BNG 331 – Cell-Tissue Material Interactions

Blood-Biomaterial Interactions and Coagulation I
Course update

• Homework 1 due Wednesday
• Paper distributed today for LBL this Friday
Blood-biomaterial interactions

• Blood is the first “tissue” that any surface of most implants will contact

• Implanting a device causes injury which results in bleeding thus starting the wound healing cascade
  – Injury -- Coagulation -- Inflammation -- Repair and Remodeling

• Goal for today: describe the roles of blood-borne cells and chemicals in the process of coagulation
Blood cell source

• Blood is a mixture of:
  – Plasma
    • The non-cell containing component of blood – water, salts and proteins
  – Numerous cell types
    • Platelets (P), leukocytes (L), erythrocytes (red blood cells; E)

• Blood cells originate from the bone marrow by a process known as hematopoiesis

Bars = 5 μm

http://ncifrederick.cancer.gov/
Bone marrow

- At birth, marrow is red and produces blood cells
  - As one ages, much of the marrow changes to yellow (adipose) tissue and stops creating new blood cells
- In the marrow there is stroma, sinuses, and sinusoidal capillaries

http://www.lab.anhb.uwa.edu.au/mb140/corepages/bone/images/bon02he.jpg
Multipotent hematopoietic stem cells

- All the cells that are circulating in the blood come from one type of cell in the marrow:
  - Multipotent Hematopoietic Stem Cell (HSC)
    - Contrary to what the book says, these cells are not pluripotent!
ES cells are **pluripotent**

Embryonic stem cells

- **endoderm**: lung, liver, pancreas, thymus, endocrine glands
- **mesoderm**: blood, vessels, muscular tissue, connective tissue
- **ectoderm**: skin and its derivatives, nervous system

All stem cells on this level will be **multipotent**
Erythrocytes (red blood cells, RBCs)
Erythrocytes

- Play minimal role in wound healing and blood-biomaterial interactions
- Have no nucleus or cytoplasmic organelles needed for protein synthesis
- Do not proliferate
- Mature RBCs do not synthesize hemoglobin
- Purely function to transport oxygen and carbon dioxide

Fun fact: there are probably about 25,000,000,000,000 \((25 \times 10^{12})\) RBCs in your body right now!

Average volume of one RBC: \(~90\) femtoliters \((90 \times 10^{-15} \text{ L})\)

Total volume of RBCs? Calculate it!

How do RBCs get their hemoglobin if they don’t have a nucleus, and thus, don’t have any DNA to code for it?
Erythrocytes: Red Blood Cells

- RBC progenitors produce hemoglobin during the differentiation process from proerythroblast to reticulocyte
  - i.e., while they still have nuclei
- Only after nuclear extrusion does the reticulocyte become an RBC and stop producing hemoglobin
- (will see the structure of hemoglobin later in the lecture)
Erythrocytes: Red Blood Cells

• Can survive for 120 days before they wear out and are removed by macrophages

• Macrophages engulfing red blood cells:
  – http://www.youtube.com/watch?v=GTigHRQFGqE
Erythrocytes: Red Blood Cells

• Biconcave and ~7 μm in diameter (some capillaries are 5 – 7 μm)
• Pure blood plasma behaves like a Newtonian Fluid (e.g., water)
  – Constant viscosity independent of the shear stress or shear rate (shear: force applied along the surface of a material)
• Whole blood behaves like a Non-Newtonian fluid due to the flow behavior of RBCs
  – Instantaneous viscosity dependent on the shear stress or shear rate
  – [http://www.youtube.com/watch?v=aY7xiGQ-7iw](http://www.youtube.com/watch?v=aY7xiGQ-7iw) (1:10)
    • RBCs are somewhat similar; at very low flow (shear rates), RBCs aggregate into masses that act like solids (very high viscosity)
    • Hence, whole blood is “shear thinning” – lower viscosity with higher shear stress – as opposed to “shear thickening”

Let’s examine this a bit closer!
Erythrocytes: Red Blood Cells

Bingham plastic: up to a certain threshold stress or rate, behaves like a solid, then suddenly starts flowing!

http://www.youtube.com/watch?v=riScU61D6YA

Shear thickening: increase in viscosity with an increase in shear stress or shear rate (think of this guy as pulling equally hard in both cases, but faster in the second case):

http://www.youtube.com/watch?v=9k9baW_UOsw

Same as “shear rate”; think of it as shear force divided by time instead of area
Erythrocytes: Red Blood Cells

• Have no nucleus, and so are extremely deformable (due to the flow properties we just saw)
  – Membrane effects in a red blood cell: http://www.youtube.com/watch?v=Ym1rvwP-po4

• The ability to deform is crucial to the function of RBCs

• Hypoxia -- oxygen shortage

Let’s look at sickle-cell anemia as a “case study” for compromised RBC deformability
Sickle-cell anemia

Before discussing what is happening pathologically, what are the symptoms?
Sickle-cell anemia

- ...is an inherited disorder affecting hemoglobin (right)
- Caused by a single AA mutation in the beta chain
  - GAA (glutamic acid) replaced by GUA (valine)
- Causes polymerization of hemoglobin into elongated, inflexible crystals under low oxygen conditions

Hemoglobin
(each “chain” is a different subunit)

http://www.proprofs.com/flashcards/upload/q8683668.jpg

Heme groups with bound Fe atoms for oxygenation
Sickle-cell anemia (cont).

- In addition to blood vessel blockage (right), the hemoglobin crystals can rupture the RBC membrane.
- Many successive rupture events leads to elevated systemic hemoglobin.

http://www.nhlbi.nih.gov/health/health-topics/topics/sca/
Platelets (thrombocytes)
Platelets

- Originate in marrow and are fragments of larger cells derived from megakaryoblasts
- **Megakaryoblasts** can range from 15 – 50 µm in diameter
- Differentiate to multiply the amount of DNA in the nucleus by ~30 times before becoming a **megakaryocyte** (35 – 150 µm in diameter)
Megakaryocyte

http://student.nu.ac.th/wuth_web/pic.htm
Megakaryocyte-Platelet

Stem cell

Developmental pathway

Hemocytoblast → Megakaryoblast → Promegakaryocyte → Megakaryocyte → Platelets

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Platelets

- Also called “thrombocytes”
- \( \sim 2 - 4 \text{ µm} \) in diameter
- Have no nucleus and cannot proliferate
- Half-life of 8-10 days, get cleared out of the spleen by macrophages
- Most prominent features are granules containing a variety of chemicals necessary for coagulation
- Contain actin, myosin, and thrombosthenin contractile proteins
- Platelets are active, while RBCs are passive
Platelets

- Platelets interact with the specific short peptide sequence arginine-glycine-aspartic acid (RGD)
  - Remember our discussion of fibronectin last week
- RGD is part of the sequence of collagen, located in the basement membrane that is normally covered by healthy endothelial cells (right)

http://www.rci.rutgers.edu/~uzwiak/AnatPhys/Blood_Vessels.html

How does platelet adhesiveness to RGD (fibronectin) mediate clotting upon injury?
Aggregation and coagulation

- Upon injury, platelets adhere to a surface to contractile proteins (actin and myosin) tighten to platelet flattens and forms pseudopodia ("legs")
  - Typically platelets adhere to the connective tissue exposed when the endothelium of a blood vessel is ruptured (right)
  - However, they also adhere to the surface of many man-made biomaterials

- Contraction causes platelets to degranulate, releasing ADP and thromboxane A$_2$
  - These are potent activators of platelets, recruiting more locally
  - glycoprotein IIb/IIIa is also upregulated for each platelet

"two-pronged approach": more platelets, plus a higher affinity per platelet
The mass of aggregated platelets further contract actin/myosin, drawing the edges of the injury together.

An end product of coagulation is production of sticky threads of the protein fibrin:
- Fibrin threads attach to and help consolidate the platelet plug
- Also trap nearby erythrocytes

At this point the platelet plug is a blood clot.
Platelet activation

(A) actin filaments capped by capping protein in unactivated blood platelet

(B) severed actin filaments capped mostly by Ca$^{2+}$-activated gelsolin and some capping protein

(C) gelsolin and capping protein removed, and rapid actin filament growth from many short fragments

(D) activated platelet spreads out, attaches to blood clot, and contracts
Platelet activation

Platelet Aggregation

Flowing disc-shaped platelet → Rolling ball-shaped platelet → Hemisphere-shaped platelet → Spreading platelet

FIRM, BUT REVERSIBLE ADHESION

IRREVERSIBLE ADHESION

Scanning electron micrograph of discoid, dormant platelets

Activated, aggregating platelets illustrating fibrin strands


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Biomaterials, devices, and thrombosis

• Normal, healthy endothelium does not induce coagulation!
• Such is the goal of implantable blood-contacting biomaterials
  – Think of deleterious effects of an implant as in two categories:

<table>
<thead>
<tr>
<th>Harmful to the implant or its function</th>
<th>Harmful to the patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Adsorption of blood components (e.g., proteins) onto the biomaterial</td>
<td>• Processes of coagulation and fibrinolysis</td>
</tr>
<tr>
<td>• Adsorption of blood cells onto the biomaterial</td>
<td>• Formation of clots on the surface of the material</td>
</tr>
<tr>
<td>• Tissue growth around the biomaterial</td>
<td>• These can migrate and clot vessels elsewhere!</td>
</tr>
<tr>
<td></td>
<td>• Injury to blood cells, causing low levels of blood-borne cells and clinical problems</td>
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