Molecular Basis of Mechanotransduction in Endothelial Cells

Shu Chien, M.D., Ph.D.
Departments of Bioengineering and Medicine, and
The Whitaker Institute of Biomedical Engineering
University of California San Diego, La Jolla, CA

May 16, 2006
Hemodynamic Forces Acting on The Blood Vessel

- Normal Stress
- Blood flow
- Shear Stress
- Stretch
Mechanotransduction is a fundamental homeostatic process in health and disease.
Hemodynamic Factors

Cell turnover

LDL

ECs

Blood

Monocyte

MCP-1*

Macrophage

Foam cell

Vessel wall

SMCs

* Monocyte Chemotactic Protein-1

ox-LDL

* LDL
Atherosclerotic Lesions are Preferentially Located at Regions with Complex Flow Patterns
Rectangular Flow Chamber to Study the Effects of Laminar Flows on EC Monolayer
Effects of Laminar Shear Stress (12 dyn/cm²) on MCP-1 Gene Expression in HUVECs
Transcription Factor
(e.g., AP-1 for MCP-I gene)

AP-1 is composed of cJun and cFos

Cis element in the promoter region of a gene
(e.g., TRE for MCP-I gene)
Roles of Ras and MAP Kinases in Shear-Induced Signal Transduction and Gene Expression

Shear stress

Membrane

Cytoplasm

GDP-Ras

GTP-Ras

ERK → cFos

JNK → cJun

Nucleus

AP-1 → MCP-1

TRE

Phosphorylation cascade.
Roles of Adapter Molecules Shc, Grb2 and Sos in Shear-Induced Signal Transduction and Gene Expression

Receptor Tyrosine Kinases (RTKs), e.g., the Vascular Endothelial Growth Factor (VEGF) Receptor Flk-1, can mediate the shear-induction of signaling.
Mediation of Shear-induced Signal Transduction by Integrins and Focal Adhesion Proteins

Shear stress

Integrins

e.g., $\alpha_v\beta_3$

FN, VN
Extracellular matrix

$\alpha_v\beta_3$ integrin interacts specifically with fibronectin and vitronectin, but not laminin or collagen.

Shear-induced integrin activation leads to its association with FA proteins.
Mediation of Shear-induced Signal Transduction by Integrins and Focal Adhesion Proteins

Shear stress

Flk-1
GTP
GDP
Tyr-P
Sos
Ras
Signaling pathways
Trans
Gene Exp.
Protein Exp.

Integrins

e.g., αvβ3
FN, VN
Extracellular matrix

Study of temporal and spatial characteristics of Src activation by Fluorescence Resonance Energy Transfer (FRET)
Design Strategy for a Novel Reporter for Src Activation by Fluorescence Resonance Energy Transfer (FRET)

CFP(1-227) SH2(from c-Src) Linker Substrate YFP

Src Substrate

Strong FRET

433 nm

527 nm

Src Activation

QuickTime™ and a Microsoft Video 1 decompressor are needed to see this picture.
Design Strategy for a Novel Reporter for Src Activation by Fluorescence Resonance Energy Transfer (FRET)

**CFP^{(1-227)}** SH2(from c-Src) **Linker** Substrate **YFP**

433 nm

**Strong FRET**

527 nm

**Weak FRET**

490 nm

Src Activation

QuickTime™ and a Microsoft Video 1 decompressor are needed to see this picture.
FRET Response of Src Reporter Induced by Pervanadate
(A phosphatase inhibitor that increases phosphorylation)

 Src Activation: YFP → CFP

Time → (2min between images)

Pervanadate

The results indicate that Src activity is increased by pervanadate.
FRET Response of Src Reporter Is Induced by Shear Stress

The results indicate that Src activity is increased by shear.
Pulling Fibronectin-coated Beads induced
A directional propagation of Src activation

(Color changes indicate increases of Src activity after pulling. Time interval between images = 2 min)
Effects of Physico-chemical Stimuli on Signal Transduction and Gene Expression

Functions: Secretion, Migration, Remodeling, Proliferation, Apoptosis, etc.
Effects of Laminar and Disturbed Flows on Wound Closure in EC Monolayer
**Effects of Flow Endothelial Cell Migration**

Laminar flow enhances wound healing, which is much slower under Disturbed flow.
Measurement of Cell Traction Force by Using the Beads-in-Membrane Technique

Cell

Fibronectin

Polyacrylamide Gel Embedded with Fluorescent Beads (D = 0.2 µm)

Glass Cover Slip

80 µm

Side View (not in proportion)
EC Traction on Silicon Membrane Containing Fluorescent Beads

A. No Flow
EC Traction on Silicon Membrane Containing Fluorescent Beads

B. 10 min. at 12 dyn/cm²
Flow
Force Balance in Mechanically Induced Cell Migration
Multi-factorial Mechanotransduction in Shear-sensing, Signal Transduction and Gene Expression

Functions: Secretion, Migration, Remodeling, Proliferation, Apoptosis, etc.
Laminar Flow $\rightarrow$ p53 $\rightarrow$ GADD45 $\rightarrow$ p21$^{cpl}$ $\rightarrow$ P-Rb $\downarrow$ P-Rb $\rightarrow$ Cell cycle arrest in G$_0$/G$_1$
Long-term Laminar Shear Stress Causes EC Arrest

![Graph showing the effect of static and shear stress on cell phases.](image-url)
Effects of Flow Patterns on Cell Turnover and Permeability

Straight part of the aorta is subjected to sustained laminar shear that leads to cell cycle arrest and hence reduced endothelial permeability.

Branch points have unsteady flow pattern, which accelerates cell turnover and increases permeability.
Endothelial Cell Mitosis (○) and Albumin Leaky Spots (■) In Rabbit Thoracic Aorta
Lipid Accumulation and Atherosclerotic Lesions around Intercostal Orifices

Lipid accumulation
(Rabbit Aorta)
D.C. Schwenke & T.E. Carew
Arteriosclerosis 9:895-918, 1989

Atherosclerotic lesions
(Human Aorta)
MCP-1 Staining and Subintimal WBC Localization around Intercostal Orifices

Histochemical Staining of MCP-1 (Rat Aorta)
G. Norwich & S. Chien

Intimal Distribution of WBC (Rabbit Aorta)
Malinauskas et al., Atherosclerosis 115:145, 1995
Oscillatory Shears with Different net forward Flows

Relative KLF2 mRNA Level (% of control)

- Pulsatile Shear: (12 ± 4 dyn/cm²)
- Reciprocating Shear: (0.5 ± 4 dyn/cm²)
Differential Regulation of KLF2 at Branch Points in Vivo
Effects of Local Constriction on KLF2 Expression in Vivo
# Blood Flow Patterns in Relation to Endothelial Cell Functions and Pathology

<table>
<thead>
<tr>
<th></th>
<th>Straight Part</th>
<th>Branch Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow Pattern</td>
<td>Laminar</td>
<td>Disturbed</td>
</tr>
<tr>
<td>Monocyte Adhesion</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>EC turnover &amp; LDL Permeability</td>
<td>↓</td>
<td></td>
</tr>
<tr>
<td>Effects on Atherogenesis</td>
<td>Anti-Athero.</td>
<td>Atherogenic</td>
</tr>
</tbody>
</table>
Atherosclerotic Lesions are Preferentially Located at Vessel Bifurcations
Hemodynamic Forces Acting on The Blood Vessel

- **Normal Stress**
- **Shear Stress**
- **Stretch**
Uniaxial and Biaxial Stretch Devices

Uniaxial Stretch

Biaxial Stretch
Uniaxial Stretch

Biaxial Stretch

10% linear strain

Cell Borders (β-Catenin mAb / Alexa 488 anti-mouse)

F-Actin (Rhodamine Phalloidin)
Effects of Uniaxial Stretch on Stress Fiber Orientation

Cell Images

Frequency Distribution of Angle of Orientation

Polar Plots
Effects of Uniaxial Stretch Magnitude on Stress Fiber Orientation

Cyclic Uniaxial Stretch

Static

1%
P-value: 0.22

3%
P-value: 0.04

5%
P-value: <0.001

7.5%
P-value: <0.001

10%
P-value: <0.001
Effects of Inhibition of Downstream Effectors of Rho: Rho Kinase (ROCK) and MDia on Stress Fiber Orientation Induced by 10% Stretch

Inhibition of ROCK (Y27632) or MDia (F1F2Δ1) changed the 10% stretch-induced stress fiber orientation from perpendicular to parallel.
Effects of Active Mutant RhoV14 on Stress Fiber Orientation

<table>
<thead>
<tr>
<th>Transfection</th>
<th>GFP (control)</th>
<th>GFP + RhoV14</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Stretch</td>
<td>-</td>
<td>2.5%</td>
</tr>
<tr>
<td></td>
<td>10%</td>
<td>-</td>
</tr>
</tbody>
</table>

Cell Images

Orientation distribution
Effects of Active Mutant RhoV14 on Stress Fiber Orientation

<table>
<thead>
<tr>
<th>Transfection</th>
<th>GFP (control)</th>
<th>GFP + RhoV14</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Stretch</td>
<td>-</td>
<td>2.5%</td>
</tr>
<tr>
<td></td>
<td>10%</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2.5%</td>
<td></td>
</tr>
</tbody>
</table>

Cell Images

Orientation distribution
### Effects of Active Mutant RhoV14 on Stress Fiber Orientation

<table>
<thead>
<tr>
<th>Transfection</th>
<th>GFP (control)</th>
<th>GFP + RhoV14</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Stretch</td>
<td>- 2.5% 10%</td>
<td>- 2.5% 10%</td>
</tr>
</tbody>
</table>

**Cell Images**

![Cell Images](image1)

**Orientation distribution**

![Orientation distribution](image2)
Effect of Active Rho Mutant (RhoV14) on Stretch-Induced Stress Fiber Orientation

Random → 0

Orientation Parameter

GFP

GFP + RhoV14

Aligned → 1

kTime™ and a decompressor this picture.
Effect of Active Rho Mutant (RhoV14) on Stretch-Induced Stress Fiber Orientation

RhoV14 has an effect on stress fiber orientation equivalent to an additional 2.9% stretch.
JNK Activation is Transient in Response to Uniaxial Stretch, but Sustained with Biaxial Stretch
### Hypothesis
Remodeling in response to uniaxial stretch leads to the subsidence of JNK activation.

### Significance
Sustained, but not transient, JNK activation causes apoptosis.
6 hr
6 hr

90° turn

0.5 hr
6 hr

90° turn

0.5 hr
6 hr

90° turn

0.5 hr

6 hr
Regulation of Stress Fiber Orientation and JNK Activation by 10% Uniaxial Stretch: Effects of Change in Stretch Direction

JNK activation subsided following stress fiber realignment
The Rho-mediated stress fiber orientation perpendicular to the direction of stretch represents a mechanism by which cells adapt to mechanical strain that involves molecular and biomechanical responses.

<table>
<thead>
<tr>
<th></th>
<th>Uniaxial Stretch</th>
<th>Equibiaxial Stretch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell and Actin Filament</td>
<td>Perpendicular to Stretch</td>
<td>Random</td>
</tr>
<tr>
<td>Orientation</td>
<td>Transient</td>
<td>Sustained</td>
</tr>
<tr>
<td>Time Course of JNK Activation</td>
<td>Transient</td>
<td>Sustained</td>
</tr>
<tr>
<td>Apoptosis</td>
<td>Protected</td>
<td>Enhanced</td>
</tr>
</tbody>
</table>
Conclusions:
Importance of Directionality in Mechanotransduction

Laminar flow with a net forward direction is atheroprotective, whereas disturbed flow with little net forward direction is athergenic.

Uniaxial stretch with a definitive direction is anti-apoptosis, whereas biaxial stretch without a net direction leads to apoptosis.

The directionality of the mechanical stimuli and the consequent directional remodeling of the cytoskeleton play important roles in the modulation of cell functions in response to mechanotransduction.