Blood-Biomaterial Interactions and Coagulation II

“I’m concerned about his platelets.”
Course update (a couple not on your handout)

- Homework 1 due today (please hand in at the start of class)
- Homework 3 distributed today (due Monday)
  - Please double space (ok if you didn’t for first HW)
- LBL Friday
  - Discussion questions and glossary for Friday LBL posted on the course website
  - Remember to put together one sentence summary for each figure (to hand in)
    - example from the current LBL paper
    - Please hand your summary in to me directly, before class starts!
Recap of previous class

• Injury -- platelet adheres to a surface and contractile proteins (actin and myosin) tighten and platelet flattens to form pseudopodia

• Contraction causes platelets to degranulate, releasing ADP and thromboxane A₂
  – These are potent activators of platelets: stimulants include expression of glycoprotein IIb/IIIa, which cause other platelets to adhere

A platelet pseudopodium stained for two different kinds of granules (red and green color)

Blair P, Flaumenhaft R. Blood Rev 2009
Recap of previous class (cont.)

• These newly recruited platelets degranulate, continuing the cascade of platelet aggregation and resulting in a platelet plug ➔

• Actin and myosin contraction of the platelet plug help to compact and stabilize the plug drawing the edges of the injury together

• An end product of coagulation is production of sticky threads of the protein fibrin

We will examine aggregation and coagulation in the context of blood vessel injury ➔ but where do biomaterials fit in?
Platelet adhesion on biomaterials

- Activation of platelets with artificial surfaces is a key event in the **thrombogenic complications** of prosthetic devices in contact with the blood (Park and Park 1989)
- Platelets are seen to adhere rapidly to various surfaces upon contact with blood, either as a **monolayer** or as **aggregates**...this process is influenced by a combination of factors including surface smoothness, surface charge, wettability, surface tension and the flow conditions (Mohammed et al 1974)
- **Note**: some biomaterials are **designed** to elicit a certain response upon implantation – just not coagulation!
  - e.g., Nayak S, et al. The promotion of osseointegration of Ti surfaces by coating with silk protein sericin. *Biomaterials* 2013

We will discuss thrombosis in the context of biomaterials and devices further at the end of the lecture
Platelet aggregation — the accumulation of platelets to the site of a wound to form a platelet plug.

Coagulation — the activation of clotting factors following platelet aggregation, ultimately resulting in a thrombus or a blood clot.

Biomaterials can be affected both by aggregation and coagulation — let’s first examine each in the context of injury.
Platelet aggregation: #1 mechanism

- Chemicals produced by the arachidonic acid cascade (AAC) within the platelet influence subsequent platelet aggregation
- AAC stimulated by presence of chemicals released by other platelets
- Some products of AAC promote platelet aggregation, while some inhibit platelet aggregation

# : promote further platelet aggregation
* : inhibit further platelet aggregation
Platelet aggregation: #2 mechanism

Contributions to coagulation (clotting)

- Factor V released by platelets and is activated (Va) by contact with an enzyme called thrombin.
- Factor Va and Xa combine to create a compound called prothrombin activator, which splits the plasma protein prothrombin into fragments, make more thrombin (positive feedback).
- Thrombin cleaves peptide fragments away from fibrinogen → produces fibrin monomers → polymerize into fibrin.

Contributions to platelet aggregation

- Fibrinogen facilitates platelet-platelet adhesion.
- Roles of thrombin in aggregation:
  - Facilitates platelet-platelet adhesion.
  - Stimulates platelet activation and degranulation.

To see where the thrombin comes from, let’s look at the coagulation cascades!
Aggregation versus coagulation

Platelet aggregation – the accumulation of platelets to the site of a wound to form a platelet plug

Coagulation – the activation of clotting factors following platelet aggregation, ultimately resulting in a thrombus or a blood clot

Biomaterials can be affected both by aggregation and coagulation – let’s first examine each in the context of injury
Coagulation cascades – introduction

- Small cuts can bleed copiously, whereas large cuts might clot quickly → why is this??
  - Put another way, how do tissues and blood vessels interpret and respond to trauma?

- Vascular spasm – the constriction of blood vessels that stops or dramatically slows blood flow
  - Stimulated by nervous reflexes
  - Augmented by short-acting metabolites such as endothelin

- Think of these spasms as an “outside-in” counterpart to clotting “inside-out”

http://www.medivisuals.com/
Coagulation cascades – introduction

• The same trauma leads to the formation of a hemostatic blood clot (the bottom image on slide 9)

• Coagulation $\rightarrow$ the result of cascading chemical reactions called **clotting factors**
  – Factors circulate in the bloodstream and are activated either by enzymatic cleavage or surface contact (e.g., with an activated platelet or biomaterial surface)

• Two primary cascades:
  - **extrinsic** pathway
  - **intrinsic** pathway
Extrinsic coagulation cascade

- Is initiated by **trauma** to the vascular walls and surrounding tissues (extrinsic):

  Collection of several factors, including phospholipids from damaged cell membranes.

  Starting here, the rest overlaps with reactions in the intrinsic cascade (discussed next)!
Introduction

• The same trauma leads to clotting (what we studied last time)

• Coagulation → the result of cascading chemical reactions called **clotting factors**
  – Factors circulate in the bloodstream and are activated either by enzymatic cleavage or surface contact

• Two primary cascades:

  - **extrinsic** pathway
  - **intrinsic** pathway
Intrinsic coagulation cascade

• Is initiated by either:
  – exposure of blood to a foreign surface (anything besides a healthy endothelial cell)
  – trauma to platelets within the blood (possibly due to chemical interactions with a foreign surface)

• Collagen/fibronectin exposed upon endothelium injury count as the former!

• However, so do any biomaterials
  – Severity of response depends on the biomaterial and environmental properties mentioned earlier
  – However, the coagulation response does always occur
Intrinsic coagulation cascade
Hemostatic Plug Formation

Primary
- Aggregation
  - Platelet Aggregation

Secondary
- Coagulation
  - Thrombin
  - Fibrin


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Platelet Adhesion and Activation

Normal platelets in flowing blood

Platelets adhering to damaged endothelium and undergoing activation

Aggregation of platelets into a thrombus


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Not only chemical, but also mechanical factors (shear stress), facilitate this process!
An aside – thrombus versus thrombosis

A thrombus is:

normal in the case of **acute injury**

Pathologic in the case of **thrombosis** (vessel **blockage**)

https://s3.amazonaws.com/
Anticoagulants

• **Nonspecific** biologic control mechanisms:
  – **Blood flow**: passing blood volume dilutes coagulants and removes them from the local injury area
  – Several steps within the coagulation cascade are favored only when catalyzed on the surface of activated platelets or at a local stimulus
Anticoagulants and fibrinolysis

- **Specific anticoagulants**
  - **endothelium**: smooth and “slimy” nature of glycocalyx mucopolysaccharide layer on the blood contacting surface
  - **thrombomodulin**: binds thrombin, making it unavailable for further coagulation cascades
  - **fibrin**: ~85 – 90% of thrombin formed during coagulation absorbs to the newly polymerized fibrin threads, localizing the coagulation
  - **antithrombin III (ATIII)**: serum protein that binds and inactivates free thrombin
Removal of a blood clot via fibrinolysis

• Clots are actively removed by enzymatic digestion that includes a time delay of 24 – 48 hours
  – During coagulation, **plasminogen**, which is circulating in the plasma, is trapped in the forming clot
  – Injured tissue releases **tissue plasminogen activator** that converts plasminogen to **plasmin** over 1 – 2 days
  – **Plasmin** is a proteolytic enzyme that digests the fibrin threads of the clot and other pro-coagulants
Blood Clotting

Summary
Biomaterials, devices, and coagulation

• The healthy endothelium is the perfect non-thrombogenic blood contacting material!

• Deleterious impact of implants:
  – Remember our two categories from the end of last lecture:
    • Effects on the patient (e.g., formation of a clot on the surface of a biomaterial that migrates to block a vessel → “embolus”)
    • Effect on the implant (e.g., layer of tissue growth around the device, possibly affective its function)

• Strategies to mimic the endothelium:
  – Use of implant materials with smooth surfaces
  – Surface modification with negative charges
  – Imitation of glyocalyx
  – In general, hydrophilic > hydrophobic for “stealthiness”

Please read page 82 of book (will read pp 83 – 85 for HW) → when doing the assignment, always keep in mind the book is >10 years old!
So, what’s really important to know (for an exam or otherwise???)
References