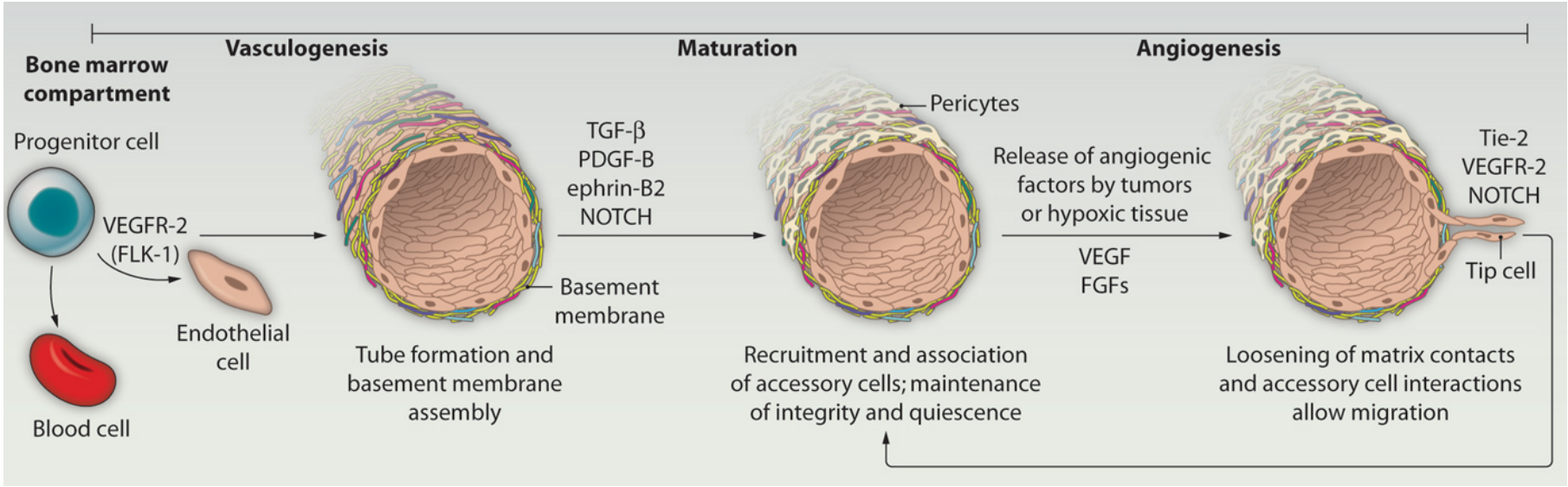
Lecture #14: Vascularization

The Need for Vascularization

- Bioreactor/culture conditions provide nutrients *in vitro*
- Vascularization eventually provides nutrients *in vivo*
- Nutrient supply is constrained during initial period following implantation, prior to full vascularization

Vasculogenesis & Angiogenesis



Bae et al., Sci Trans Med, 2012

Angiogenesis

- In order for your new tissue to incorporate into the tissue, new blood vessels must form for nutrient delivery and removal of waste

Angiogenesis Facts

- Blood vessels are comprised of cells called endothelial cells. The total surface area covered by these cells in an adult is 1000 m^2 -- roughly the size of a tennis court.
- If all the blood vessels in the body were lined up end-to-end, they would form a line that could circle the earth twice.
- Blood vessel cells do not normally grow in the healthy adult they are normally inactive, or quiescent.
- There are at least 20 different known angiogenic growth factors.
- Angiogenic growth factors are being tested in humans for growing new blood vessels to heal wounds and to restore blood flow to the heart, limbs, and brain.

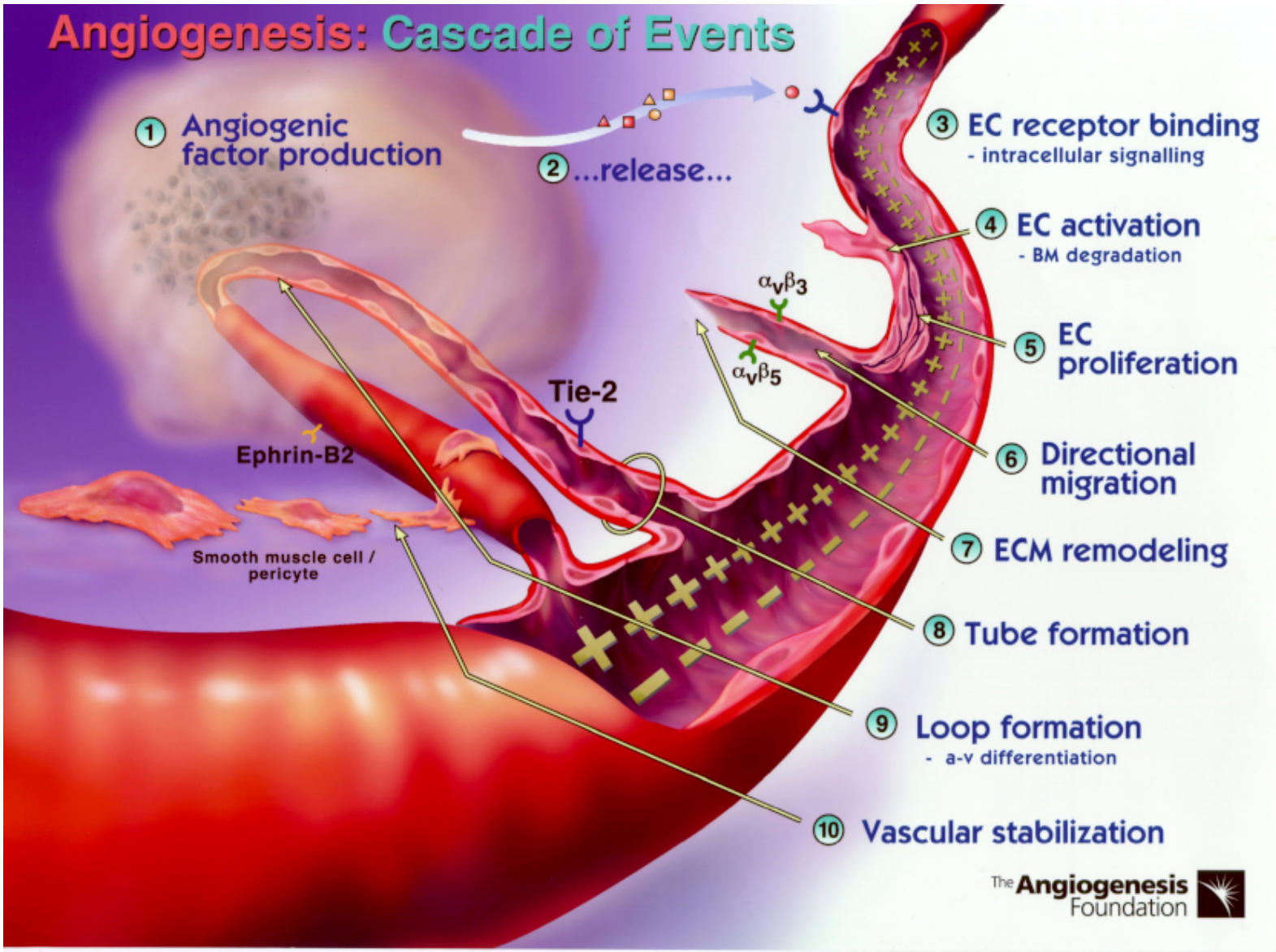
Angiogenesis Facts

- There are at least 30 known natural angiogenesis inhibitors found in the body.
- The first angiogenesis inhibitor molecule was discovered in 1975 by Dr. Judah Folkman and Dr. Henry Brem in a study of cartilage.
- Angiogenesis inhibitors have been discovered from natural sources, including tree bark, fungi, shark muscle and cartilage, sea coral, green tea, and herbs (licorice, ginseng, cumin, garlic).
- In total, more than 300 angiogenesis inhibitors have been discovered to date.
- The first successful treatment of an angiogenesis-dependent disease occurred in 1989, when the drug interferon alpha2a, an angiogenesis inhibitor, was used to regress the abnormal blood vessels growing in the lungs of a boy with a benign disease called pulmonary hemangiomatosis.

Angiogenesis Facts

- The first FDA-approved device to stimulate new blood vessels to grow in diseased hearts is a laser used in a technique called Direct Myocardial Revascularization, or DMR (sometimes called transmyocardial revascularization, TMR).
- The first FDA-approved blood vessel therapy for eye disease is a type of photodynamic therapy called Visudyne (QLT Therapeutics/CibaVision), which has shown effectiveness for treating macular degeneration.
- The first angiogenesis-stimulating medicine is a prescription gel called Regranex (recombinant human platelet-derived growth factor-BB, Ortho-McNeil) that became FDA-approved to heal diabetic foot ulcers in December 1997- last lecture *black box malignancy warning.
- More than $4 billion has been invested in the research and development angiogenesis-based medicines, making this one of the most heavily funded areas of medical research in human history.

Angiogenesis Process



Modifying Angiogenesis

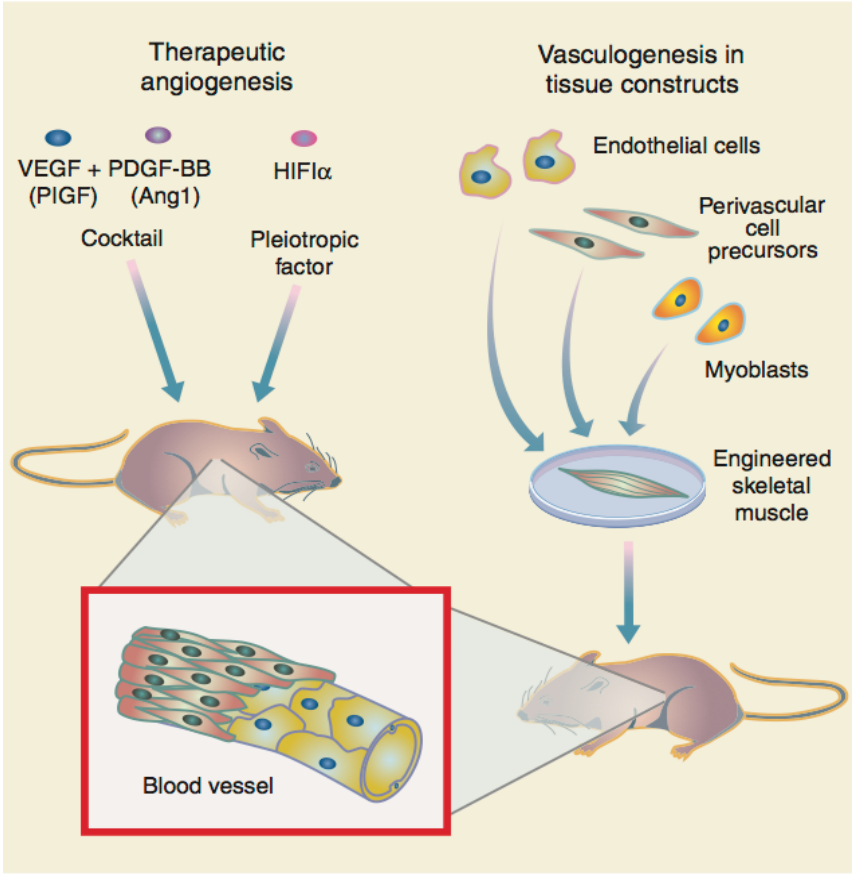
- Induction
  - Induction is necessary in new tissues when transport is limited to the tissue (engraftment, wound healing)
- Suppression
  - Especially useful in cancer due to increased angiogenesis near tumors

Complex interactions with immune system

Acceleration of Vascularization

A. Deliver angiogenic growth factors
   1. VEGF, PDGF, etc.
   2. Controlled release from the scaffold
   3. Genetically modify cells to increase expression of factors

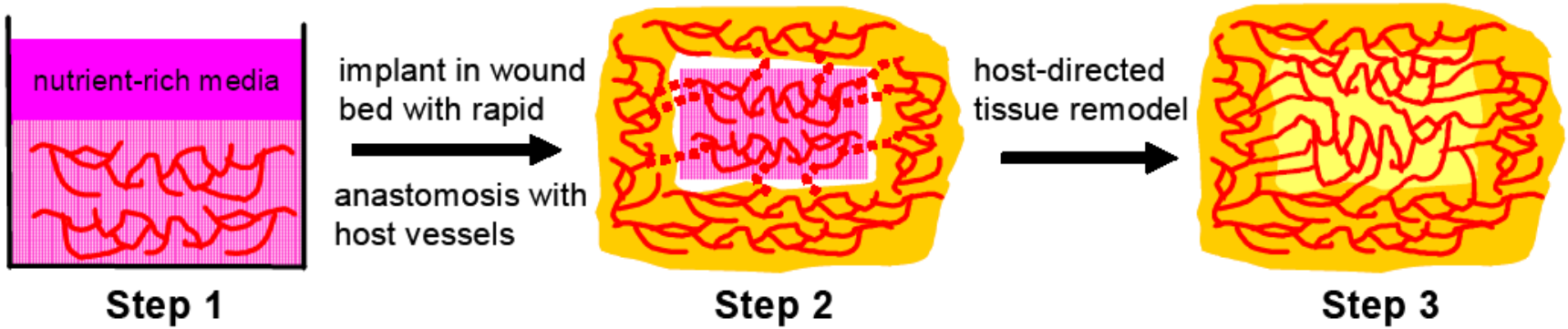
B. Pre-vascularize the construct in vitro



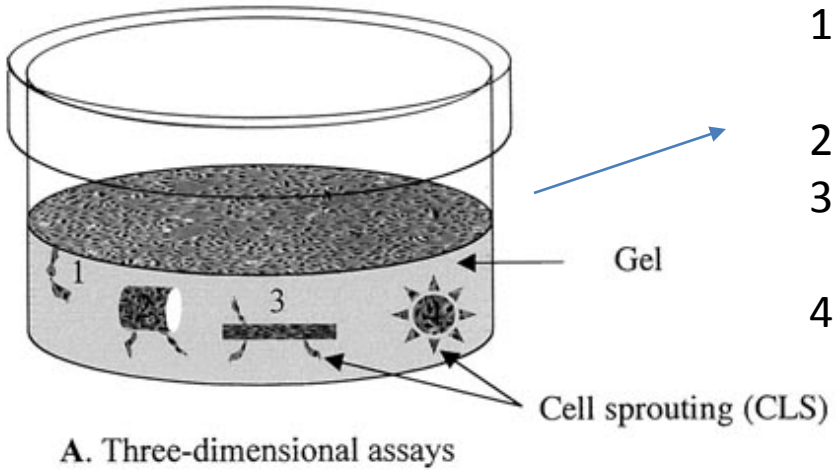
re 1 Strategies for promoting neovascularization and for engineering vascularized muscle tissu

From Jain, et al., Nat. Biotech., 2005

Prevascularized Constructs

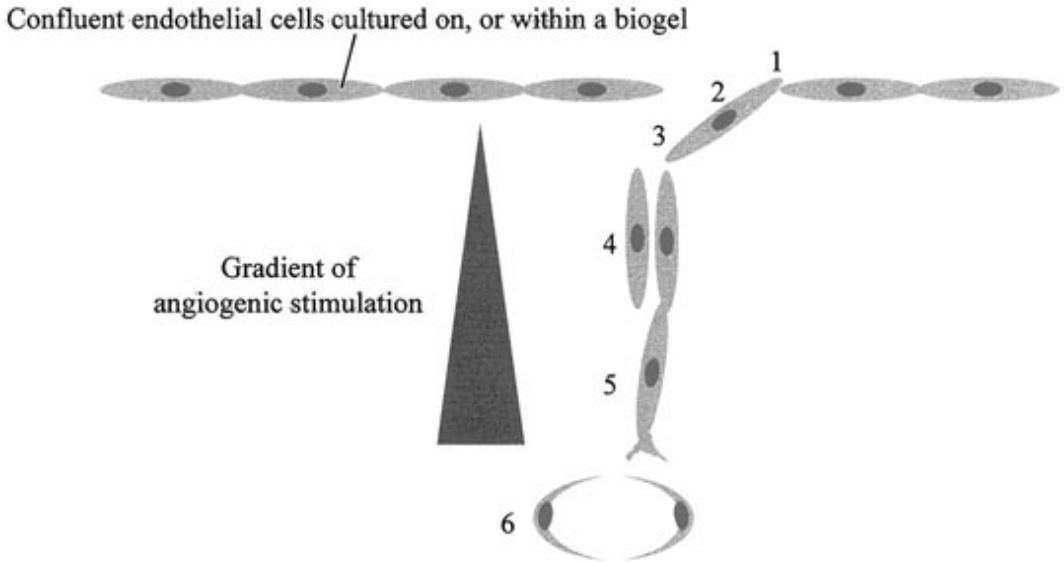


In Vitro Angiogenesis Models



A. Three-dimensional assays

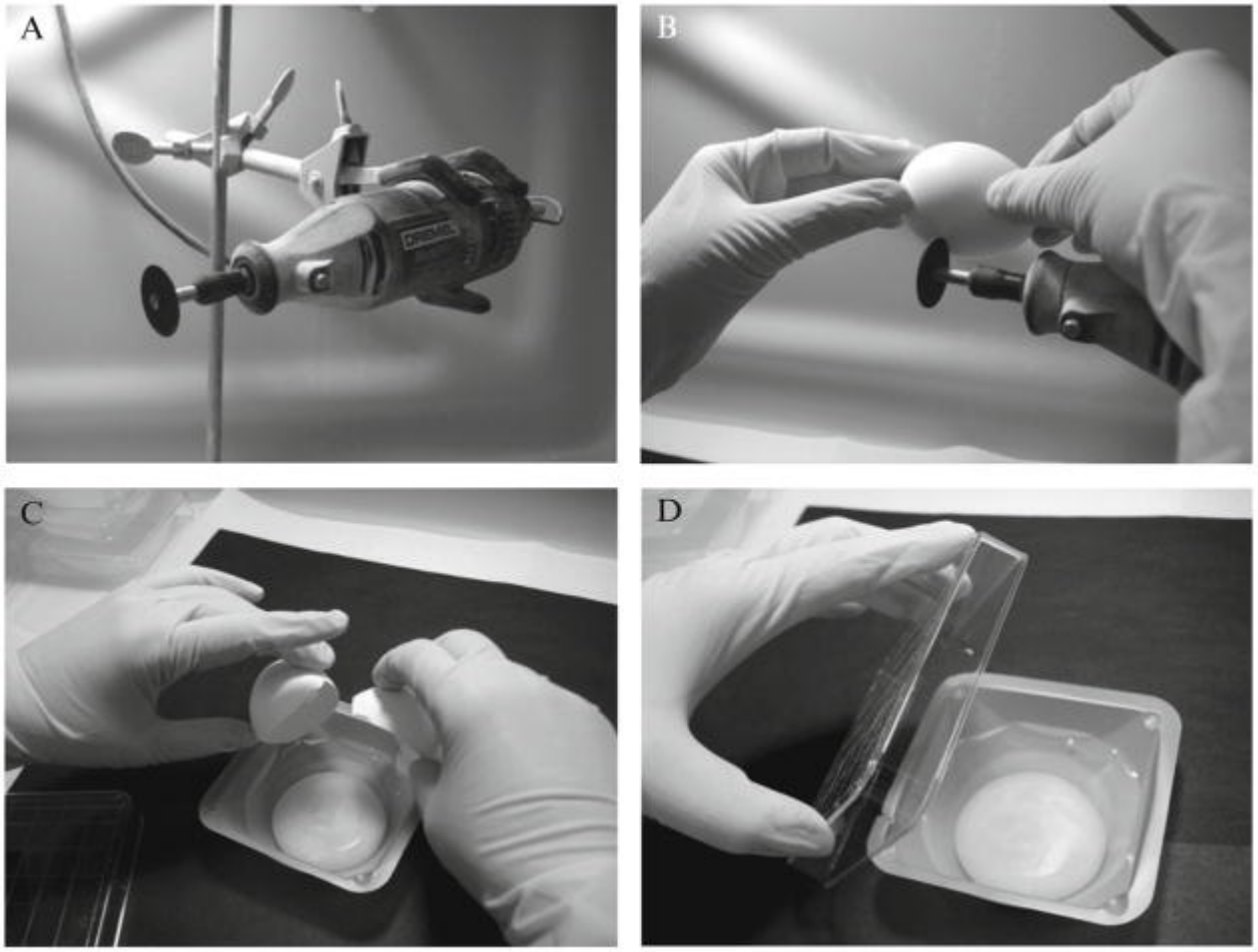
1. Growth into gel from top monolayer
2. Explant outgrowth
3. Explant outgrowth (different size vessel)
4. Outgrowth from solid material surface



Vailhe et al, Lab Invest, 2001

In Vivo Angiogenesis Models

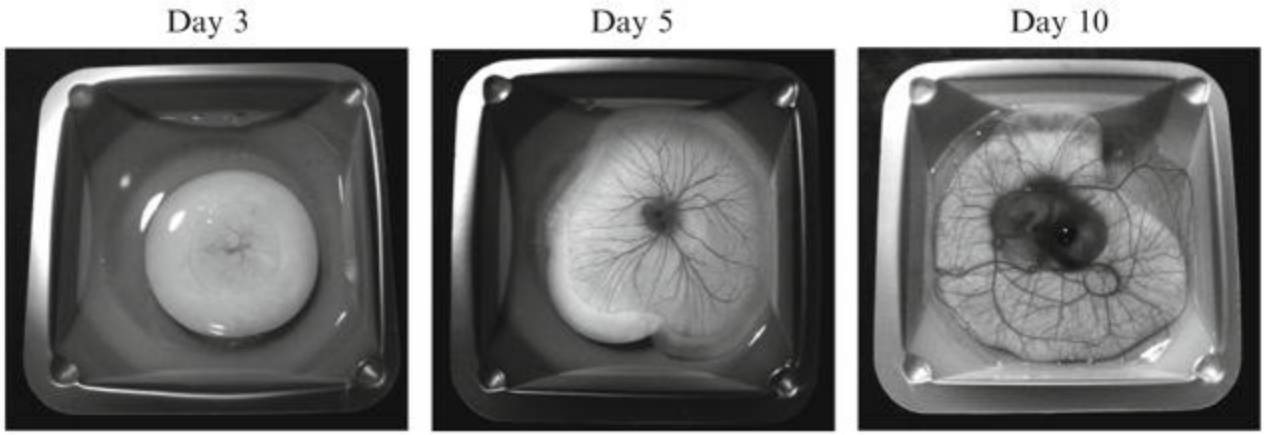
Chick Chorioallantoic Membrane (CAM) Assay



Deryugina, Methods Enzymol, 2009

In Vivo Angiogenesis Models

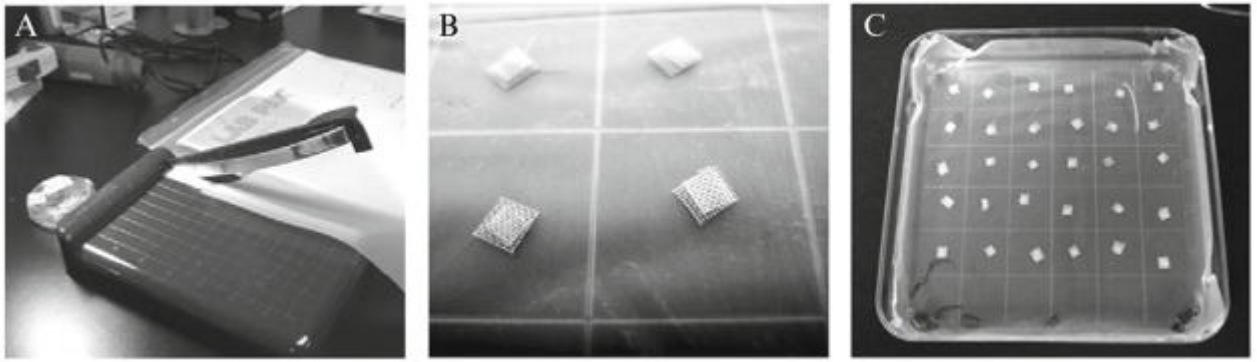
Chick Chorioallantoic Membrane (CAM) Assay



Deryugina, Methods Enzymol, 2009

In Vivo Angiogenesis Models

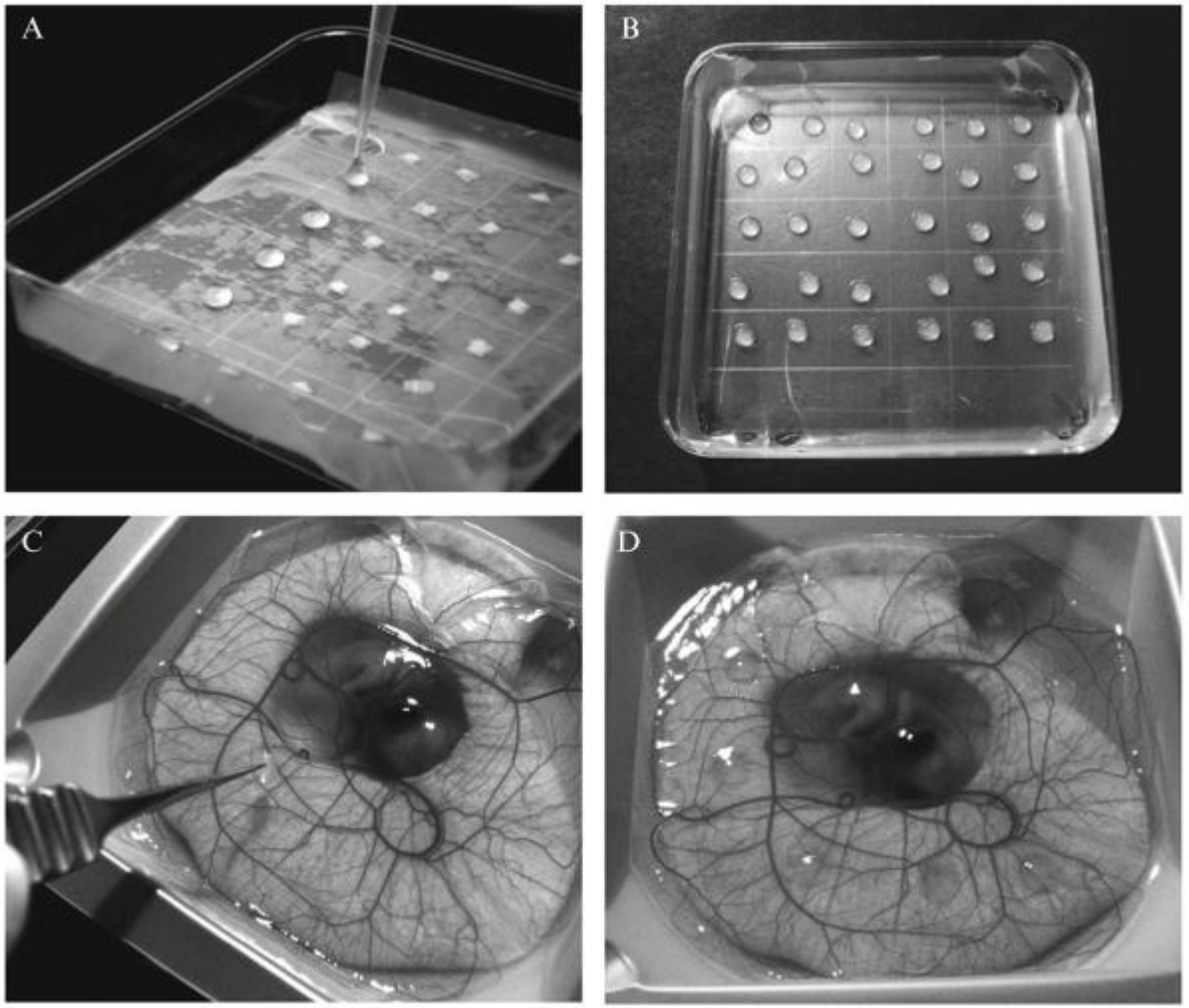
Chick Chorioallantoic Membrane (CAM) Assay



Deryugina, Methods Enzymol, 2009

In Vivo Angiogenesis Models

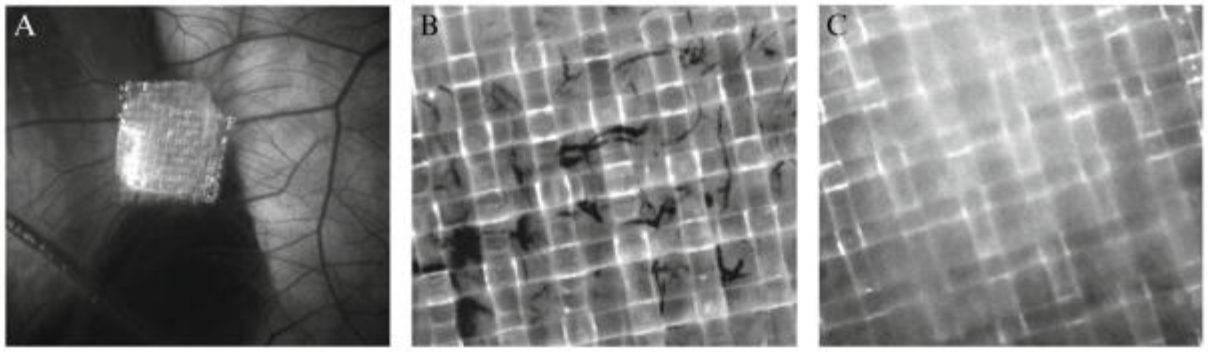
Chick Chorioallantoic Membrane (CAM) Assay



Deryugina, Methods Enzymol, 2009

In Vivo Angiogenesis Models

Chick Chorioallantoic Membrane (CAM) Assay



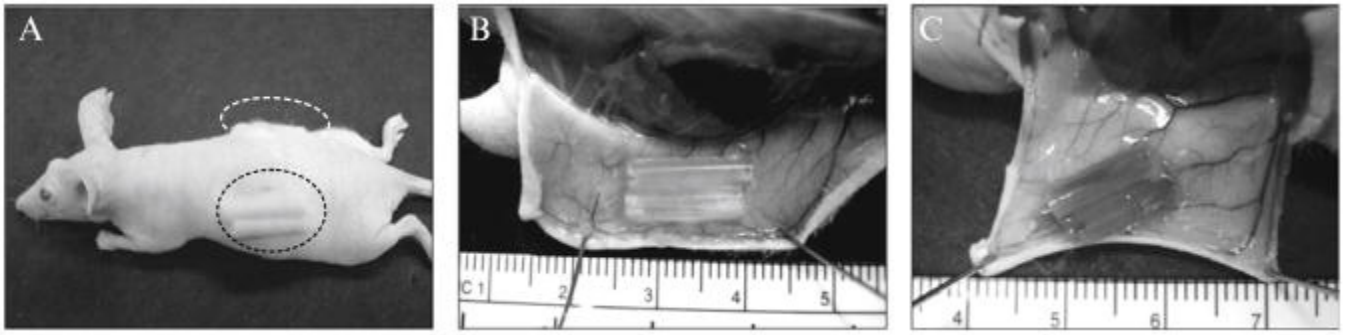
+ angiogenic stimuli (e.g. VEGF)

no stimuli

Deryugina, Methods Enzymol, 2009

In Vivo Angiogenesis Models

Subcutaneous Mouse Model

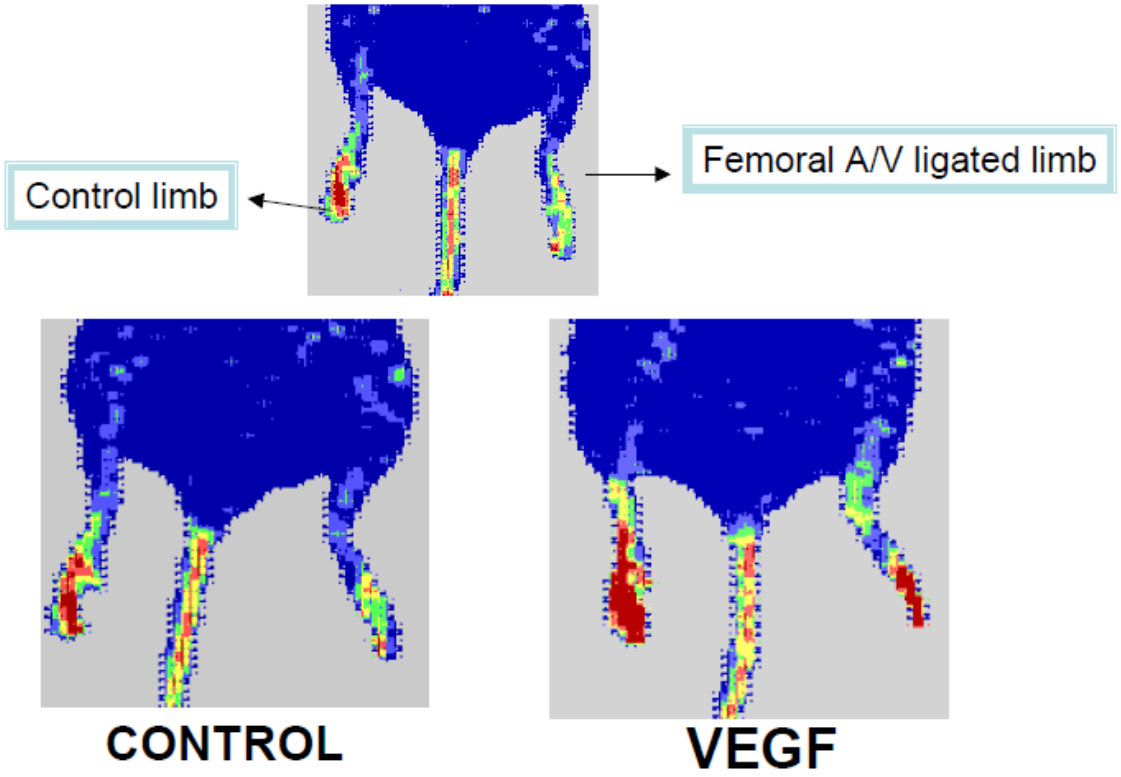


Deryugina, Methods Enzymol, 2009

Assess In Vivo Angiogenesis

Doppler Perfusion Imaging

Moving Blood Cells Shift the Frequency of Incident Light According to the Doppler Principle



David Mooney

Pre-Vascularized Tissues

nature biotechnology

Engineering vascularized skeletal muscle tissue

Shulamit Levenberg[1,2], Jeroen Rouwkema[3], Mara Macdonald[2], Evan S Garfein[4], Daniel S Kohane[5], Diane C Darland[6], Robert Marini[7], Clemens A van Blitterswijk[3], Richard C Mulligan[8], Patricia A D'Amore[6] & Robert Langer[2]

NATURE BIOTECHNOLOGY VOLUME 23 NUMBER 7 JULY 2005

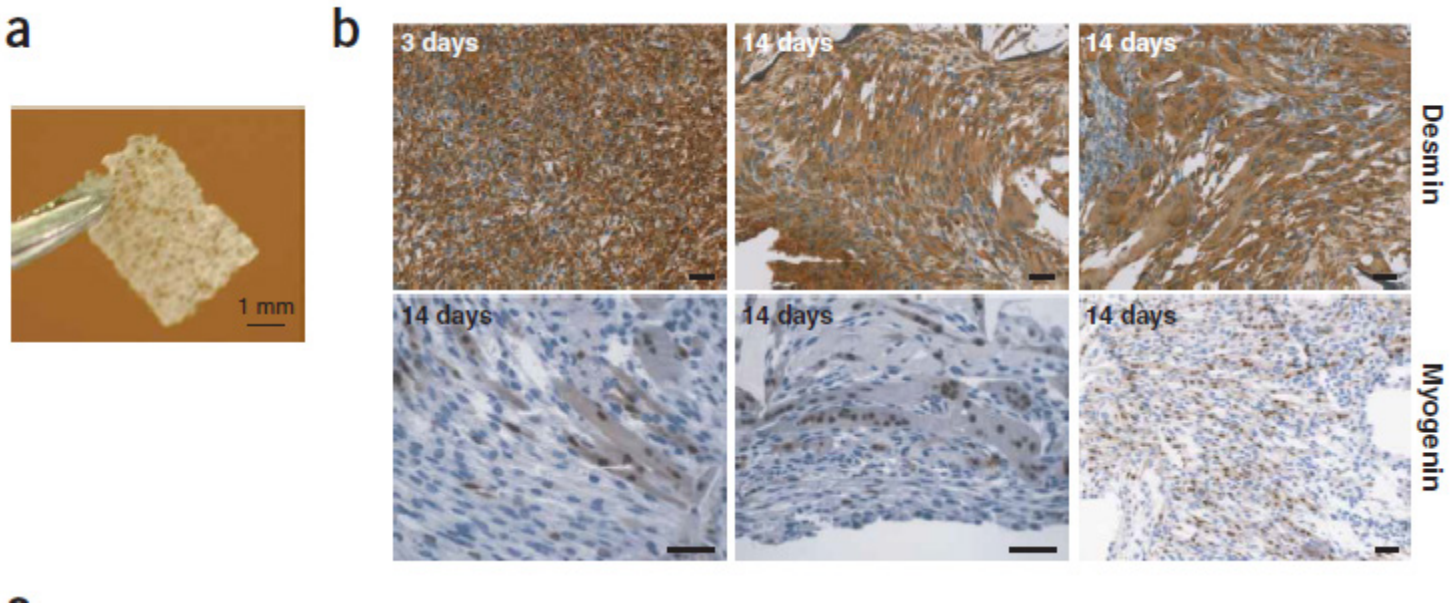

a
1 mm
b
3 days
14 days
14 days
Desmin
14 days
14 days
14 days
Myogenin

Figure 1a,b

Porous Scaffold: Salt Leaching

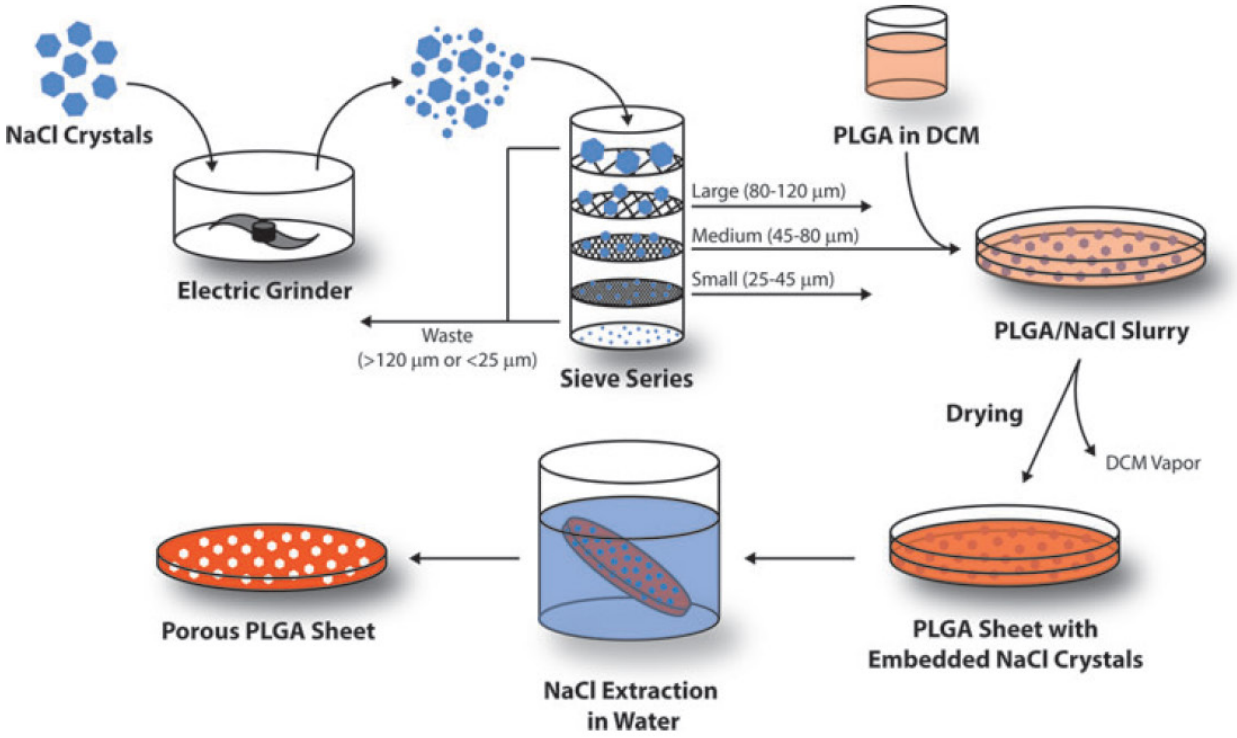


FIG. 1. Schematic of the salt leaching/solvent casting method used to fabricate poly(lactic-co-glycolic acid) (PLGA) scaffolds with various pore sizes.

Worthington et al. J Ocular Pharm & Ther, 2016

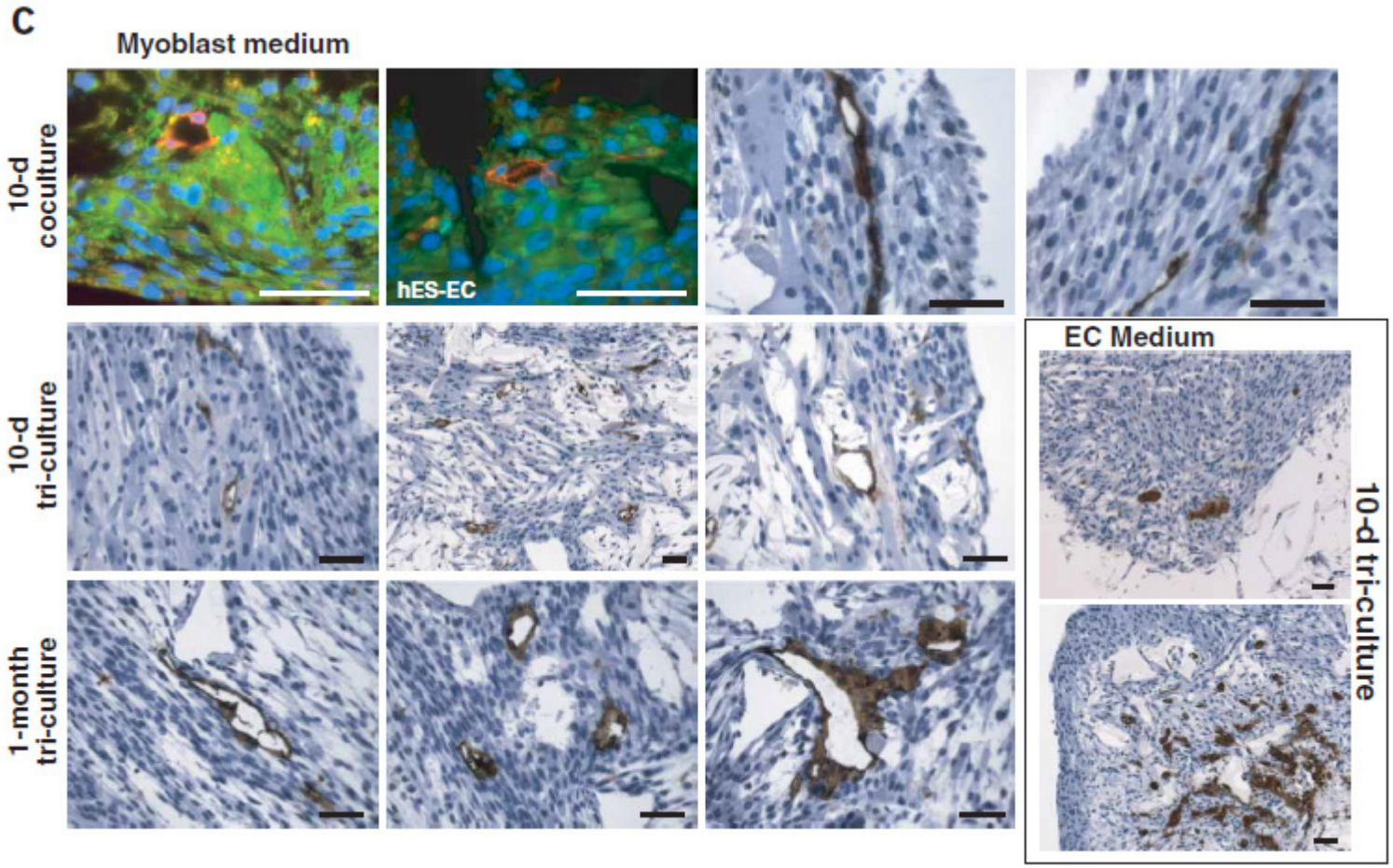

C
Myoblast medium
10-d coculture
hES-EC
10-d tri-culture
1-month tri-culture
EC Medium
10-d tri-culture

Figure 1c

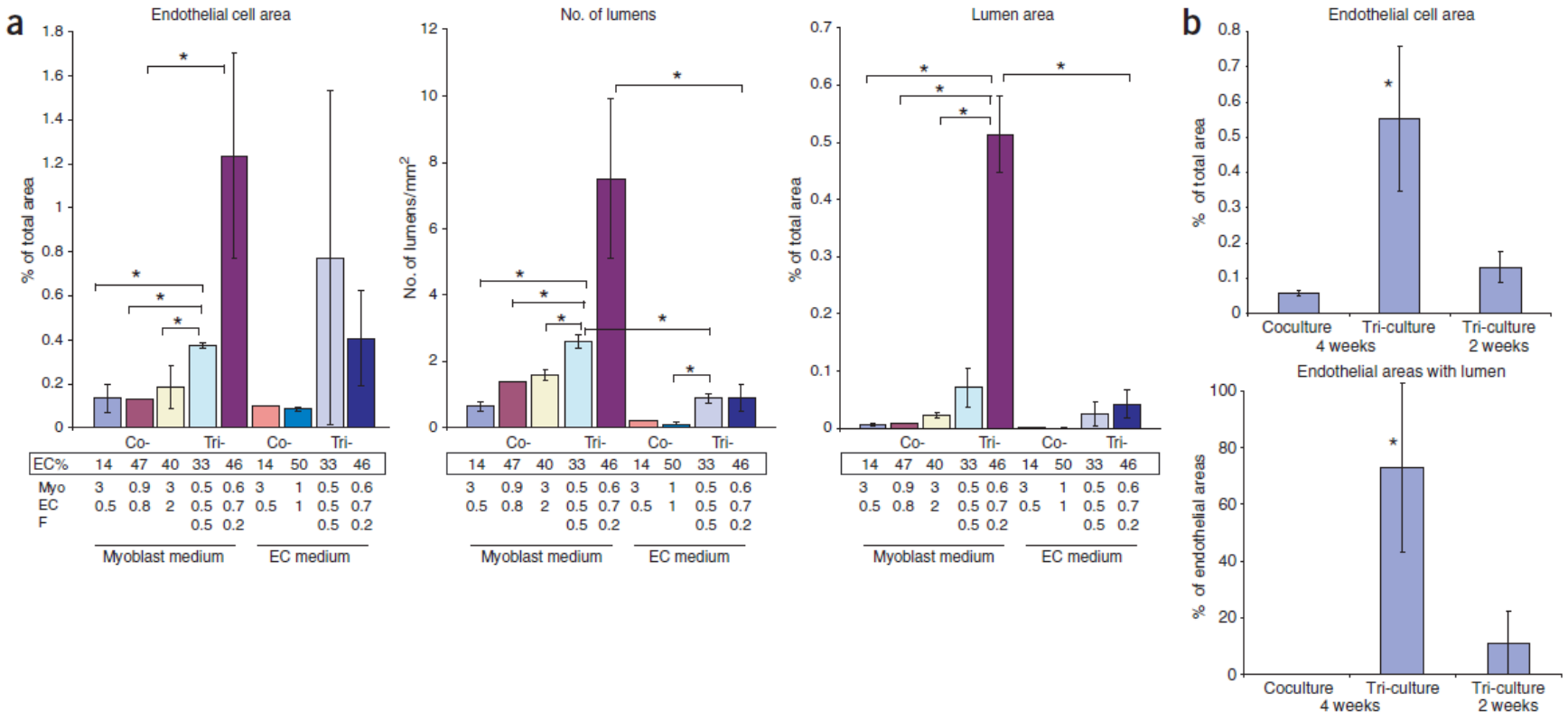
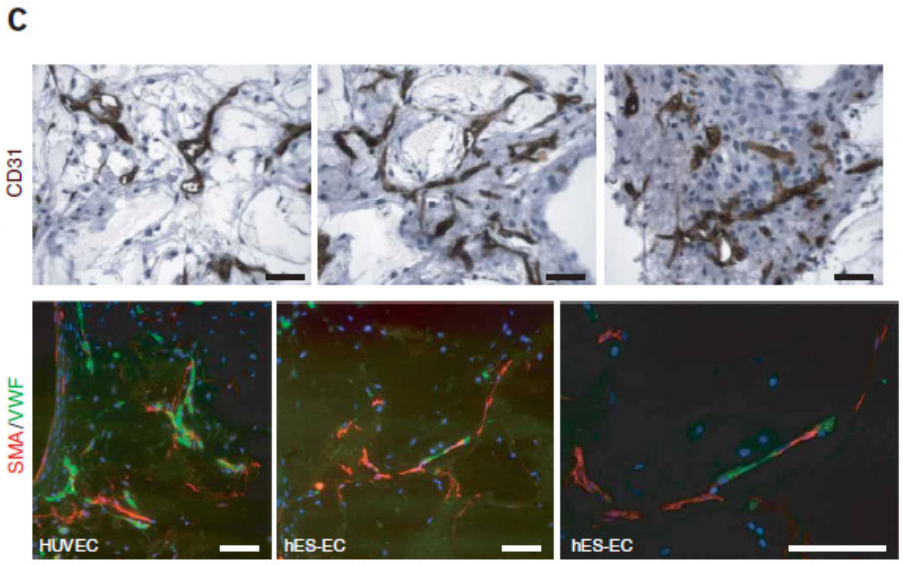

a
Endothelial cell area
% of total area
No. of lumens
No. of lumens/mm2
Lumen area
% of total area
Co-
Tri-
Co-
Tri-
EC%
14 47 40 33 46 14 50 33 46
Myo
3 0.9 3 0.5 0.6 3 1 0.5 0.6
EC
0.5 0.8 2 0.5 0.7 0.5 1 0.5 0.7
F
0.5 0.2 0.5 0.2
Myoblast medium
EC medium
b
Endothelial cell area
% of total area
Coculture 4 weeks
Tri-culture
Tri-culture 2 weeks
Endothelial areas with lumen
% of endothelial areas
Coculture 4 weeks
Tri-culture
Tri-culture 2 weeks

Figure 2a,b


c
CD31
SMA/VWF
HUVEC
hES-EC
hES-EC

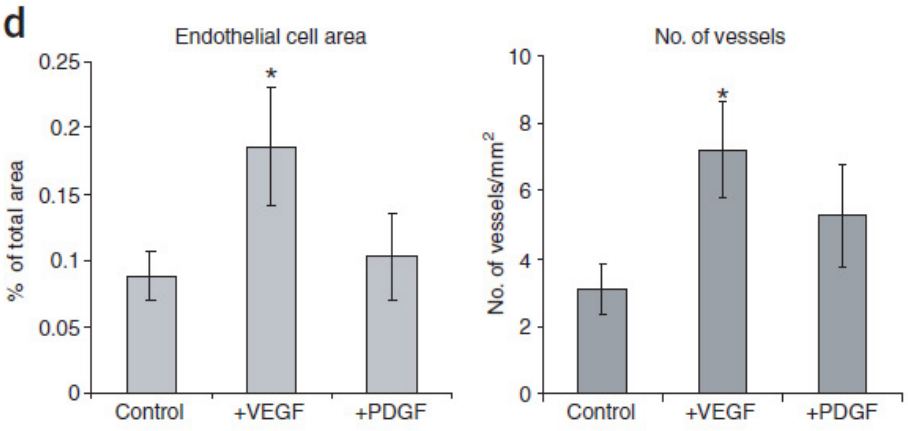

d
Endothelial cell area
% of total area
0.25
0.2
0.15
0.1
0.05
0
Control
+VEGF
+PDGF
No. of vessels
No. of vessels/mm2
10
8
6
4
2
0
Control
+VEGF
+PDGF

Figure 2c,d

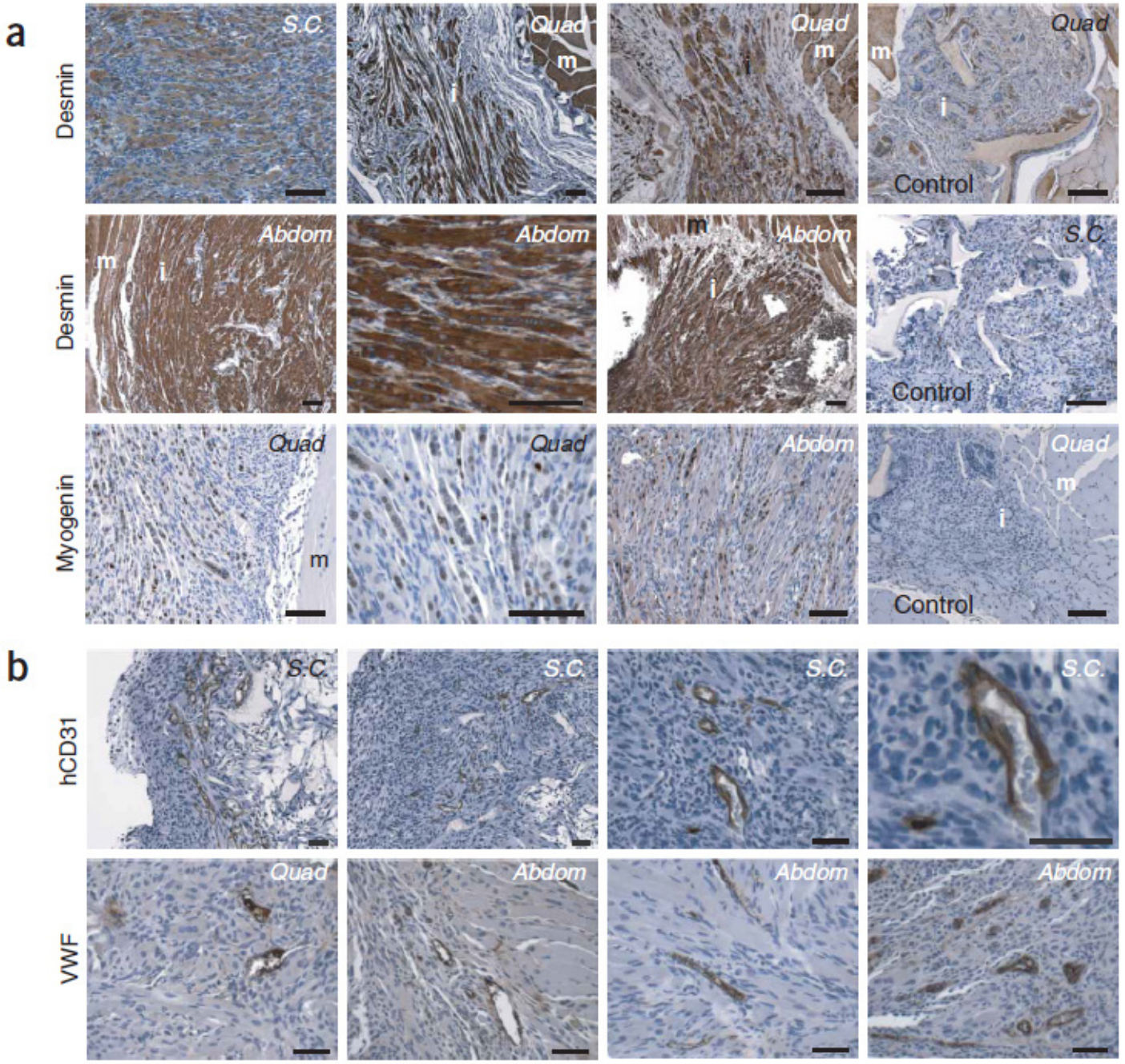

a
Desmin
S.C.
Quad
m
i
Quad
m
i
Quad
m
i
Control
Desmin
Abdom
m
i
Abdom
m
Abdom
i
S.C.
Control
Myogenin
Quad
m
Quad
Abdom
Quad
m
i
Control
b
hCD31
S.C.
S.C.
S.C.
S.C.
VWF
Quad
Abdom
Abdom
Abdom

Figure 3a,b

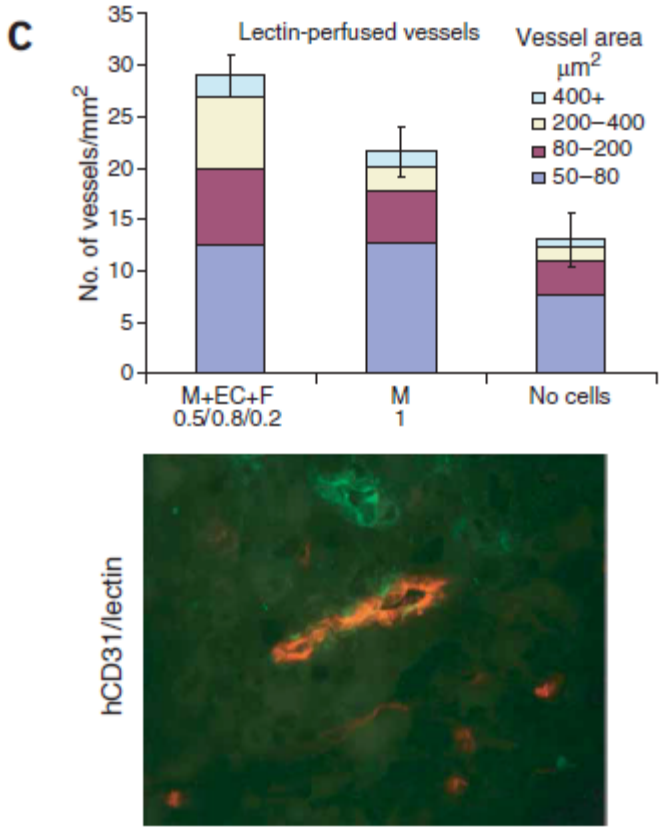
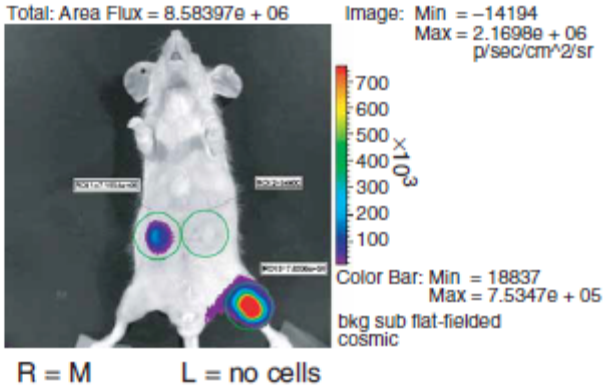
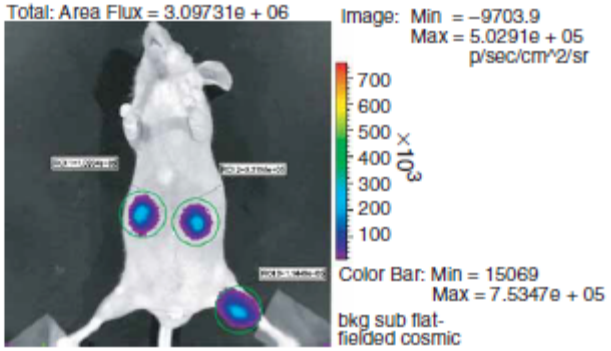

C
Lectin-perfused vessels
Vessel area
µm2
400+
200–400
80–200
50–80
No. of vessels/mm2
35
30
25
20
15
10
5
0
M+EC+F
0.5/0.8/0.2
M
1
No cells
hCD31/lectin

Figure 3c

d



R = M L = no cells



R, L = M + EC + F

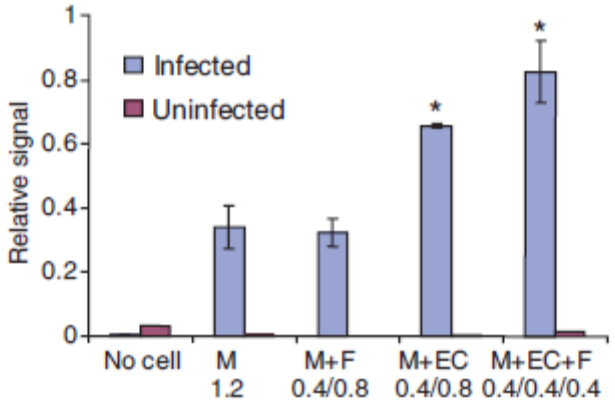
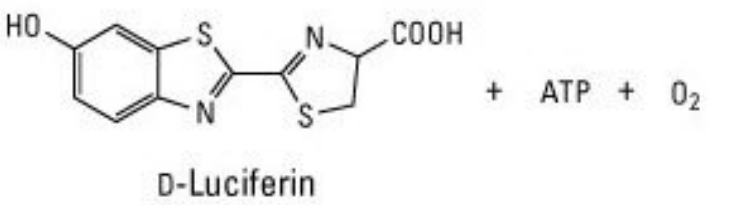


Figure 3d

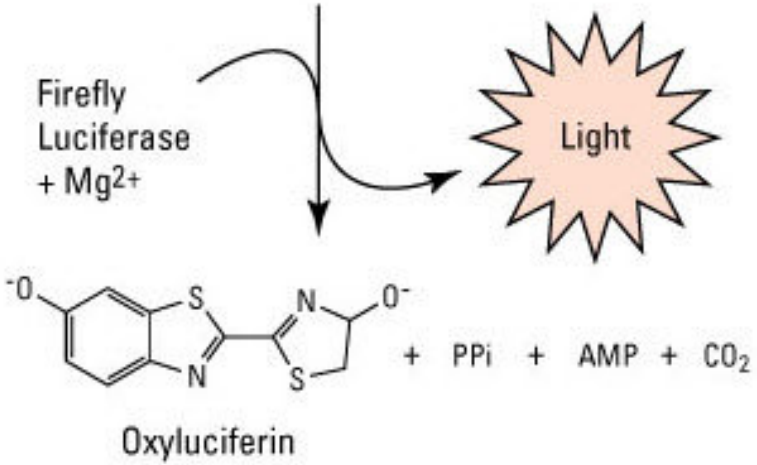
Luminescence (Luciferase-Luciferin)

Injected in blood circulation in Fig. 3d (previous slide)



D-Luciferin

Made by cells in Fig. 3d (previous slide)



Oxyluciferin

Thermo Fisher