Controlling morphogen/growth factor presentation via controlled release

David Mooney
Harvard University
CARDIOVASCULAR DISEASE

- Claimed 959,000 lives (1999)
- Leading cause death in USA
- Diseases of vessels major subset (600,000 procedures/year)
VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF)

- Secreted heparin-binding protein
- Promotes endothelial cell migration, survival, proliferation
- Initiator of angiogenic cascade

(Gerwins et al., *Critical Reviews in Oncology-Hematology*. 2000)
The Path

• Quantitative biological data (concentrations, gradients, duration)

• Factor distribution in vivo

• Delivery technologies

3D in vitro models

mathematical models

polymer systems
Role of \([V], \frac{d[V]}{dt}, \text{ and } \frac{d[V]}{dr}\):

Starting vessel

EC

ECM

Mural cell

New, immature vessel

[VEGF]

Rapid degradation

\(t_{1/2} \sim 30-90 \text{ min}\)

Diffusion away

Apoptotic ECs
3D Sprouting Assay

- Microcarriers were coated with human microvascular endothelial cells (HDMECs)
  - Beads provide a depot of cells from which sprouting occurs
- Beads were embedded in a fibrin gel and cultured with media containing growth factor(s) of interest
- Sprout formation was quantified after 5 days

**SUMMARY:**

Sprouting maximal \( 10 < [V] < 250 \text{ ng/ml} \)

Directionality \( [V] < 250 \text{ ng/ml} \)
& \( d[V]/dr > 25 \text{ pg/ml} \)
LIMITATIONS CURRENT PROTEIN DELIVERY

- poor availability with oral administration
- introduced in solution systemically, or into tissue of interest

Intravenous/intracoronary injection < 0.1% myocardium (24 hr)
Tissue directed delivery > 95% lost (days)

= Deliver supraphysiologic quantities (mg instead of ng-ug)
  - Expansion atherosclerotic lesions
  - Growth tumors
  - Neovascularization in non-target tissues
FAILURE OF CLINICAL TRIALS

<table>
<thead>
<tr>
<th>Phase I</th>
<th>10’s patients</th>
<th>VEGF\textsubscript{165}</th>
<th>SAFE, BENEFIT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase II</td>
<td>179 patients</td>
<td>VEGF\textsubscript{165}</td>
<td>LITTLE EFFECT</td>
</tr>
</tbody>
</table>

(Henry et al., 1999)

(Eppler SM et al., 2002)
Concentration; Time; Location

Local Concentration
= In - Out - Production/Consumption

- $\frac{\partial C}{\partial t} = \kappa \nabla^2 C - \nabla C_v + R$
  - $C$ = growth factor concentration
  - $\kappa \nabla^2 C$ = diffusion
  - $\nabla C_v$ = fluid flow
  - $R$ = Production/consumption
RAPID DRUG DESTRUCTION

- VEGF < 90 min
- Insulin < 25 min.
- Growth hormone < 25 min.
- Parathyroid hormone < 15 min.
DRUG CONCENTRATION PROFILE

Time (hr)

Drug concentration

desired range

next dose
DRUG CONCENTRATION PROFILE

Diagram showing the drug concentration over time (day-week) with a desired range indicated by dashed lines.
Modeling Tissue Distribution

\[ \frac{\partial c_i}{\partial t} = D_V \nabla^2 c_i + k_r c_{oi} - k_c c_i \] (simple diffusion, reaction models)
REQUIRE LOCALIZED THERAPY?

Diabetes

↓ vessel density
↓ VEGF signaling

↑ vessel density
↑ VEGF signaling
Reservoir system

- Drug in highly saturated state w/in reservoir inside of device.
- Drug diffuses through polymer membrane

\[ J = -D \frac{\partial C}{\partial r} \]

(Fick’s 1st Law)
Bioerodible matrix system

biodegradable polymers
SUSTAINED, LOCAL POLYMER DELIVERY

Bioactive molecule

PLGA → CO₂ → NaCl → foamed PLGA → H₂O → open pores

Shea et al., Nature Biotechnol. 1999

\[
\text{Average % of Total Protein Released}
\]

\[
\text{Time (Days)}
\]

In vivo

In vitro
Injectable Alginate Hydrogels
VEGF Tissue Distribution:

Sustained, Localized Delivery

Temporal distribution of VEGF$_{165}$ in tissue (gel delivered)

(Silva et al., *J Thromb Haem* 2007)
PERIPHERAL ISCHEMIA: FEMORAL ARTERY/VEIN LIGATION

Femoral A/V ligated limb

Control limb

Femoral A/V ligated limb

CONTROL

VEGF

Laser Doppler Perfusion Imaging
(Moving blood cells shift the frequency of incident light according to the Doppler principle)
ISCHEMIC HINDLIMB: VASCULARIZATION

A

B

Blank scaffold condition - 6 weeks

VEGF scaffold condition – 6 weeks
PERFUSION

INCIDENCE OF NECROSIS

**Graph C**
- X-axis: Days post-surgery
- Y-axis: Ischemic/non-ischemic hindlimb perfusion ratio
- Comparison between C57BL/6 and SCID models

**Graph D**
- X-axis: Days post-surgery
- Y-axis: Ischemic/non-ischemic hindlimb perfusion ratio
- Comparison between C57BL/6 and SCID models

**Bar Chart**
- X-axis: Time (weeks)
- Y-axis: Distribution of Hindlimb ischemia severity
- Comparison between Bolus VEGF and Gel/VEGF treatments
- Legend:
  - Necrotic foot
  - Multiple necrotic toes
  - One necrotic toe
  - Tips of toes (black)
  - Normal
Require multiple, sequential factor delivery?

Starting vessel

New, immature vessel

Mural cell association

Mature vessel with ECM

ECM

EC

Mural cell

VEGF, Ang2

PDGF, Ang1

TGF-β

VEGF

Apoptotic ECs

Ang 1/2 = angiopoietin 1 and 2; PDGF = platelet derived growth factor; TGF=transforming growth factor.
DYNAMIC FACTOR PRESENTATION

![Graph showing dynamic factor presentation]

- **Factor 1**: Exhibits a peak concentration at around 10 days, followed by a rapid decline.
- **Factor 2**: Shows a more gradual increase and then a steady decrease over time.
- **Factor 3**: Consists of numerous data points distributed over the 60-day period, indicating a consistent, albeit low, concentration level.
SEQUENTIAL DELIVERY OF FACTORS
Gene Therapy

(1) ex vivo followed by cell tx.

(2) in vivo

“The challenge is to develop gene therapy as an efficient and safe drug delivery system.”
Method of Gene Delivery

**Viral**
- High expression levels
- Potential long term expression
- Safety concerns

**Non-Viral**
- Minimal safety concerns
- Manufacturing cost
- Low expression levels

- Sept. 1999: 18 year old Jessie Gelsinger dies after receiving adenovirus intended to cure liver defect

- X-linked SCID: 14/15 French boys cured with gene therapy
  - 2 diagnosed with cancer 2002
  - 1 diagnosed cancer 2005
Gene Delivery from Scaffolds

Based on approach of Bonadio and co-workers
Sustained, controlled release of plasmid DNA

Cumulative DNA release (% of incorporated)

Time (days)

75:25 (i.v. = 0.2)
75:25 (i.v. = 0.7)
85:15 (i.v. = 1.4)
In vivo - nt β-gal plasmid
X-gal stain - 4 weeks

40X

400X
Non-Viral Gene Delivery Using Condensation

- Plasmid DNA
- Carrier Molecules
- DNA Condensate
- Plasma Membrane
- Lysosome
- Endocytosis
- Endosomal Escape
- Nuclear Uptake
Localized Gene Therapy

Poly(ethylenimine) (PEI)
- MW 25kDa, branched
- Proton sponge effect

(Huang et al., Human Gene Therapy 2005, & Gene Therapy 2005)
Sequential VEGF and PDGF Delivery

Blank
65+10/mm²
65% (+)

PDGF
81+12/mm²
78% (+)

VEGF
116+9/mm²
43% (+)

VEGF+PDGF
108+7/mm²
77% (+)

Rat Myocardial Infarct

Before treatment                4 weeks after treatment

* * *

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ANGIOGENESIS ON DEMAND:
FUNDAMENTAL TOOL REGENERATIVE BIOLOGY

Nerve regeneration

Bone regeneration

Therapeutic angiogenesis

Cell transplantation
**Students/Post-docs**
Ruth Chen
Eduardo Silva
Lonnie Shea
Yen Chen Huang
Darnell Kaigler

**Collaborators**

**Angiogenesis**
Mike Grossman (U Michigan)
Bob Guldberg (Georgia Tech)
Karen Hirschi (Baylor)
Peter Polverini (U Michigan)
Sanjay Rajagopalan (Ohio State)
Christer Sylven (Karolinska University)

**Bone**
Paul Krebsbach (U Michigan)