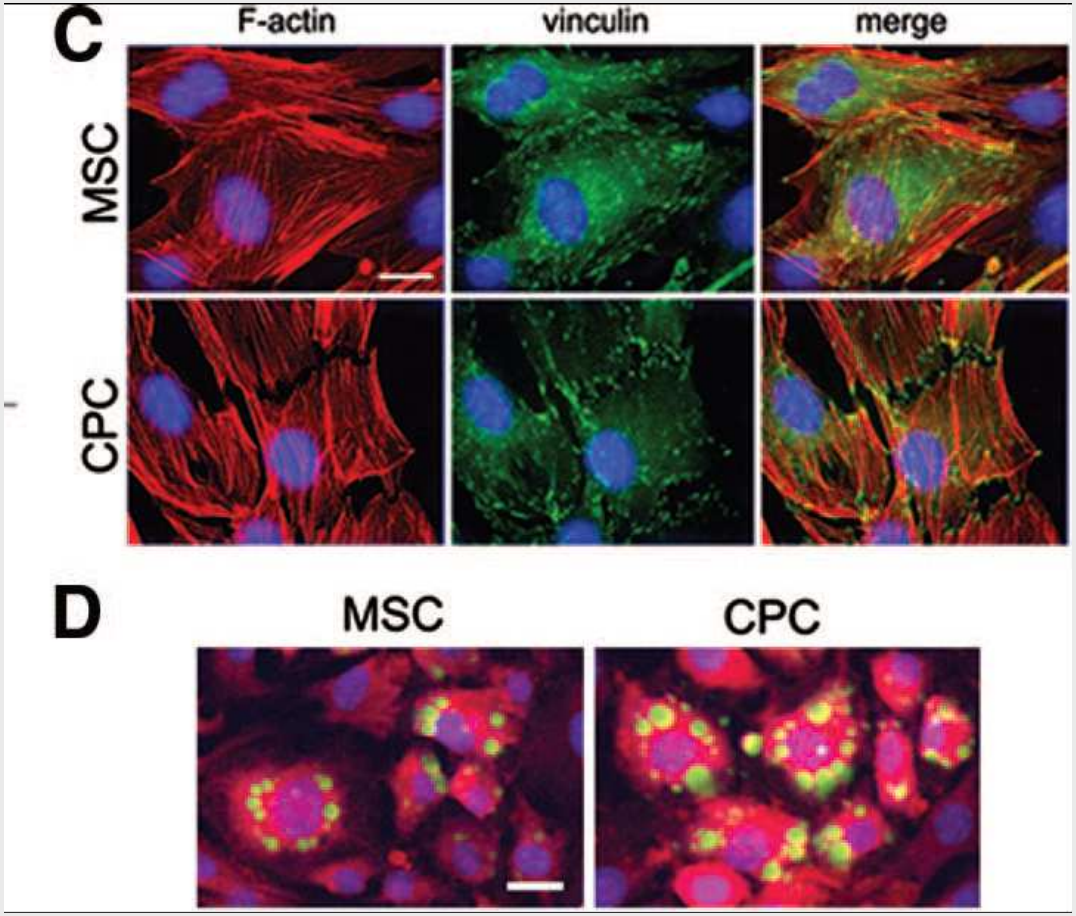
MSC

CPC

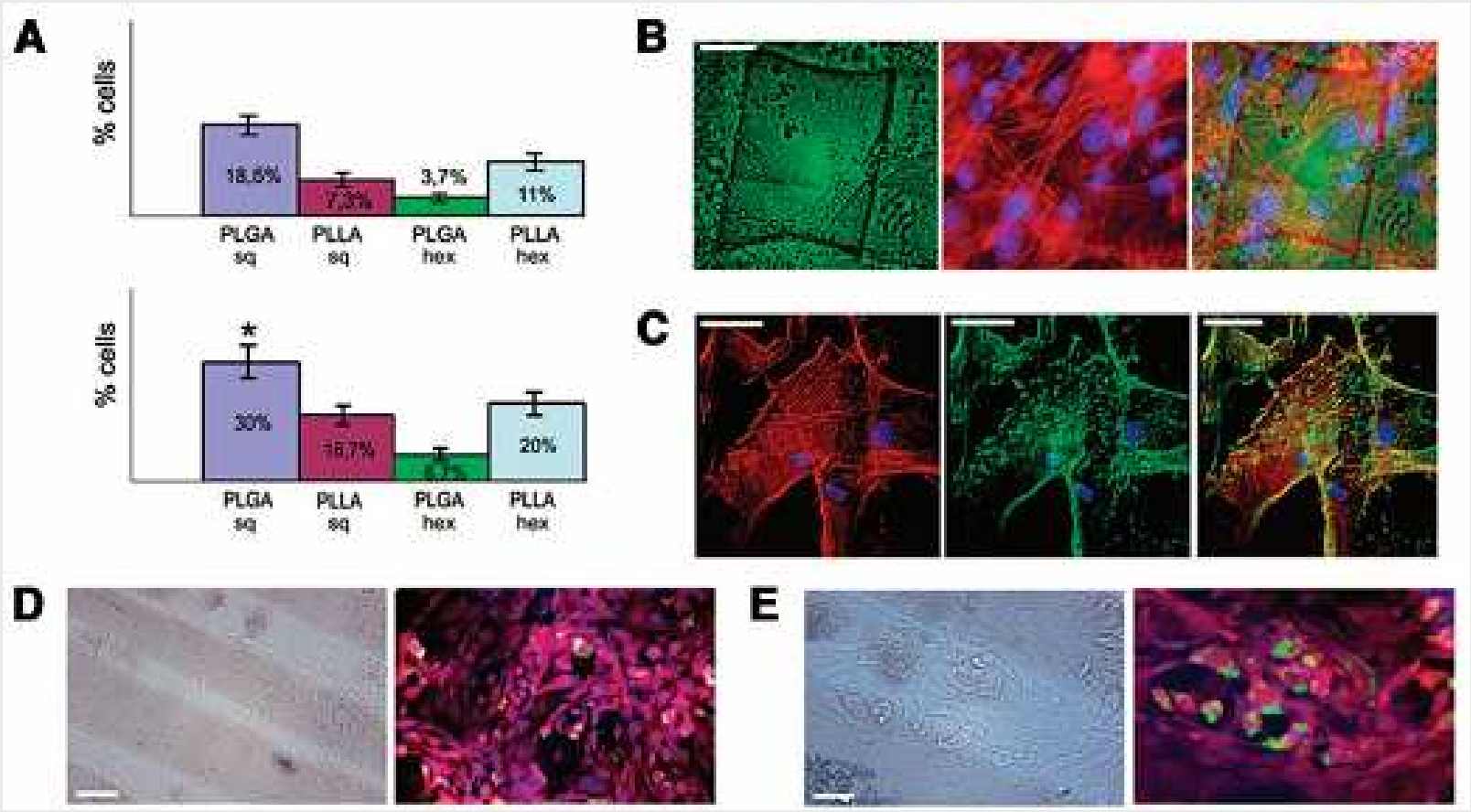
adhesion

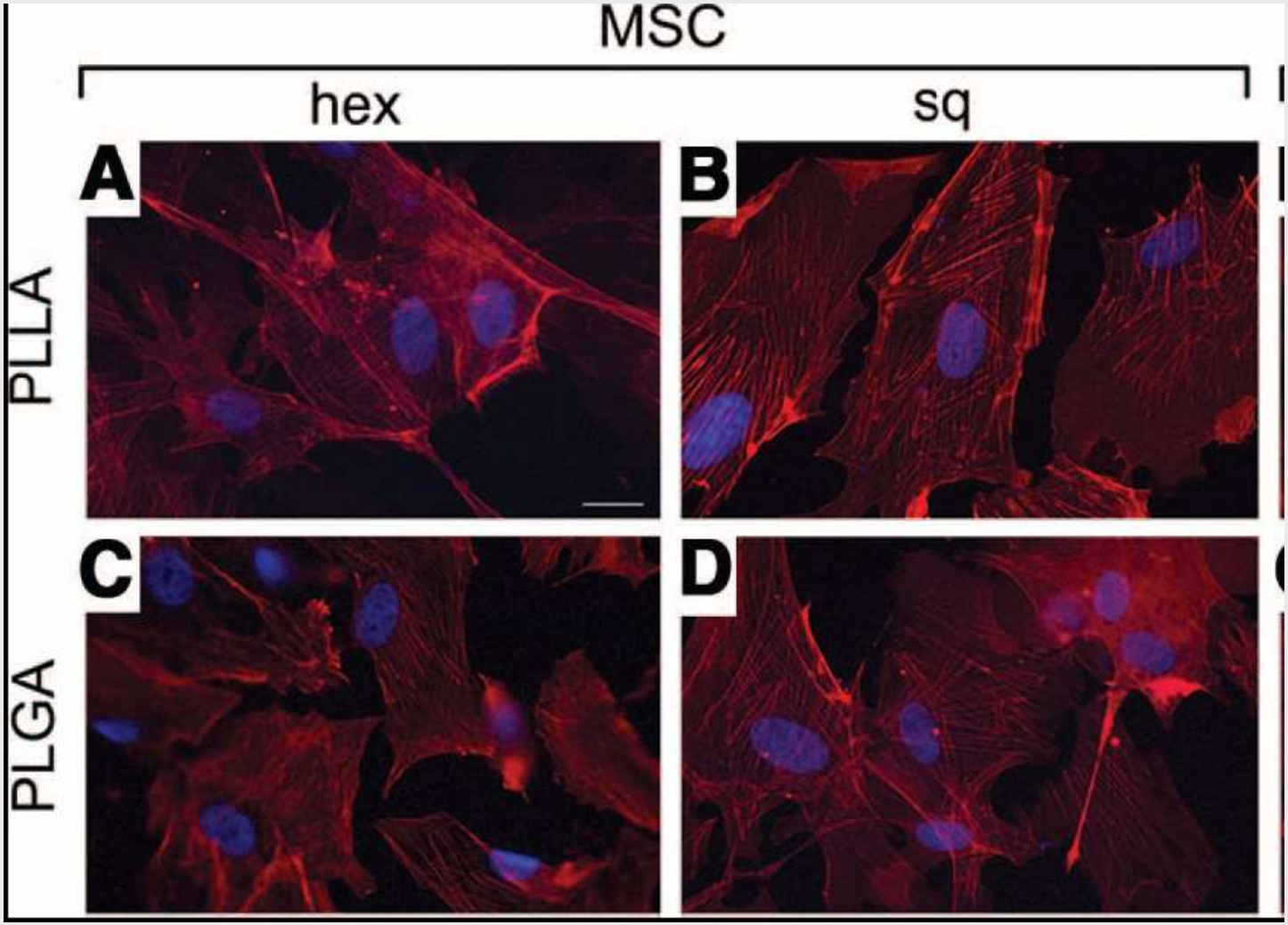
growth




C
F-actin
vinculin
merge
MSC
CPC
D
MSC
CPC

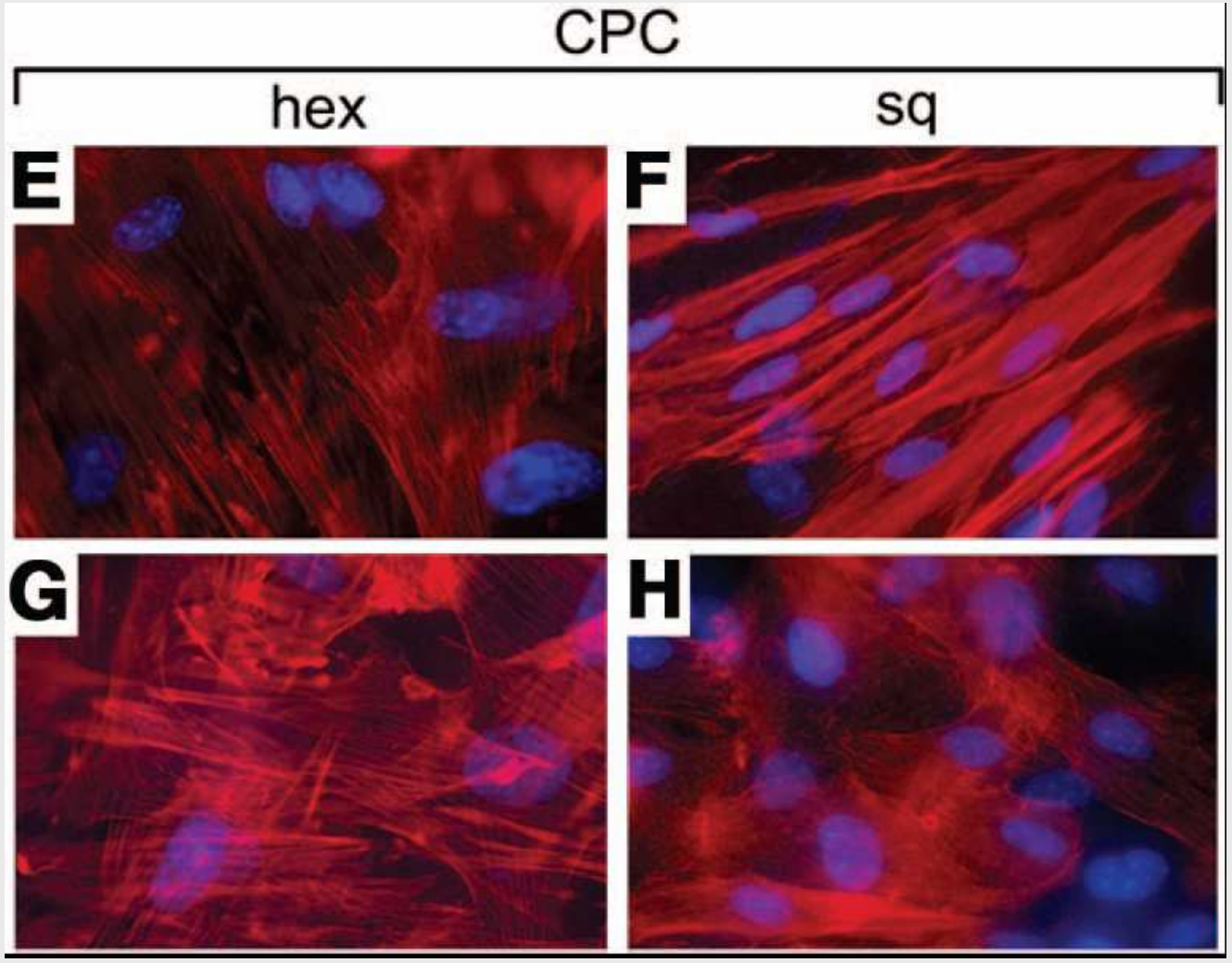
CPC on PLLA 3D




MSC
hex
sq
A
B
C
D
PLLA
PLGA

PLLA

PLGA



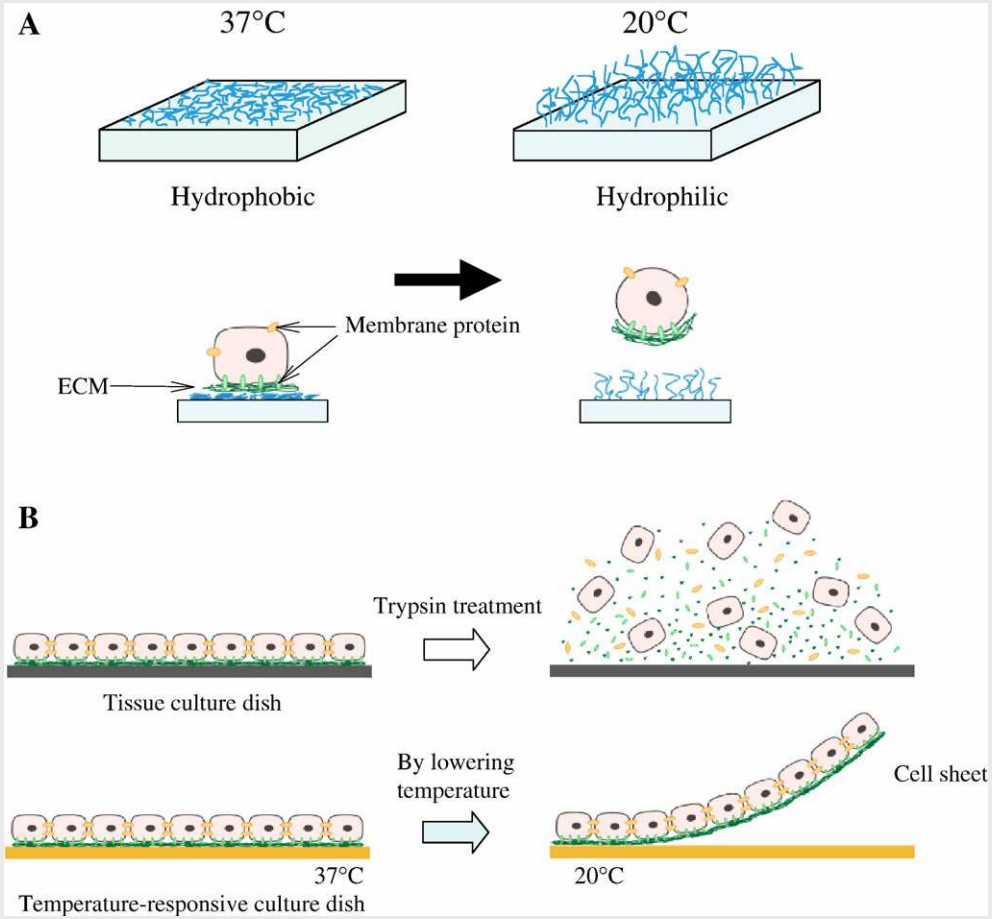
Cell sheet engineering

- High cellularity
- No foreign material/scaffold
- Layers with different cell types could be combined (x vascularization)

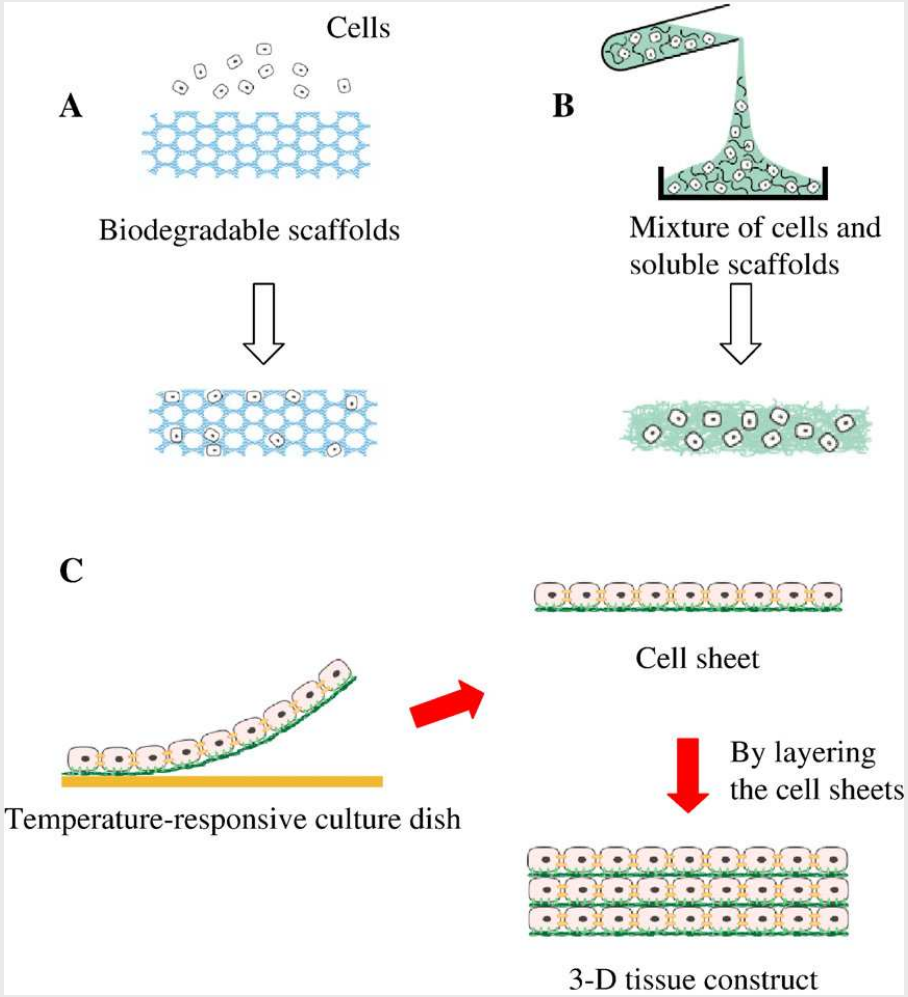
Masuda et al., Advanced Drug Delivery Reviews, 2007

- needle injection has some disadvantages
  - loss of transplanted cells by leakage, poor survival of grafted cells, myocardial damage resulting from injury by the needle, subsequent acute inflammation, potential lethal arrhythmia
  - three-layered sheets of cardiomyocytes could be vascularized in subcutaneous tissue without necrosis, but that sheets with four or five layers have areas with disorganized vasculature and primary ischemi
  - increasing the number of layered myoblast sheets to estimate the optimal number of myoblast sheet layers for regeneration of host heart

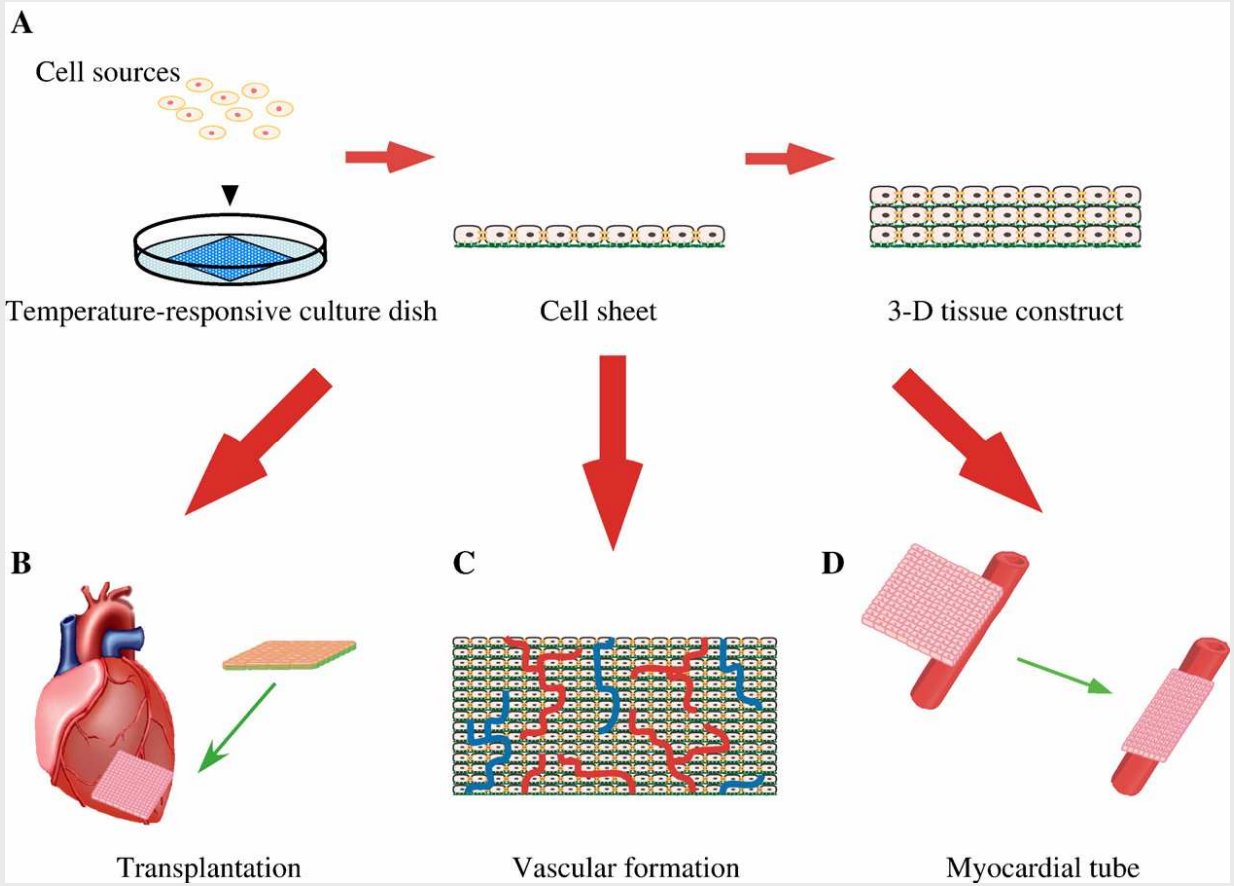
Smart scaffold: poly (N-isopropylacrylamide) (PIPAAm)



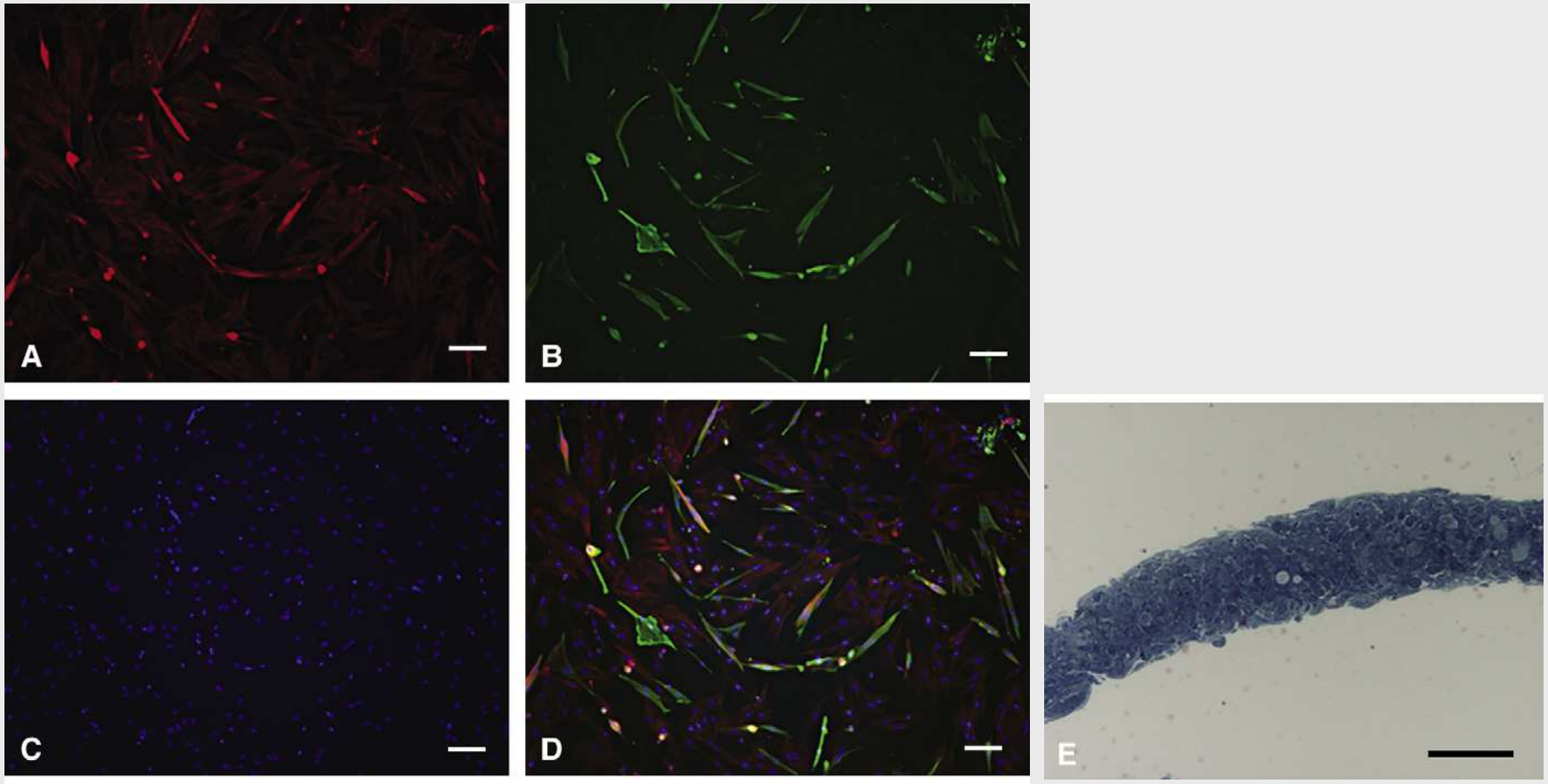
Myocardial tissue engineering methods.



myocardial tissue reconstruction by using cell sheet engineering



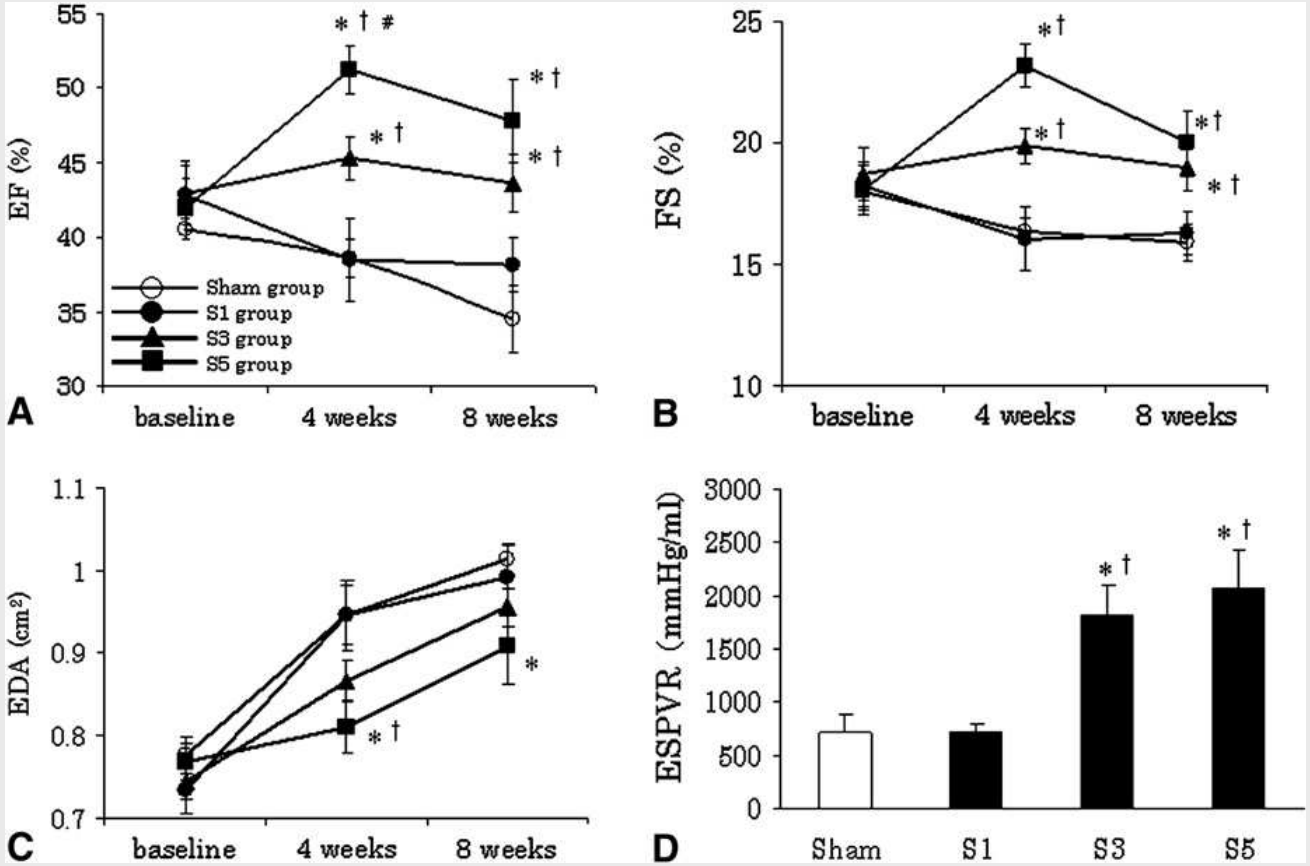
desmin-positive muscle lineage cells



Cardiac function after layered myoblast sheet implantation

Ejection fraction

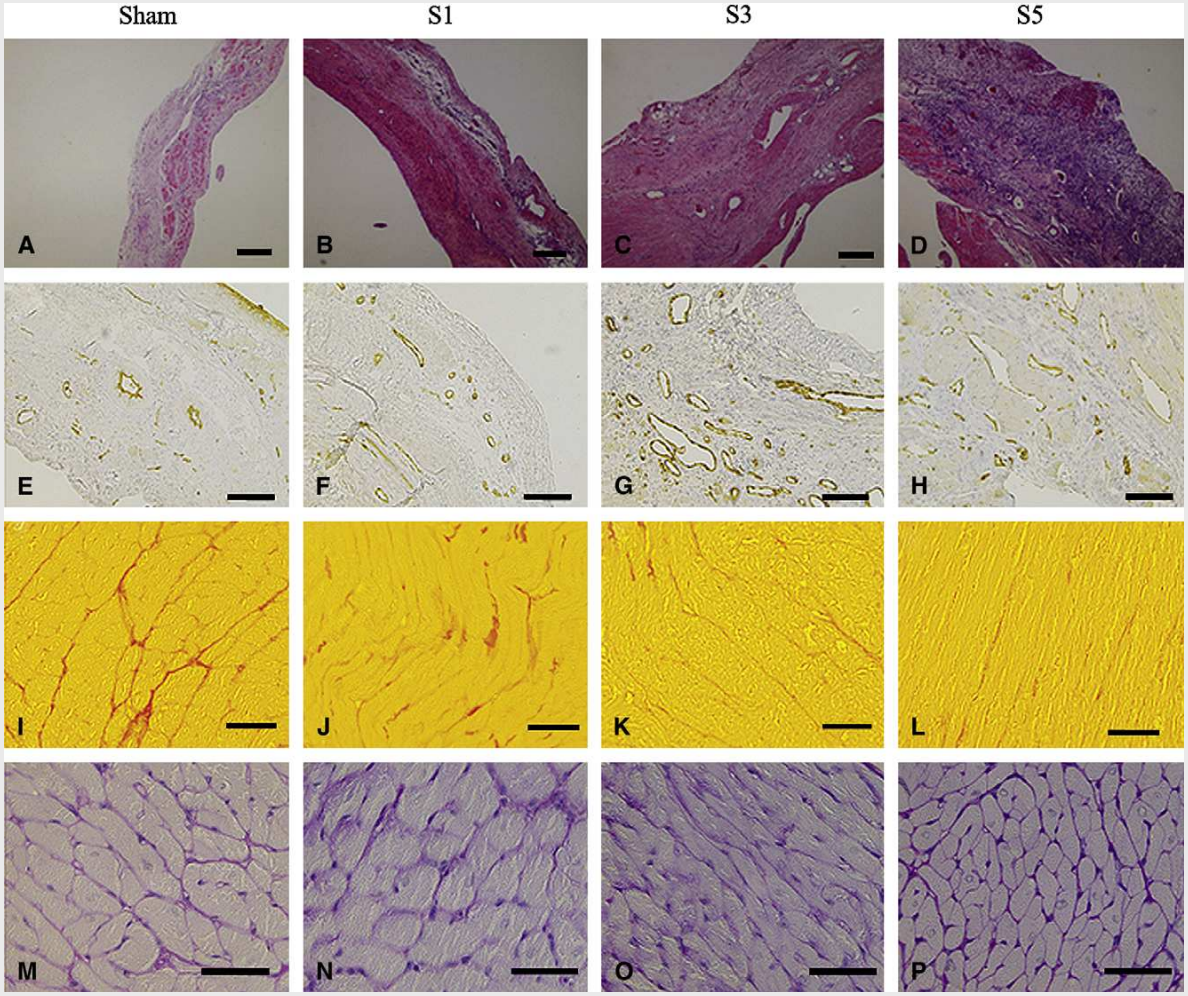
fractional shortening



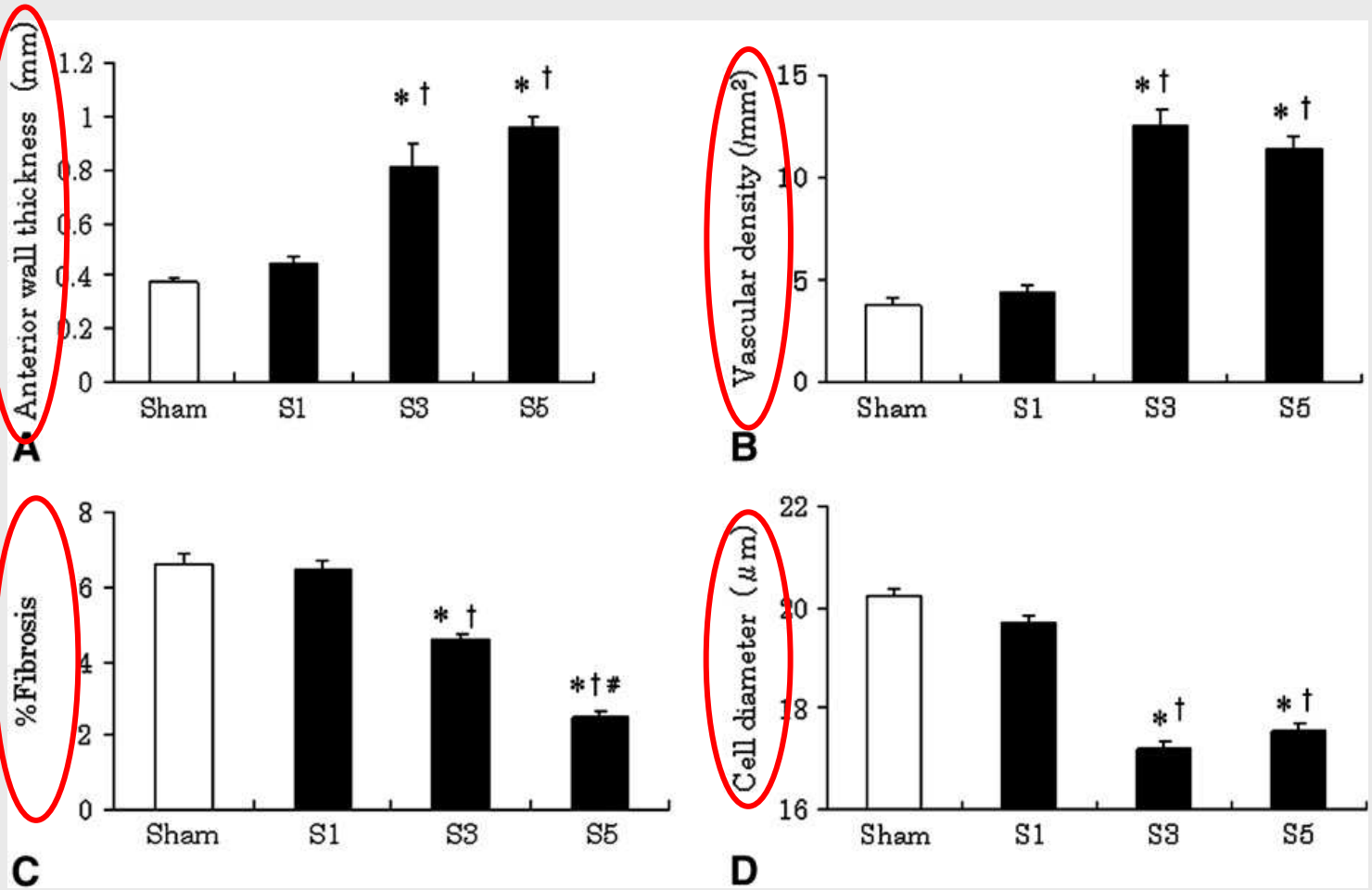
End diastolic area

end-systolic pressure-volume relationship

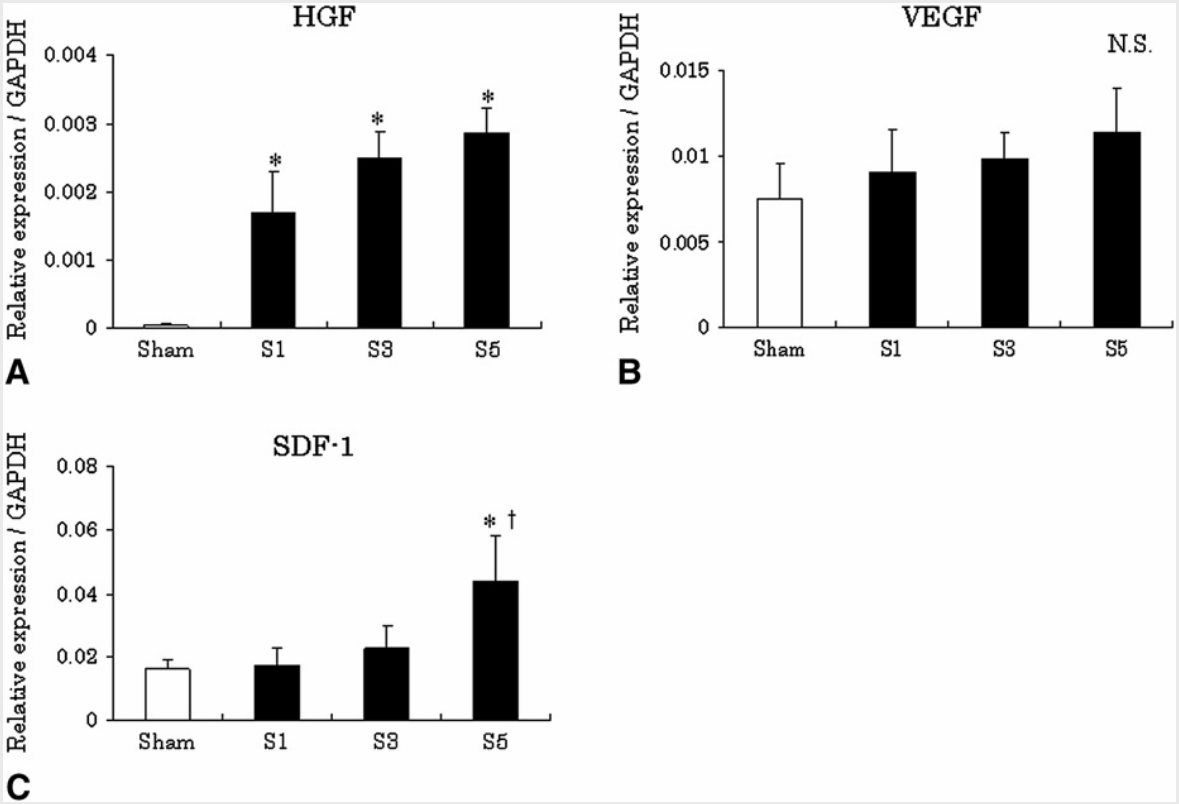
Histology of implanted host hearts



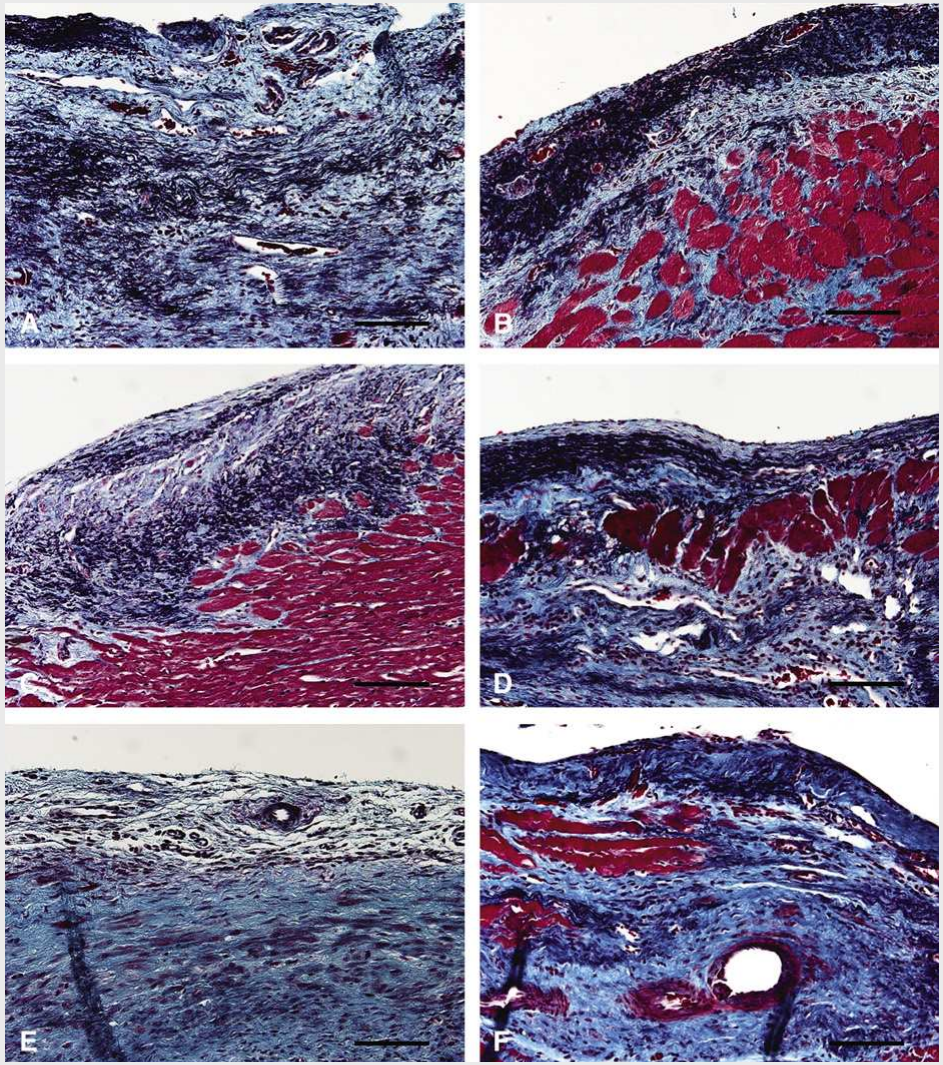
Quantitative assessment of histologic findings

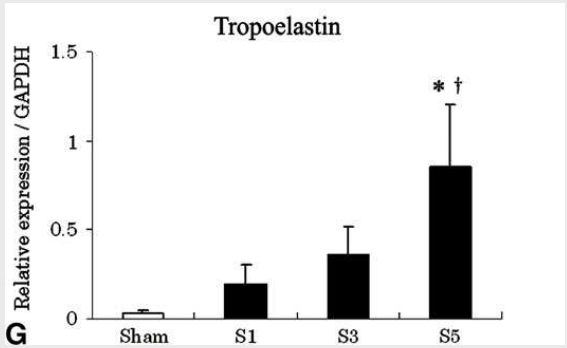


Relative expression of growth factors



Elastic fibers were constructed after implantation, with expression of mRNA.





Ott et al: Perfusion-decellularized matrix: using nature’s platform to engineer a bioartificial heart

NATURE MEDICINE VOLUME 14
[NUMBER 2 [FEBRUARY 2008

background

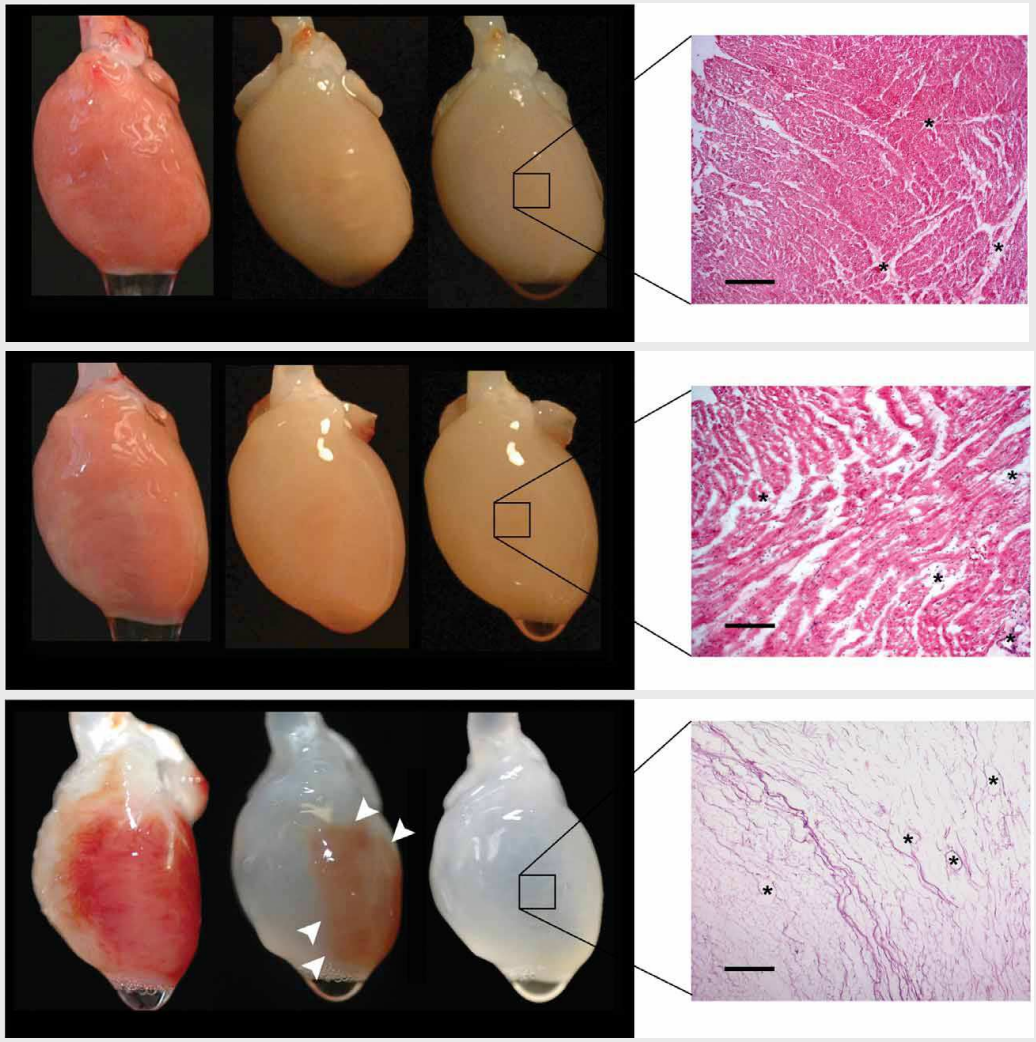
- supply of donor organs is limited
- lifelong immunosuppression and often heart failure for hypertension, diabetes and renal failure
- ‘thick’ (4100–200 mm) cardiac patches cannot create the geometry necessary to support the high oxygen and energy demands of cardiomyocytes at a depth greater than 100 mm from the surface

experiment

- decellularized hearts by coronary perfusion with detergents, preserving the underlying extracellular matrix → produced an acellular, perfusable vascular architecture
- reseeded these constructs with cardiac or endothelial cells.
- maintained eight constructs for up to 28 d by coronary
- perfusion in a bioreactor that simulated cardiac physiology.
- day 4 → macroscopic contractions.
- day 8, under physiological load and electrical stimulation, constructs could generate pump function (equivalent to about 2% of adult or 25% of 16-week fetal heart function) in a modified working heart preparation.

- Perfusion decellularization of cadaveric hearts
  - : SDS, anterograde, 12 h, <4% original DNA, GAGs unchanged
- roperties of the decellularized construct
  - Collagens I and III, laminin and fibronectin; fiber composition and orientation of the myocardial ECM preserved, cardiac cells removed → compressed construct: valves OK

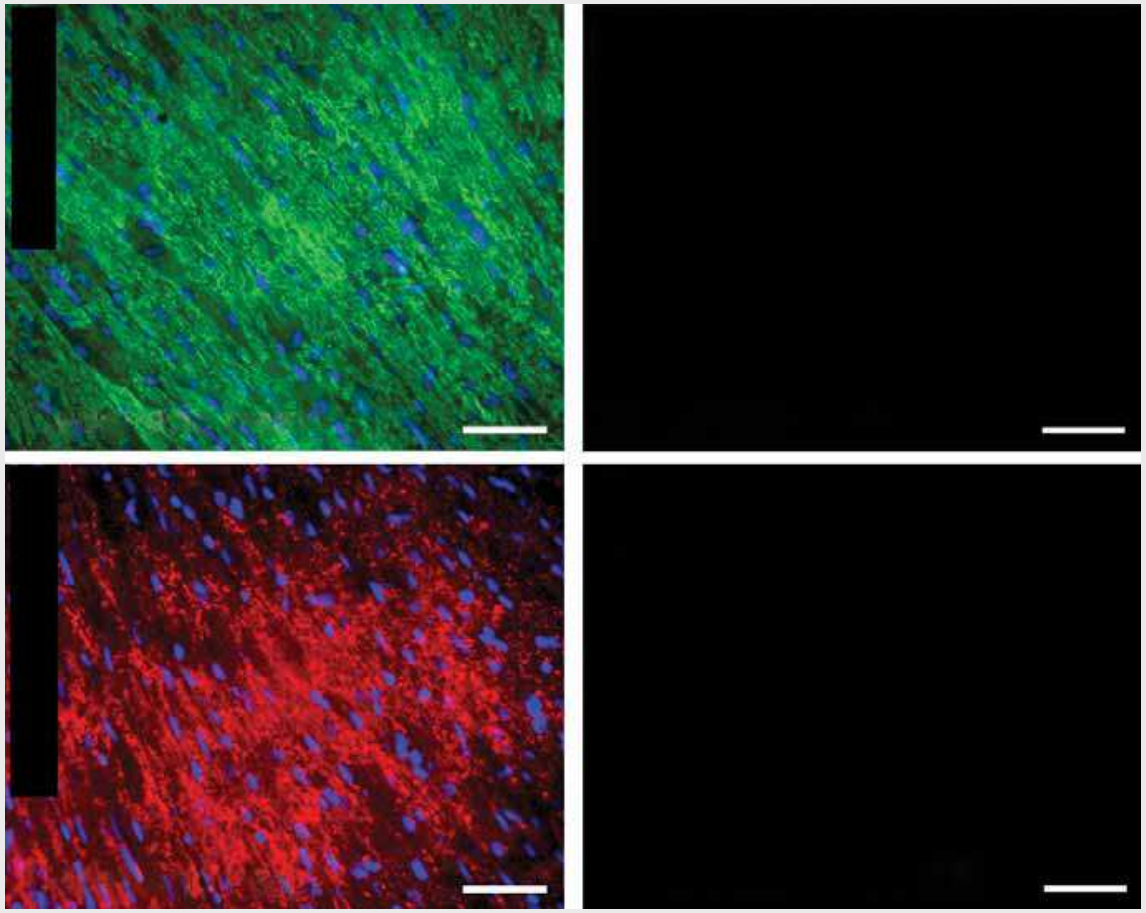
Perfusion decellularization (12h)



1% PEG

1% TX-100

1% SDS



a-MHC

DAPI

Fig 2

- Physical properties (stiffness, elasticity) OK
- ECM proteins (collagen, laminin, fibronectin) OK
- larger cardiac vessels and the smaller third- and fourth-level branches & valves OK, endothelial cells missing (direct perfusion of the coronary vasculature)
- Epicardium still present

Recellularization of decellularized cadaveric hearts – Fig 3

- Perfused in 2D cultures: → viable cardiac muscle with myofibers 0.25-1.10 thick
- In bioreactor and seeded with freshly isolated neonatal cardiac cells through intramural injection
- Induced pulsatile contraction → then spontaneous rythmic depolarization **Fig 4**

- Histology: 1mm thick, confluent cellularity, >95% alive cells
- Everywhere expression of sarcomeric actin, cardiac MHC, some areas vWF+,
- From d8: immature X-striated fibers, connexin 43

- Engineered heart
- Decell & recell → maturation

Electrical stimulation systems for cardiac tissue engineering

Nature Protocols, 2009; 4:155-163

- Cardiac cells cultured with the application od a pulsatile electrical field mimicking those in native heart