Topics: Blood and Contact Activation of Blood Coagulation

Class notes available on http://www.ems.psu.edu/~vogler/EAVogler.htm

Click on “403 Guest Lecture” on 1st page for PPT slides
Quick Review of 9/25 Lecture

- Blood: Hematology. The fluid that circulates through the heart, arteries, veins, and capillaries in humans and other vertebrates, serving chiefly to carry oxygen and nutrients to the body cells and to remove carbon dioxide and other waste products from the cells. In humans, it consists of a pale yellow liquid, plasma, in which the solid elements (red blood cells, white blood cells, and platelets) are suspended.
Plasma is the fluid phase of whole blood

Test tube of whole blood

Blood separated into fluid and cell phases

Centrifuge

Plasma

Packed cell layer

hematocrit
Blood Coagulation and Hemostasis

**hemostasis** (hē’mō-stā’sis, hē-mōs’stē-) also **hemostasia** (hē’mō-stā’zhē, -zhē-ə, -zē-ə) -- **n.** 1. The stoppage of bleeding or hemorrhage. 2. The stoppage of blood flow through a blood vessel or body part.

- Acellular (humoral) component
- Cellular component

**humoral** (hyŏō’mōr-əl) adj. 1. Physiology. Relating to bodily fluids, especially serum. 2. Relating to or arising from any of the bodily humors.
Composition of Blood

General breakdown of blood:

- 55% plasma

  plasma = water, salts, plasma proteins, substances transported by blood

Plasma contains all of the proteins necessary to support COAGULATION.
Plasma is the fluid phase of whole blood

Test tube of plasma

Coagulate

Coagulated plasma forms a gelatinous mass

some shrinkage from original volume

A “clot” = Gel network
The liquid fraction that can be separated from clotted plasma is serum.

Clotted plasma forms a gelatinous mass. Allow to stand, the clot shrinks. The fluid phase that forms is called serum.
Plasma Coagulates through a Cascade of Self-Amplifying Zymogen-Enzyme Interconversions

Thrombin is central to hemostasis, cleaving fibrinogen to fibrin fragments that polymerize and cause plasma to coagulate.
Enzymes of the coagulation cascade are identified as "Factors".

Thrombin is central to hemostasis

Cellular Aspect of Coagulation

- Platelets (thrombosis)
- Protein C (fibrinolytic)
- Fibrinogen (procoagulant)
- FV (procoagulant)
- FVIII (procoagulant)
- FXIII (procoagulant)

Thrombin
Fibrinogen is the blood protein “substrate” for the enzyme thrombin.

The disulfide rings are regions containing three disulfide bonds cyclically linking homologous segments of the α, β, and γ chains. N-linked polysaccharide are represented by filled hexagons. The Arg-Gly bonds that are cleaved by thrombin in fibrin activation are indicated.
Thrombin hydrolysis of Arg-Gly releases fibrinopeptides A and B allowing intermolecular association of fibrinogen into fibrin.
Animation showing formation of fibrin network in blood capturing a blood cell.
Lecture 9/2701

Contact (Surface) Activation of the Blood Coagulation Cascade
And Relevance to Biomaterials
Plasma Coagulates through a Cascade of Self-Amplifying Zymogen-Enzyme Interconversions

"Procoagulant" Surface Activation

INNISIC PATHWAY

EXTRINSIC PATHWAY

BLOOD PLASMA COAGULATION
Multiple proteins and mediators

THE COAGULATION CASCADE

INTRINSIC PATHWAY

EXTRINSIC PATHWAY

COMMON PATHWAY

Contact Activation by a Procoagulant Surface
- The Players -

THE COAGULATION CASCADE

INTRINSIC PATHWAY

Factor XII

Procoagulant Surface

KEY

HMWK high molecular weight kininogen
α₂ M alpha₂ macroglobulin
C₁ INH C₁ esterase inhibitor
AT III antithrombin III

The Players

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Contact Activation by a Procoagulant Surface - The Players -

THE COAGULATION CASCADE

INTRINSIC PATHWAY

Factor XII

Autoactivation or catalysis

Procoagulant Surface

KEY
- HMWK: high molecular weight kininogen
- $\alpha_2$ M: alpha$_2$ macroglobulin
- C$_1$ INH: C$_1$ esterase inhibitor
- AT III: antithrombin III

Phospholipid

$\text{Ca}^{++}$

AT III

C$_1$ INH

$\alpha_2$ M
Discovered as a blood deficiency in an individual with the surname Hageman.

FXII is involved with several different plasma protein cascades including activation of the intrinsic cascade...

Activation of FXII leads to production of kinins through the activation of kallikrein...

Contact Activation by a Procoagulant Surface
- The Players -

Kallikrein and kininogen

Kallikrein is a serine proteinase that cleaves kininogens yielding the kinins bradykinin and plasmin. Kallikrein has direct chemotactic activity; plasmin is involved in fibrinolysis.
kal.li.kre.in Pronunciation: "ka-l&-'krE-&n, k&-'li-krE-&n
Function: noun a hypotensive protease that liberates
kinins from blood plasma proteins and is used
therapeutically for vasodilation.

ki·nin (kī’nĭn) n. Any of various structurally related
polypeptides, such as bradykinin, that act locally to induce
vasodilation and contraction of smooth muscle. [Short for
bradykinin: brady- + Greek kinein, to move; see kei-²
below + -in.]

va·so·dil·a·tion (vā’zō-dĭ-lā’shən, -dĭ-) also
va·so·dil·a·ta·tion (-dĭl’ə-tā’shən, -dĭl’ə-) --n. Dilation of
a blood vessel, as by the action of a nerve or drug.
**Some Definitions**

**chemotaxis** (kēˈmo-təkˈsis, kēmˈō-) *n.* The characteristic movement or orientation of an organism or cell along a chemical concentration gradient either toward or away from the chemical stimulus. --**chemotactic** (-təkˈtik) *adj.* --**chemotactically** *adv.*

**fibrinolysis** (fīˈbrə-nəlˈī-sis) *n.*, **pl. fibrinolyses** (-sēz′). The breakdown of fibrin, usually by the enzymatic action of plasmin. --**fibrinolytic** (-nə-līˈtik) *adj.*
Simple Summary of the Contact Activation Complex

Figure 1. Protein components of the contact phase of the Hageman factor system.

Biochemistry of Contact Activation Ascribes Contact Activation to “Molecular Assembly” on a Surface

Biochemistry of Contact Activation Ascribes Contact Activation to “Molecular Assembly” on a Surface

Contact activation of blood plasma coagulation is “catalyzed” by the presence of a surface.

Summary III

• Factor XII activation is self amplifying (through kallikrein and high molecular weight kininogen.
• Kinins are released that function as vasodialators and chemotaxic agents.
• Mechanisms of contact activation involve “molecular assembly” on procoagulant surfaces.

What’s A Surface Anyway?
What is a surface?

surface (sûrˈfəs) n. Abbr. sur. 1.a. The outer or the topmost boundary of an object. b. A material layer constituting such a boundary. 2. Mathematics. a. The boundary of a three-dimensional figure. b. The two-dimensional locus of points located in three-dimensional space. c. A portion of space having length and breadth but no thickness.

A layer with a certain thickness
What is a surface?

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What is a surface?

1. An abstract mathematical construct without volume or roughness.
2. A physical reality with thickness at least the dimensions of atoms or molecules - and a fractal dimension (a graininess) at least of the order of molecules.
   - Surface of a liquid
   - Surface of a solid
   - An every-day experience
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   - An every-day experience

Because surfaces and physical phenomena related to surfaces is an every-day experience, the beginnings of surface science are contemporaneous with early physics and chemistry.
The Strange Properties of Surfaces

• Aristotle (384-322 B.C) noted that gold leaf floated on water. But gold is denser than water and observed buoyancy conflicts with the hydrodynamic principle of Archimedes (287 - 212 BC; the average density of a water-floating body must equal the average density of water).

• Galileo (1564-1642) names the phenomenon “capillary depression”, but this surface phenomenon is not quantitatively explained until 1920 (note > 2000 year conundrum!)

The Strange Properties of Surfaces

- Phenomenon of capillary rise (capillarity) - liquid rising up narrow tubes above the level of a reservoir - remained unsolved until Laplace in 1805.


Section of a diagram illustrating capillary rise from Segner 1752.*
The Strange Properties of Surfaces

- Stable bubbles and froths in the ocean (and beer!).
- “Tears of wine” (Marangoni effect).
- Water “beads up” on some materials, not on others.
- Different surfaces induce different biological responses…
Q: What makes surfaces so unique?

A: The chemical and energetic predicament created by placing molecules at the periphery of a phase.
Chemical and Energetic Predicament of Molecules at the Periphery

In-class explanation of Irving Langmuir’s rationalization of surface energetics using the foam-packing-crate illustration.

Chemical and Energetic Predicament of Molecules at the Periphery

Irving Langmuir was one of a number of important scientists working in the early 1900’s to establish the “constitution and fundamental properties of solids and liquids”.

Irving Langmuir, 1881-1957
http://www.ge.com/ibhisil.htm
Chemical and Energetic Predicament of Molecules at the Periphery

• Langmuir was awarded the Nobel prize in 1932 for his work in area of surface science.

• Langmuir is widely regarded as the father of modern surface science.

Irving Langmuir, 1881-1957
http://www.ge.com/ibhisil.htm
“Since energy must be expended in breaking apart a solid, the surfaces of solids must contain more potential energy than do the corresponding number of atoms in the interior. Since this potential energy is probably electromagnetic energy in the field between atoms, the interatomic forces are more intense on the surface than in the interior.”

Irving Langmuir, 1881-1957

http://www.ge.com/ibhisil.htm

Chemical and Energetic Predicament of Molecules at the Periphery

- Molecules located at an interfacial boundary are at a higher energetic state than bulk molecules by virtue of being deprived of nearest-neighbor interactions.
- Excess energy state is termed surface energy or interfacial energy.
Biochemistry of Contact Activation Ascribes Contact Activation to “Molecular Assembly” on a Surface

Contact activation of blood plasma coagulation is “catalyzed” by the presence of a surface.

HOW???

Biomaterials Surface Science: Understanding the Effect of Material Surfaces on “Biocompatibility”

General Experimental Requirements to Study the Biological Response to Surfaces

• Means of systematically varying surface chemistry/energy/water wettability.

• Means of testing/rating the biological response to these materials.
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Three Routes to Surface Engineering

• Material selection
  – Judicious choice of materials with inherently different surface chemistry from the families of glass, metals, polymers…

• Surface Modification
  – Chemical modification of material surfaces to incrementally vary chemical composition of a base material.

• Self Assembled Monolayers
  – Control outermost surface functionality by synthetic methods that produce dense and well-defined layers on a substratum.
RF Gas Plasma Setup

- Vacuum chamber
- Oxygen plasma
- Plate-style internal electrodes
Reactive Gas Discharge = A Plasma

**plasma** (plāz ′ mə) also **plasm** (plāz ′ m) --n. 1.a. The
used in transfusions. 3. Protoplasm or cytoplasm. 4. The
fluid portion of milk from which the curd has been
separated by coagulation, whey. 5. **Physics**. An electrically
neutral, highly ionized gas composed of ions, electrons, and
neutral particles. It is a phase of matter distinct from solids,
liquids, and normal gases. [New Latin, from Late Latin,
image, figure, from Greek, from *plassein*, to mold. See
**pelə**-**2** below.] --**plas·mat·ic** (plāz-măt′ ĭk) or **plas·mic** (-mĭk) adj.
Three Routes to Surface Engineering
Applied to Coagulation Case Study

Permits incremental oxidation of a polymer…

“Hydrophobic” → “Hydrophilic”

• Surface Modification
  – Surface oxidation with Radio Frequency (RF) gas plasma discharge surface modification of polymers

…but the surface chemistry (as assessed by XPS) is a mixture of oxidized chemical functionalities as opposed to a single, well-controlled functionality.
There can be different types of SAMs

Examples:
- Organosilicon derivatives on hydroxyilated surfaces
- Alkanethiols on gold, silver, and copper
- Dialkyl sulfides on gold
- Dialkyl disulfides on gold
- Alcohols and amines on platinum
- Carboxylic acids on aluminum oxide and silver

Each of these have different:
- Kinetics of formation
- Chemical stability
- Thermal stability
- Chain length
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Shafrin and Zisman, 1952
Whitesides, Allara et al. 1988
Experiments show that high-surface energy materials - such as clean glass and oxidized polymers - strongly activate the cascade.
So what’s all the fuss???
Relevance of Blood Contact Phenomena in Biomaterials

• A majority of biomedical devices contact blood or blood by-products.
Medical procedure devices (needles, syringes, tubes…)

Temporary implants (catheters, pumps…)

Permanent implants (prostheses)

Diagnostic devices (specially treated labware…)

Number in Use

Sophistication
Relevance of Blood Contact Phenomena in Biomaterials

- A majority of biomedical devices contact blood or blood by-products.
- Surface of biomaterials control or mediate the biological response (e.g. blood coagulation).
- Understanding blood and the interaction of blood with materials is essential to structure-property relationships guiding biomaterial design.
Surfaces and the Biological (Host) Response to Materials
MatSE 404/517 (3cr) Spring Semesters