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

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

Angiogenesis: Cascade of Events

1. Angiogenic factor production
2. Release
3. EC receptor binding - intracellular signalling
4. EC activation - BM degradation
5. EC proliferation
6. Directional migration
7. ECM remodeling
8. Tube formation
9. Loop formation - α→→ differentation
10. Vascular stabilization
Mechanisms of Angiogenesis

Van Blitterswijk, Fig. 4.8
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

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

**Step 1**
- Nutrient-rich media
- Implant in wound bed with rapid anastomosis with host vessels

**Step 2**
- Host-directed tissue remodel

**Step 3**
In Vitro Angiogenesis Models

A. Two-dimensional, short term assay

B. Two-dimensional, long term assay

Vailhe et al, Lab Invest, 2001
In Vitro Angiogenesis Models

A. Three-dimensional assays

Cell sprouting (CLS)

Confluent endothelial cells cultured on, or within a biogel

Gradient of angiogenic stimulation

B.

1: Activation of endothelial cells (cytokines, hypoxia, extracellular matrix components)
2: Limited proteolysis of the matrix (plasmin, metalloproteases)
3: Modification of cell cell contacts (cell adhesion molecules: integrins, cadherins, PECAM-1...)
4: Cell proliferation (angiogenic growth factors)
5: Migration (chemokines, extracellular matrix, integrins)
6: Morphological differentiation and tubulogenesis (cytoskeleton, mechanical forces, cell-cell adhesion molecules)

Vailhe et al, Lab Invest, 2001
In Vivo Angiogenesis Models
Chick Chorioallantoic Membrane (CAM) Assay

Deryugina, Methods Enzymol, 2009
In Vivo Angiogenesis Models
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In Vivo Angiogenesis Models
Chick Chorioallantoic Membrane (CAM) Assay

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
Perfusion

Incidence of Necrosis
Pre-Vascularized Tissues

Engineering vascularized skeletal muscle tissue

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Figure 2a,b
Figure 3a,b
Figure 3d
Luminescence (Luciferase-Luciferin)

- Made by cells in Fig. 3d (previous slide)
- Injected in blood circulation in Fig. 3d (previous slide)