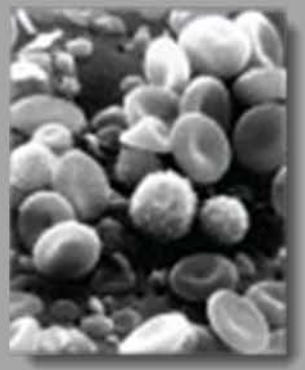

12th Annual Canadian Blood Services International Symposium

Plasma: Transfuse it, Fractionate it or Forget it?

Canadian Blood Services
it's in you to give

Rethinking the Coagulation Cascade: From Plasma Proteins to the Role of Cells

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Objectives

- Highlight the important roles of cells in directing hemostasis and thrombosis
- Clarify differences between hemostasis *in vivo* and plasma clotting in clinical laboratory tests
- Discuss the utility of clinical laboratory tests and some ways in which they can be misleading

Our group has been trying to develop a better model to help us understand coagulation

What is a Model?

- *A model is a representation containing the essential structure of some object or event in the real world.*
- The representation takes two major forms:

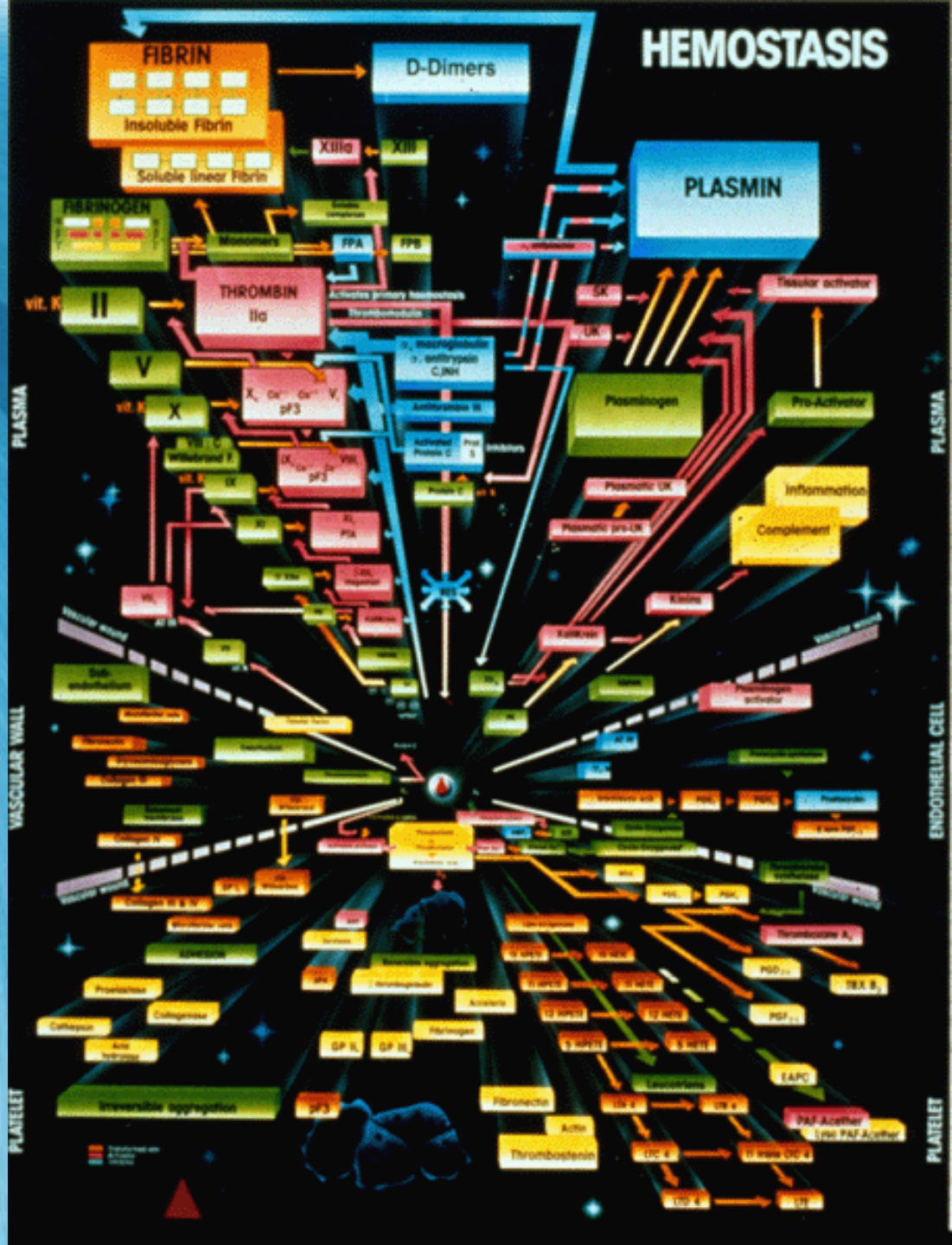
  1) Physical – a model system that can be explored experimentally

  2) Symbolic – a descriptive or conceptual construct to aid in understanding

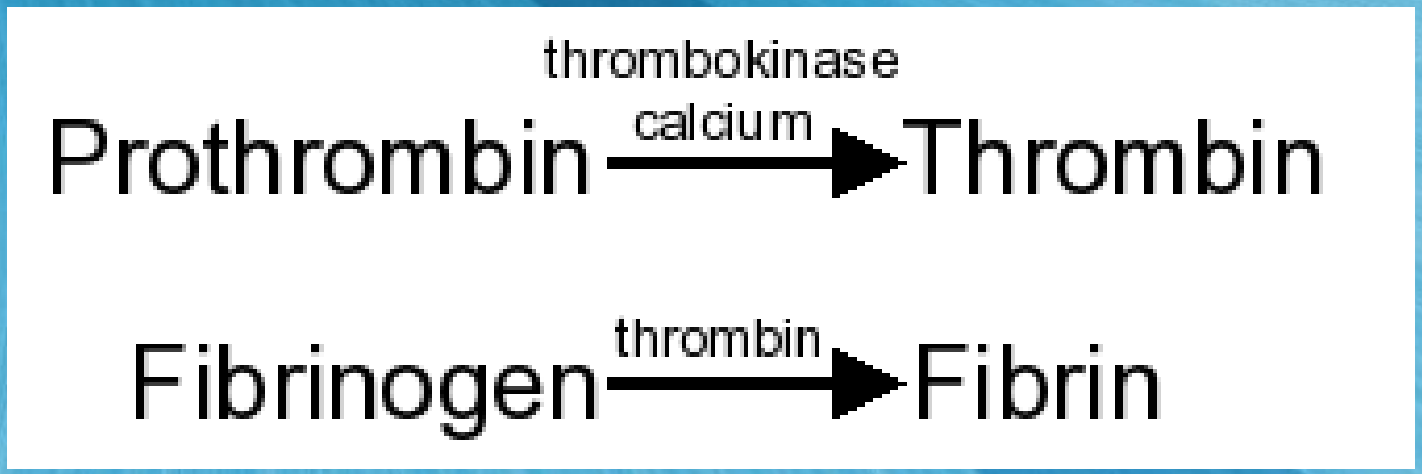
Why Bother?

A model simplifies a complicated system in order to make it easier to understand or explore experimentally, yet should remain complicated enough to reflect its essential features

How did coagulation get to be so confusing, anyway?



In 1904 Paul Morawitz proposed a model of coagulation



Morawitz, P. Beiträge zur Kenntniss der Blutgerinnung Dtsch Arch Klin Med 1904;79:1-28.

More and more factors were discovered and named different things, and it all went down hill from there…..

fibrinogen
prothrombin
accelerator (AC-) globulin
Antihemophiliac Factor
Antihemophilic Factor B
Antihemophilic Globulin (AHG)
Antihemophilic Globulin A
Autoprothrombin I
Autoprothrombin II
Autoprothrombin III
Beta cothromboplastin
Christmas Factor
Contact Factor
Cothromboplastin
Facteur Antihemophilique A
Fibrin Stabilizing Factor
Thromboplastic Plasma Component
Thromboplastinogen
Hageman Factor
Hemophilia A factor
Hemophilia B Factor
Hemophilia C factor
Labile Factor
Laki-Lorand Factor
Pavlovsky Factor
Plasma Thromboplastic Factor
Plasma Thromboplastic Factor A
Plasma Thromboplastin Antecedent (PTA)
Plasma Thromboplastin Component
Plasmakinin
Platelet Cofactor
Proaccelerin
Proconvertin
Prothrombokinase
Protransglutamidase
Prower Factor
Robbins Factor
Serum Factor
Serum Prothrombin Conversion Accelerator (SPCA)
Stable Factor
Stuart Factor
Stuart-Prower Factor
Thrombokatalysin

In 1958 the International Society on Thrombosis and Hemostasis convened a conference to standardize the nomenclature

That's how we got all those roman numerals

International Committee on the Nomenclature of Blood Clotting Factors, Rome, September 1958

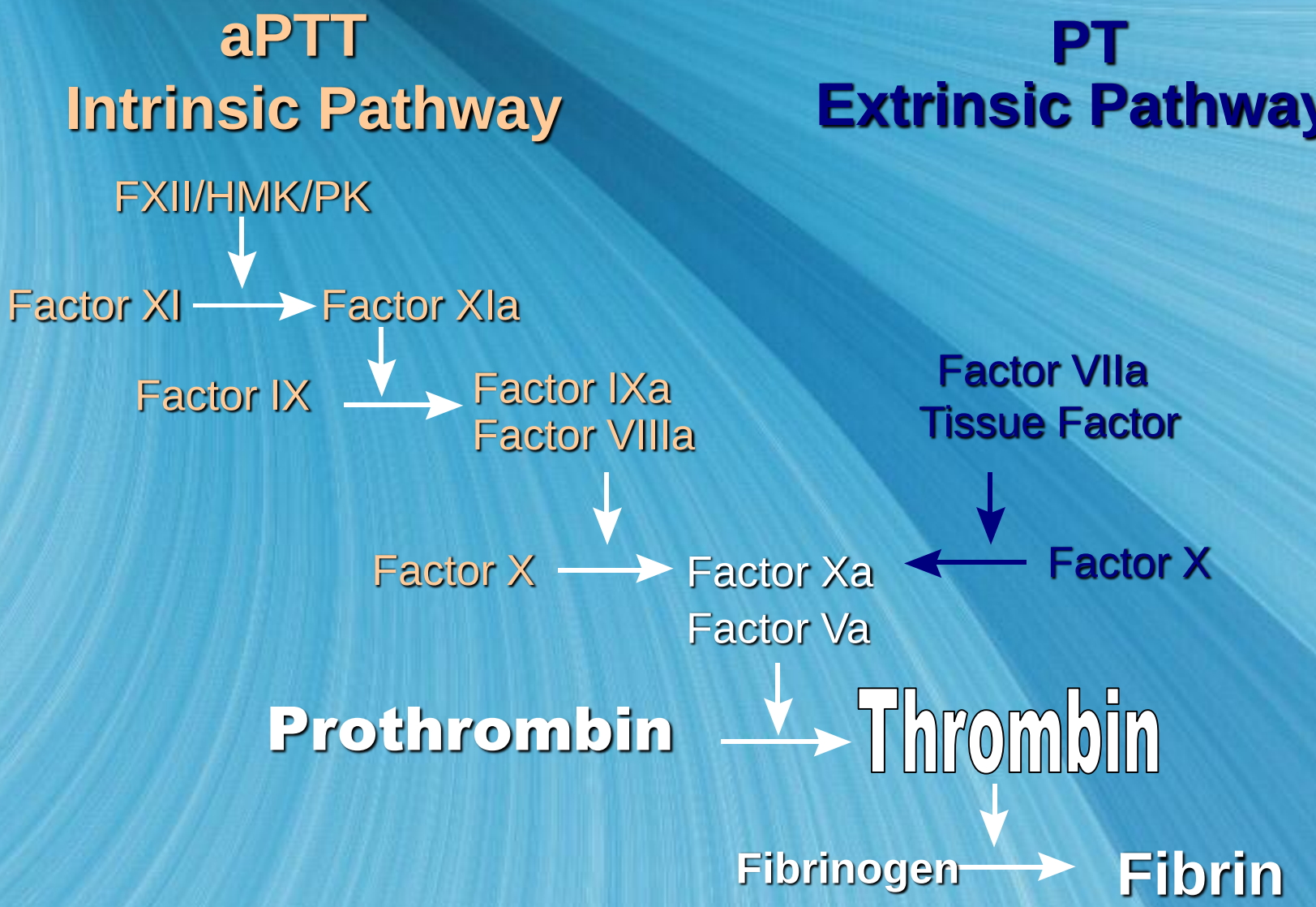
…… but nobody really knew how all those factors interacted to turn liquid plasma into a solid fibrin clot

That's why the roman numerals aren't in order in the coagulation cascade - thus making it is hard for us to remember

In the 1960's the coagulation factors were organized into a "cascade" or "waterfall" model. This evolved into the current cascade model …

1. Macfarlane RG. An enzyme cascade in the blood clotting mechanism, and its function as a biological amplifier. *Nature*. 1964;202:498-499.
2. Davie EW, Ratnoff OD. Waterfall sequence for intrinsic blood clotting. *Science*. 1964;145:1310-1312

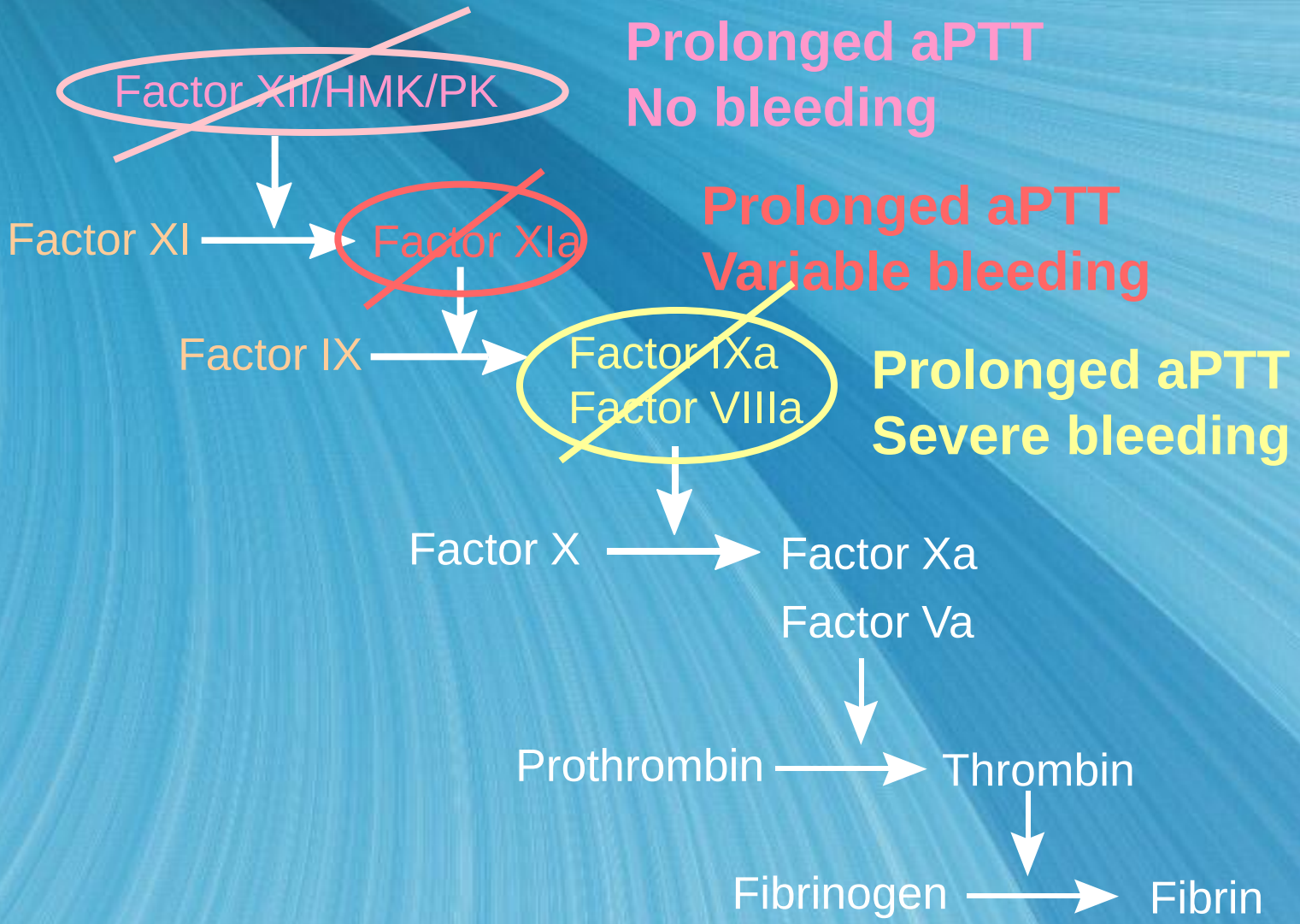
The Coagulation Cascade



The "Cascade" was intended as a model of how the coagulation proteins interact biochemically, not how hemostasis works in the body

It IS a good model of what happens in the PT and aPTT assays

aPTT Does not Correlate with Bleeding Risk



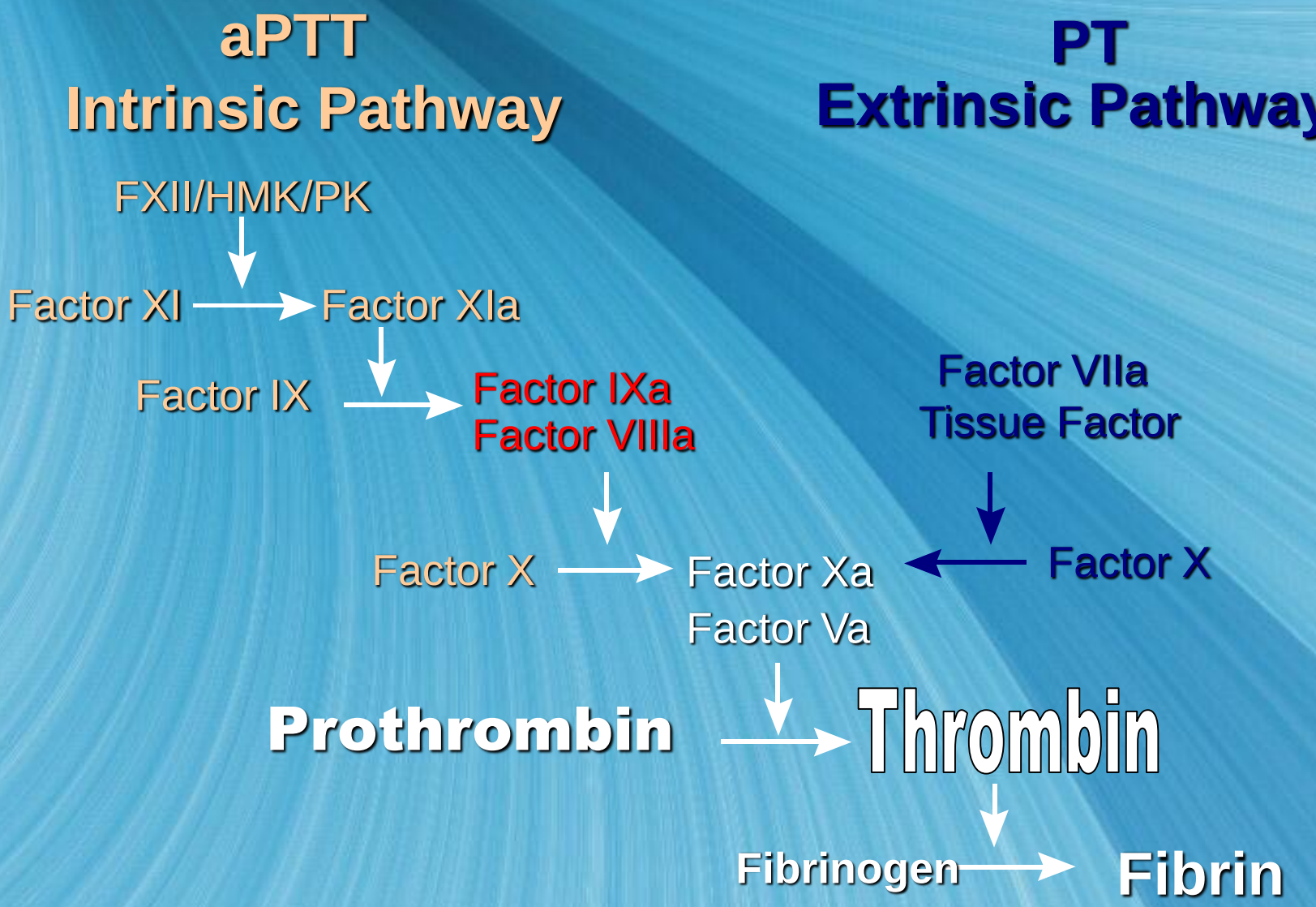
The "Coagulation Cascade" is a good model of the PT and PTT tests

It is not a good model of hemostasis *in vivo*

Why do hemophiliacs bleed?

- Hemophilia only affects the “intrinsic” pathway
- Why isn’t the “extrinsic” pathway enough for hemostasis?

The Coagulation Cascade

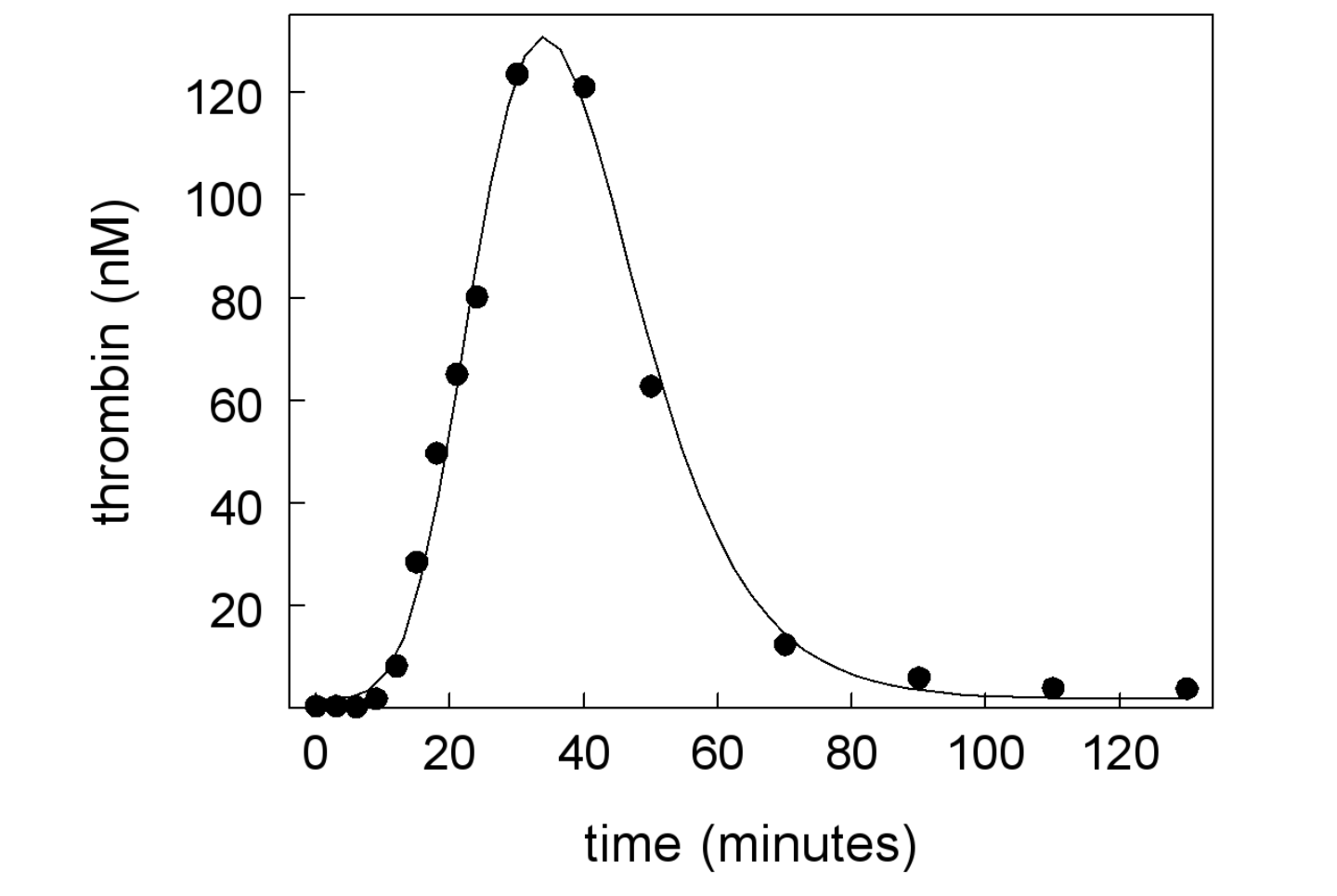


Can putting the cells back in the model explain some clinical phenomena that the "protein-centered" cascade model cannot?

Cell-based experimental model

cells	**monocytes (TF)**	**1 pM TF**	**15/uL**
	platelets		**100,000/uL**
proteins	**prothrombin**	**1400 nM**	**100 ug/mL**
	factor VII	**10 nM**	**0.5 ug/mL**
	factor IX	**70 nM**	**4 ug/mL**
	factor X	**135 nM**	**8 ug/mL**
	factor XI	**30 nM**	**5 ug/mL**
	factor V	**20 nM**	**7 ug/mL**
	factor VIII	**0.3 nM**	**0.1 ug/mL**
inhibitors	**antithrombin**	**3000 nM**	**200 ug/mL**
	TFPI	**3 nM**	**0.1 ug/mL**

Thrombin generation in the model system



Cell-based conceptual model - Hemostasis occurs on two surfaces: TF-bearing cells and platelets

1.Initiation

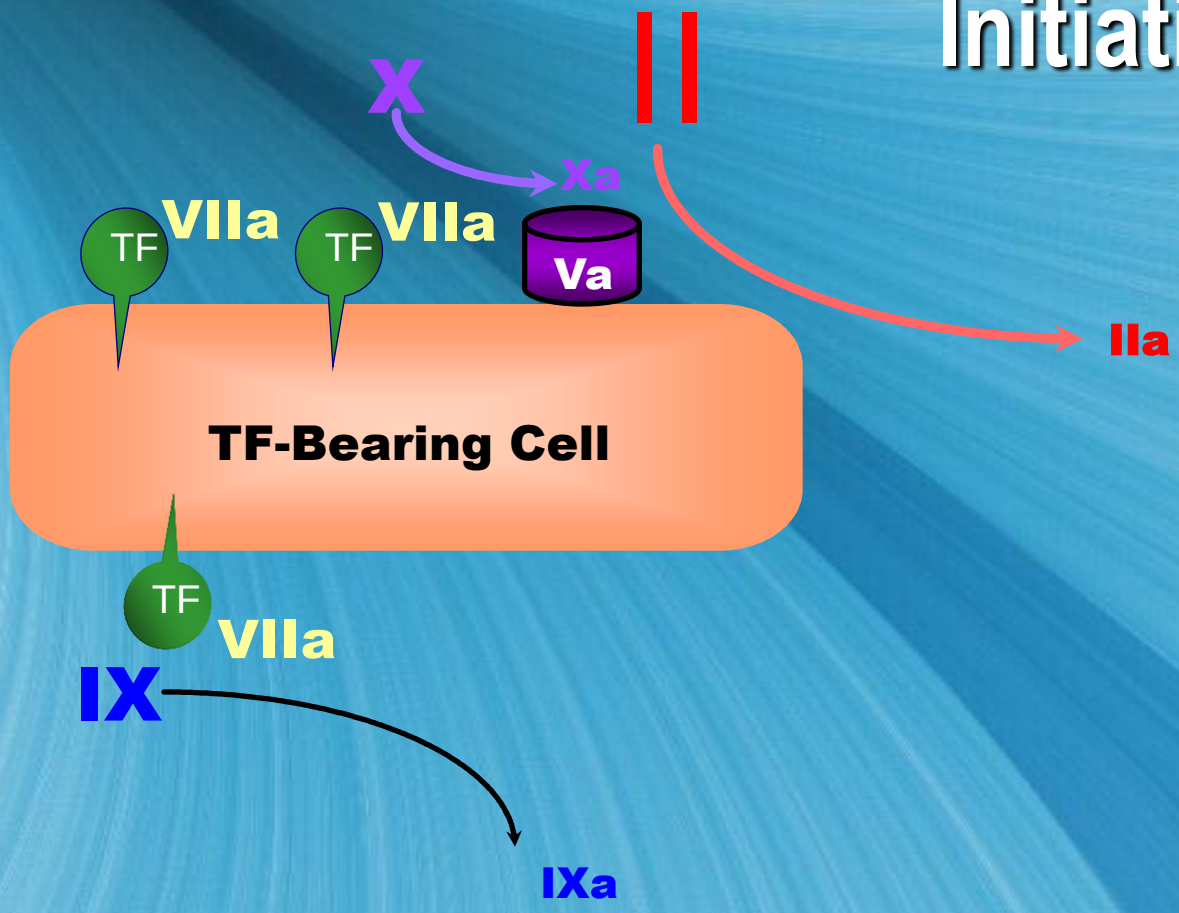
IIa

2.Amplification

3.Propagation

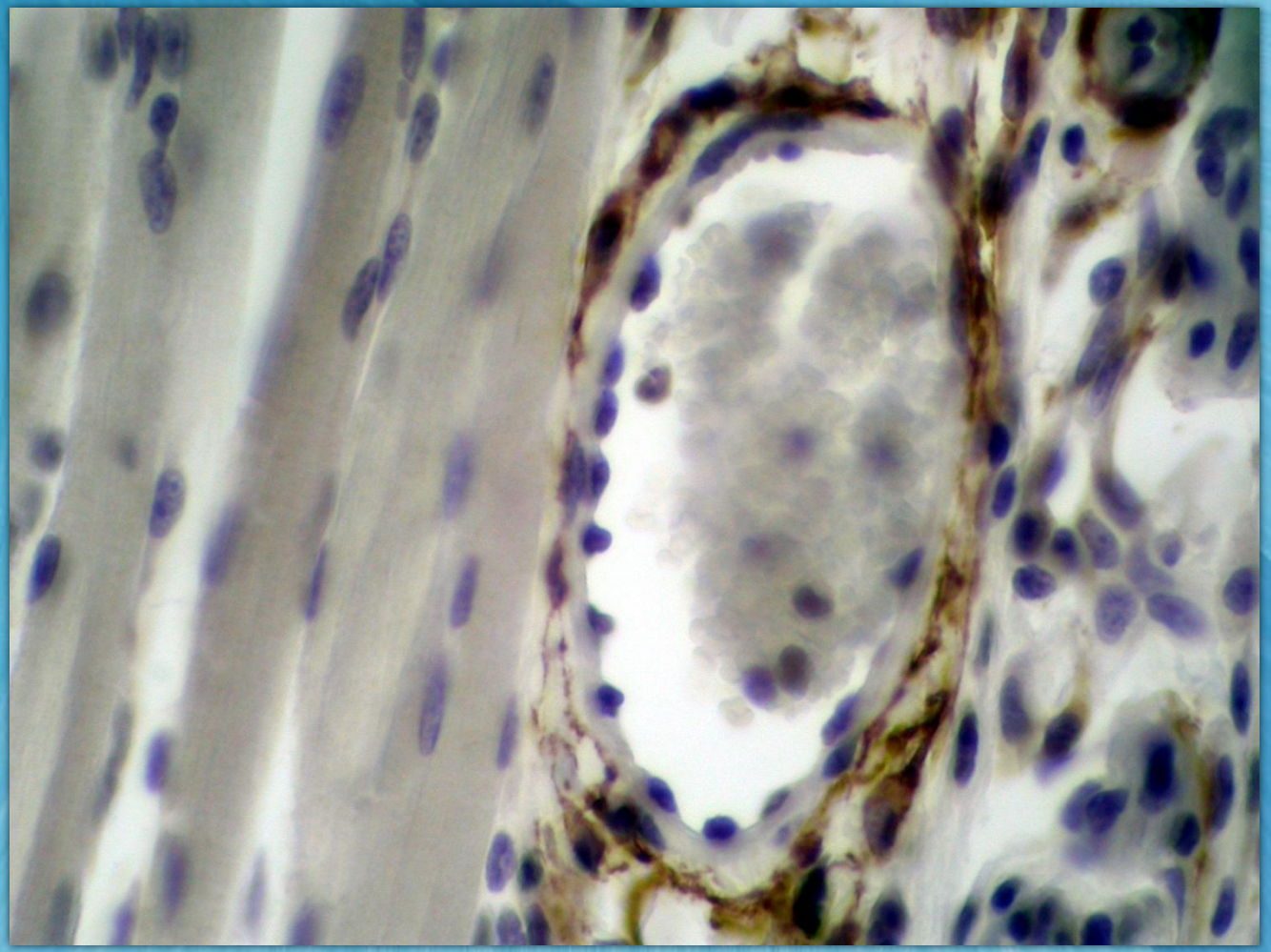
IIa

Initiation

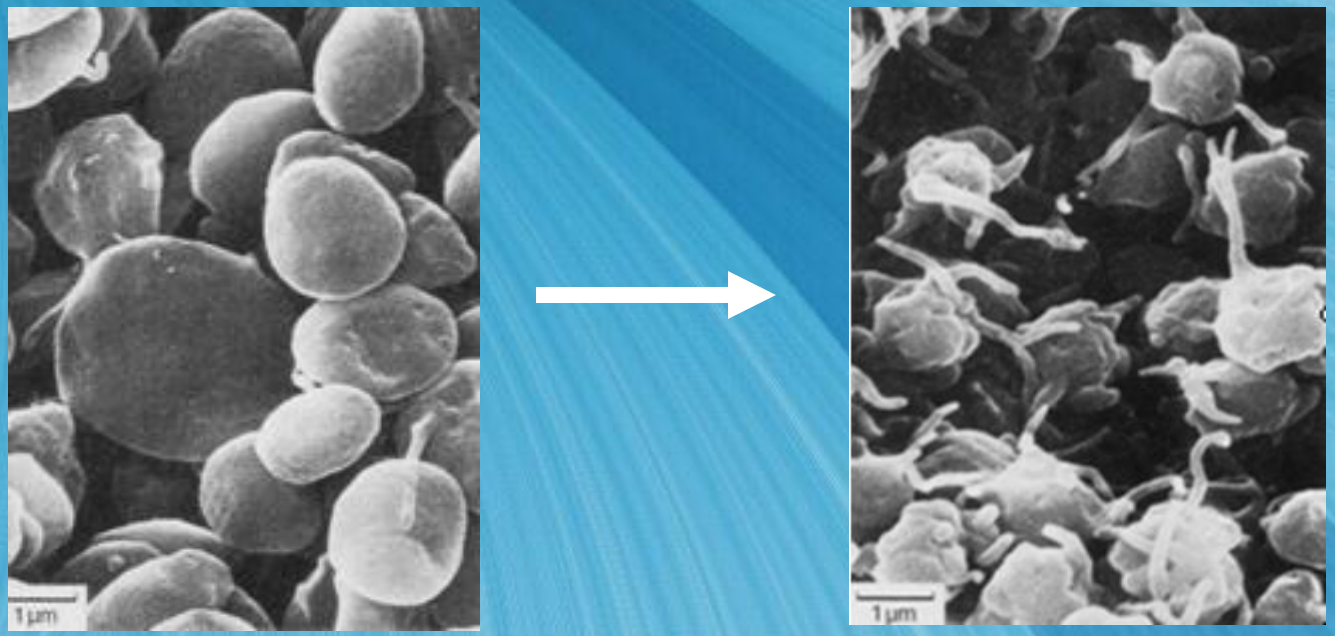


Hoffman & Monroe: A Cell-Based Model of Hemostasis.
Thromb Haemostas, 85:958-65, 2001

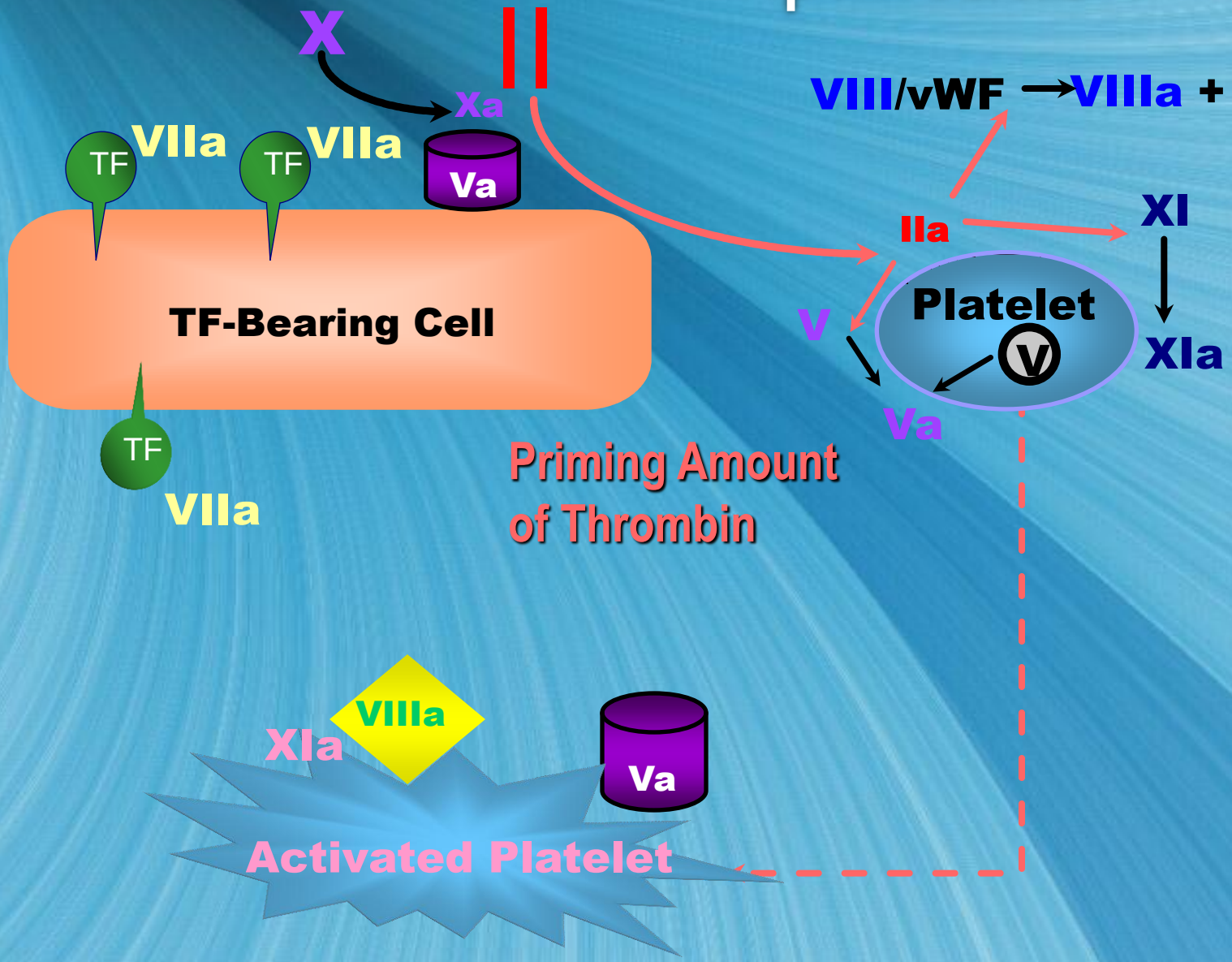
Endothelial cells don't have TF
TF is in cells outside the vessel



Platelets Adhere & Are Partially Activated at Sites of Injury

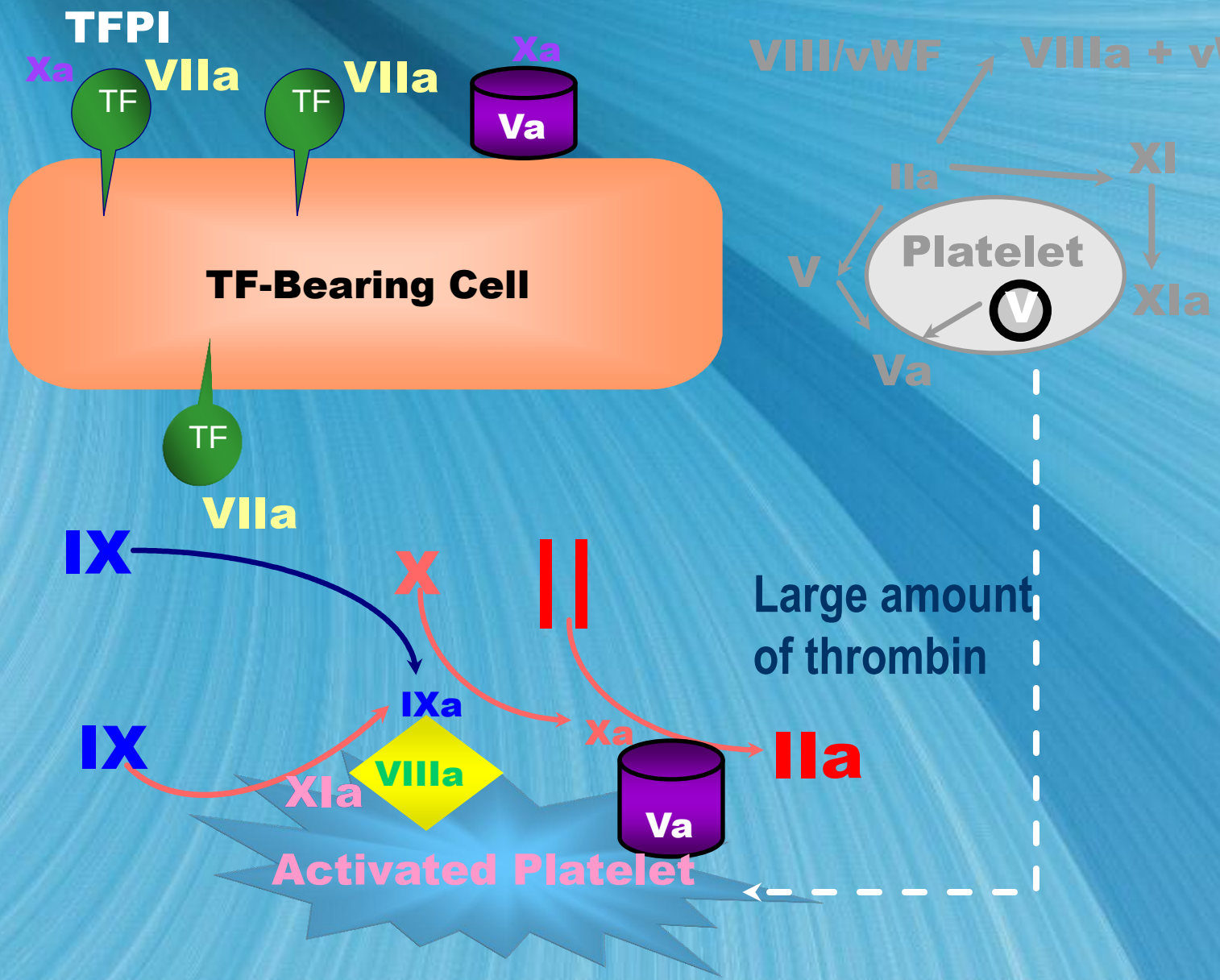


Amplification



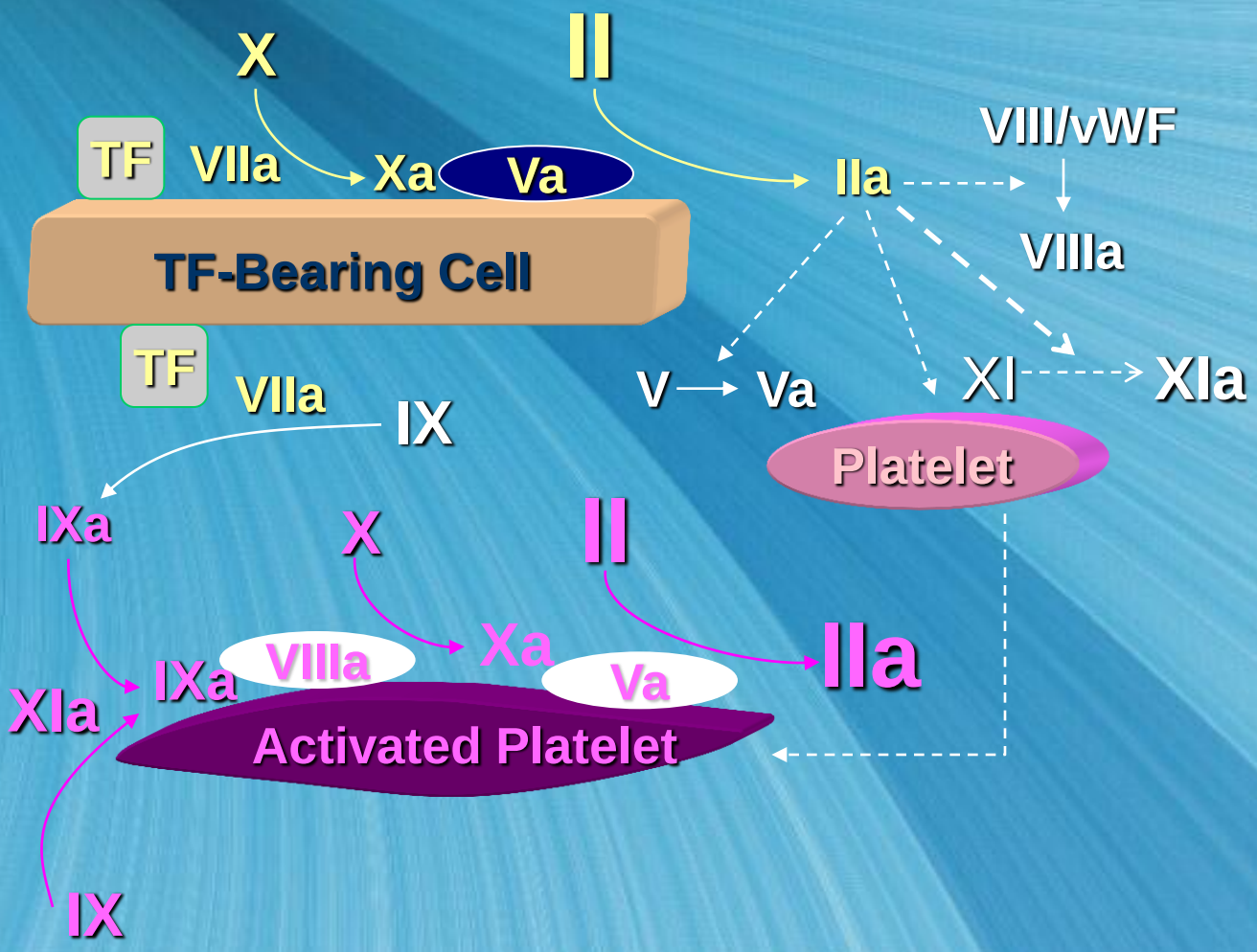
Hoffman & Monroe: A Cell-Based Model of Hemostasis.
Thromb Haemostas, 85:958-65, 2001

Propagation



Hoffman & Monroe: A Cell-Based Model of Hemostasis. *Thromb Haemostas*, 85:958-65, 2001

A Cell-Based Model of Hemostasis



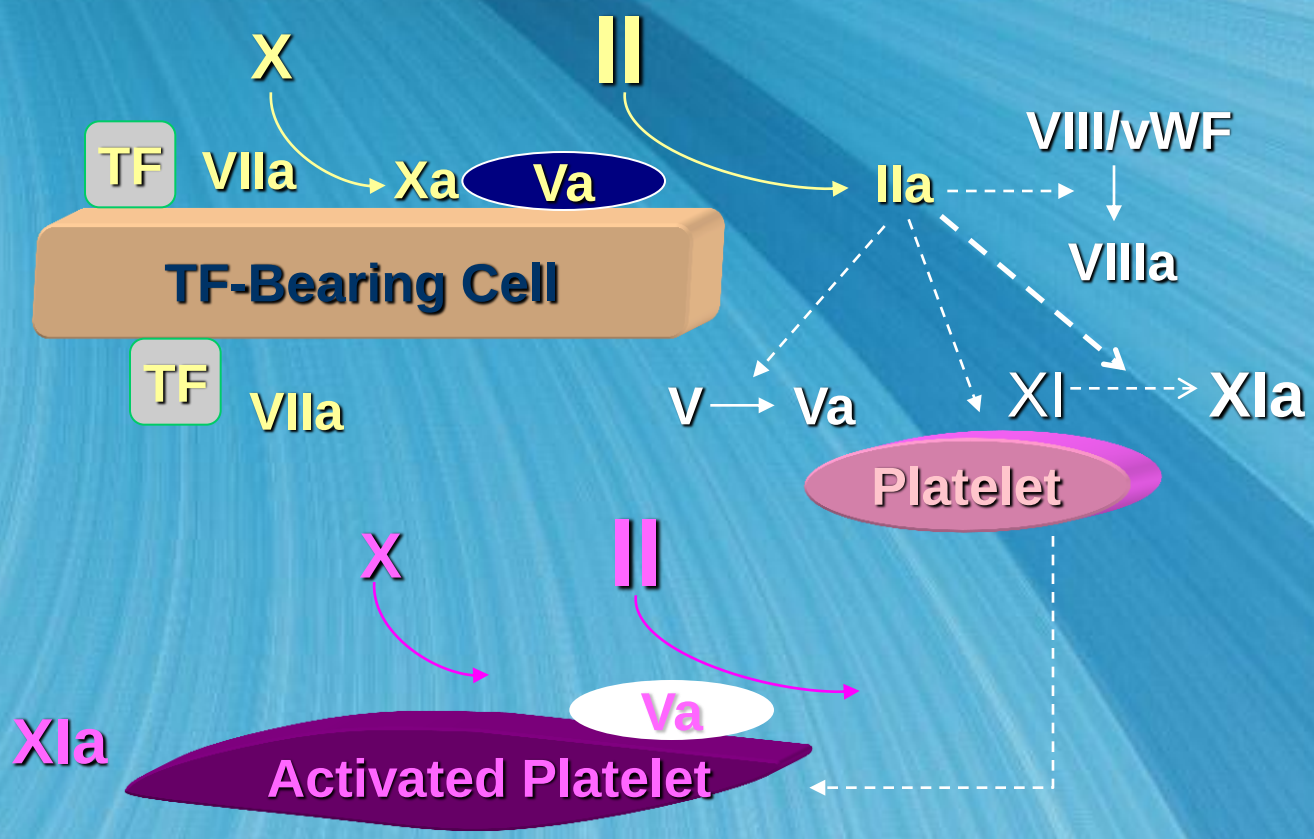
Hoffman M, et al. *Blood Coagul Fibrinolysis.* 1998;9(suppl 1):S61-S65.

There Really Are "Intrinsic" and "Extrinsic" Pathways

- They are not redundant - they operate on different cellular surfaces to fill different roles
  - The "extrinsic" or TF pathway works on the initiating cells
  - The "intrinsic" pathway works on platelets to produce the thrombin "burst"

Can a cell-based model explain why hemophiliacs bleed?

Hemophilia Is a Failure of Platelet Surface Thrombin Generation



Hoffman M, et al. *Blood Coag Fibrinolys.* 1998;9(suppl 1):S61-S65.

Our coagulation lab tests do not predict hemostasis *in vivo*!

The result of the PT or aPTT is NOT the same thing as hemostatic adequacy of the patient.

An abnormal result does not necessarily mean an increased risk of bleeding, and a "normal" result does not mean bleeding will not occur

Good News!

The common tests are useful for evaluating the cause of bleeding

- The PT and aPTT are useful if we have a bleeding patient and we want to figure out if a factor deficiency is responsible

The tests are also good for directing transfusion therapy for a bleeding patient

- Prolonged PT or aPTT = plasma
- Low fibrinogen = cryoprecipitate
- Low platelet count or defect of platelet function = platelet concentrates

What about prophylactic plasma for a patient who is not bleeding?

- It depends on the reason that the clotting times are prolonged and the clinical setting

What about prophylactic plasma for a patient who is not bleeding?

- Clearly we would not give plasma to a patient whose aPTT was prolonged because of FXII deficiency, for example

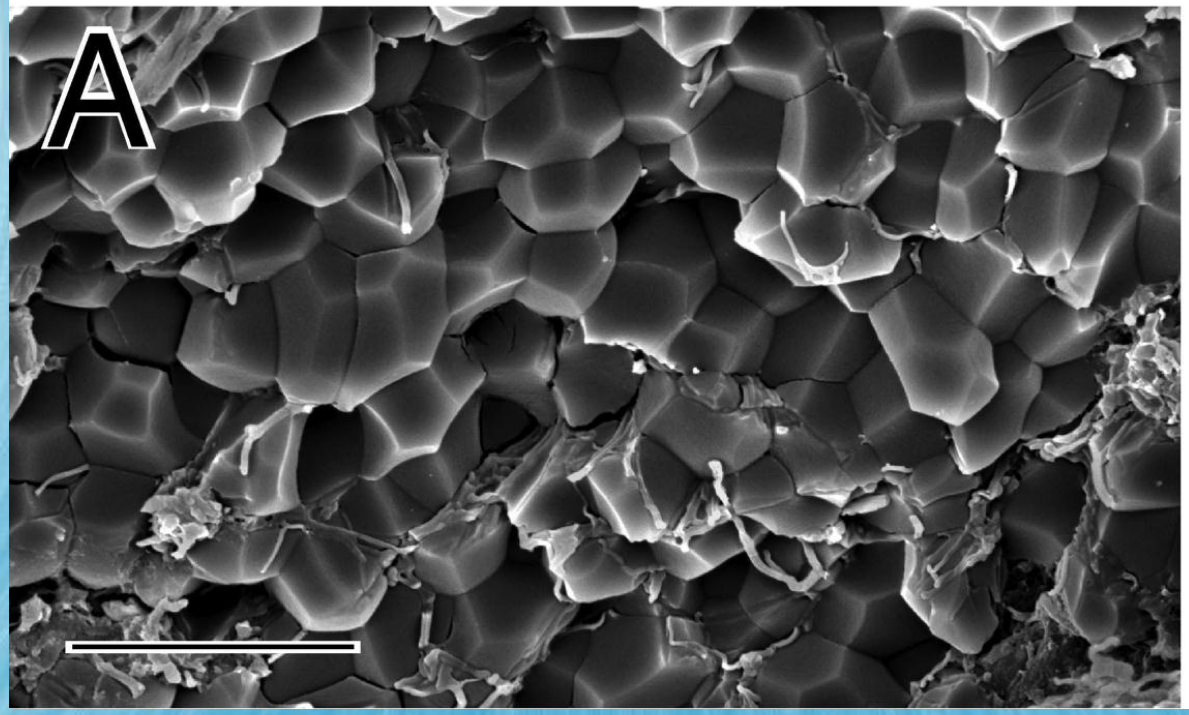
New evidence suggests that RBC have a role in hemostasis, too

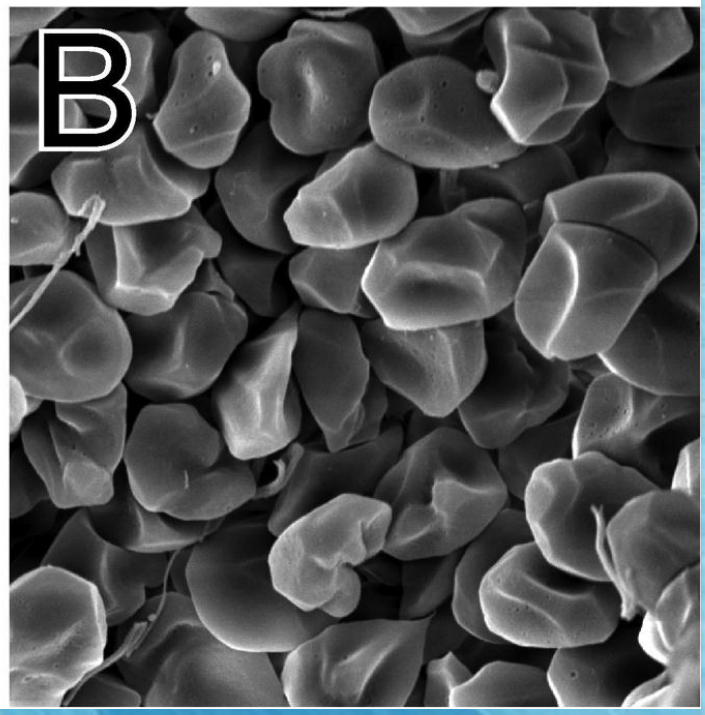
Home / March 6, 2014; Blood: 123 (10)

Clot contraction: compression of erythrocytes into tightly packed polyhedra and redistribution of platelets and fibrin

Douglas B. Cines[1], Tatiana Lebedeva[1], Chandrasekaran Nagaswami[2], Vincent Hayes[3], Walter Massefski[4], Rustem I. Litvinov[2], Lubica Rauova[3,5], Thomas J. Lowery[4], and John W. Weisel[2]

Polyhedrocytes Are Present *in vivo* in Arterial and Venous Thrombi





Polyhedrocytes in coronary artery thrombi taken by aspiration from ST-elevation myocardial infarction patients (n = 10). Erythrocytes make up about 10% of the volume of these thrombi.

Increasing RBC in the clot decreases the porocity

Table 1. Hydrogen/deuterium exchange rates

Hematocrit	Exchange time*
0%-4%	<10 s†
4%-10%	2-3 min
10%-25%	2-10 min
33%	10 min
42%	>20 min‡

*Approximate time to half the initial signal.

†This value is limited by the time necessary to do the experiment; in reality, it is likely to be milliseconds.

‡Results were variable (sometimes many hours).

FXIII not only stabilizes fibrin, but helps keep RCB in the clot

RESEARCH ARTICLE

The Journal of Clinical Investigation

Factor XIII activity mediates red blood cell retention in venous thrombi

Maria M. Aleman,[1] James R. Byrnes,[1] Jian-Guo Wang,[1] Reginald Tran,[2] Wilbur A. Lam,[2] Jorge Di Paola,[3] Nigel Mackman,[4,5] Jay L. Degen,[6] Matthew J. Flick,[6] and Alisa S. Wolberg[1,4]

✧ Implications of RBC Retention in Clots

- Hct may contribute to
  - effectiveness of hemostasis
  - severity of thrombosis
- ♦ FXIII might be a novel target for pro-hemostatic and anti-thrombotic therapies in the future

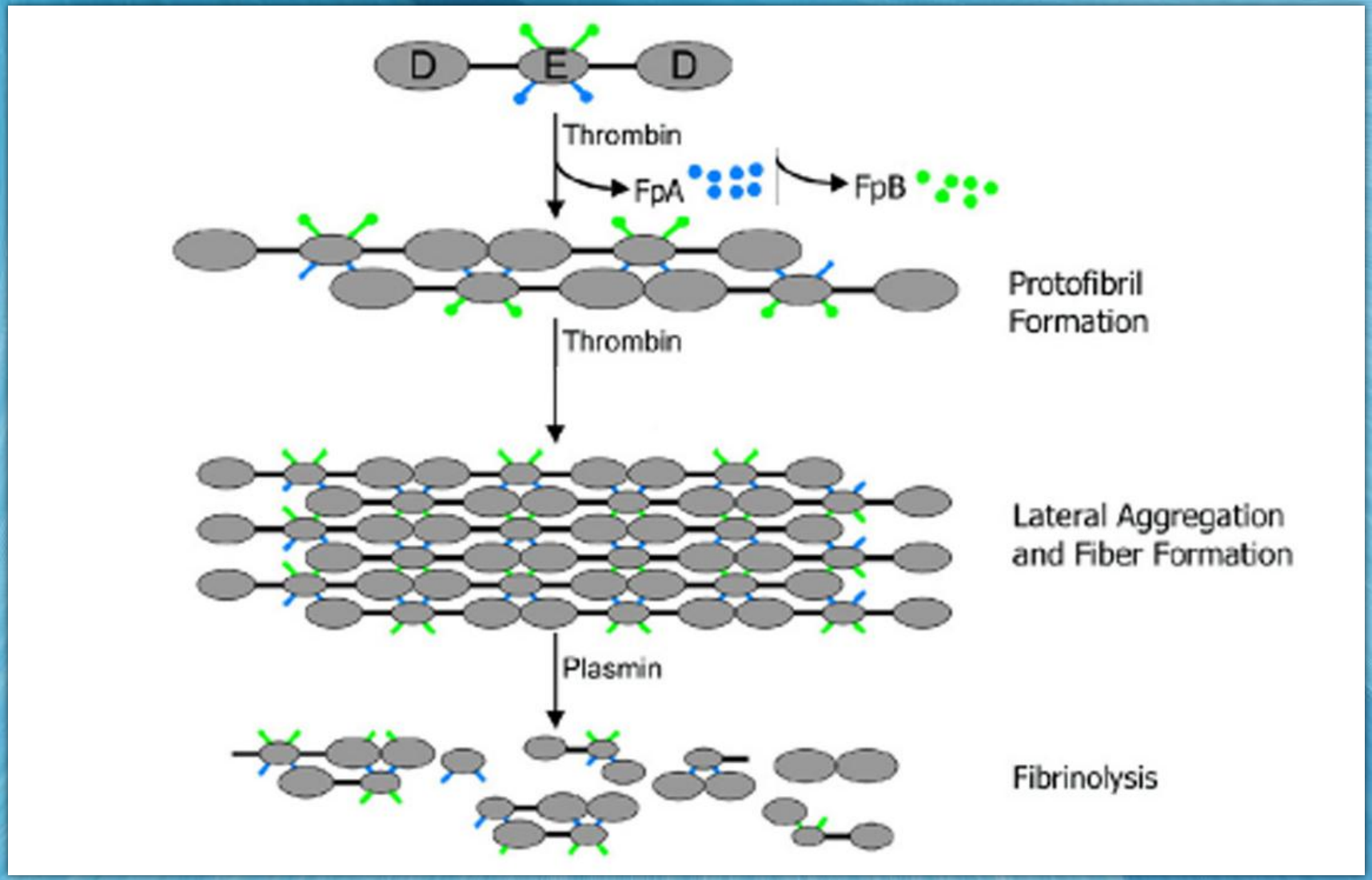
Blood component therapy does not always stop the bleeding

- The goal of transfusion is to restore the patient to "normal"
  - Replacement doesn't always work
    - FFP is always somewhat diluted
    - Platelets have a "storage defect"
    - Consumption may outstrip replacement
    - May need more volume than patient can tolerate
  - Even if we restore apparently "normal" levels, bleeding may not stop

To maintain hemostasis a sufficiently stable clot must be formed

- Primary hemostasis via platelet plug
- Stabilized by a meshwork of fibrin due to platelet surface thrombin generation
- Final clot must resist mechanical and enzymatic disruption until healing occurs

Fibrin assembly can be a race against plasmin degradation



Do any of our lab tests tell us when local fibrinolysis is winning the race?

- No - we probably have to guess
- Our lab tests only tell us about the conditions of the blood components

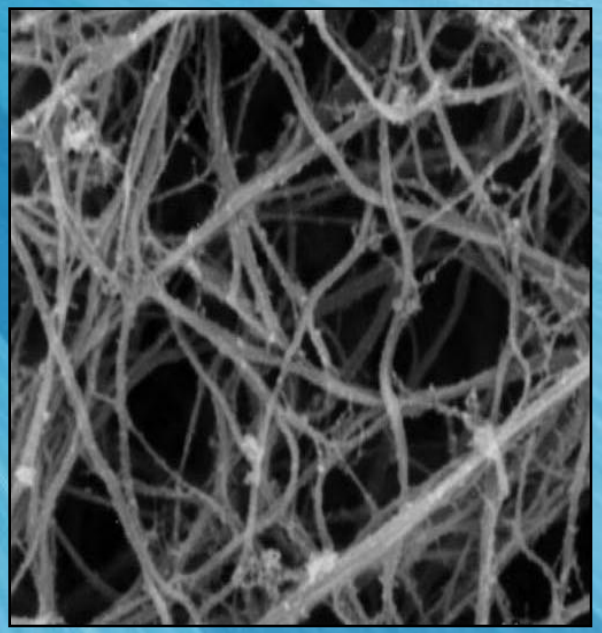
What else can we do for microvascular bleeding, when transfusion hasn't done the trick?

- Anti-fibrinolytics
- Coagulation factor concentrates - can achieve supra-normal levels of factors
  - FVIIa
  - Fibrinogen concentrate
  - Prothrombin complex concentrates

The whole idea is to get a stable platelet/fibrin clot

IIa

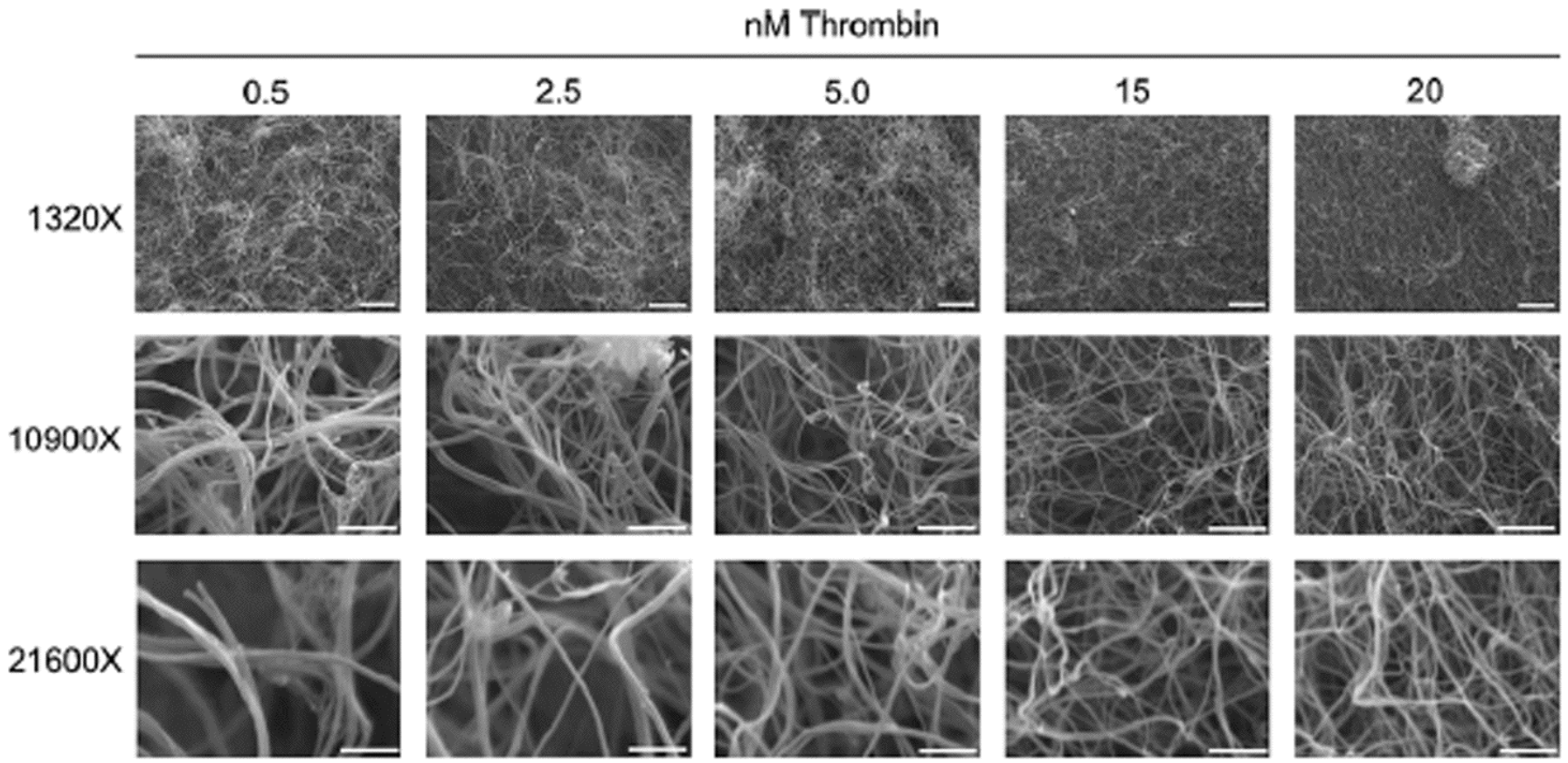
Fibrinogen →



Fibrin clot structure & stability depend on the amount/rate of thrombin generated and the amount of fibrinogen incorporated

Higher levels of each give more structurally stable clots

More thrombin gives a more tightly packed clot



Adapted from Wolberg, Blood Reviews. 2007, 21:131–142

We can enhance thrombin generation by:

- Replacing deficient factors or platelets
  - Should return thrombin generation to "normal"
    - Might not be enough
- Administration of rFVIIa or Prothrombin Complex Concentrates (PCC)
  - Note that these are off-label uses
  - Can get thrombin generation higher than "normal" in non-hemophilic patients

We can increase fibrinogen with:

- Cryoprecipitate
  - Concentrated form of fibrinogen as well as FVIII/vWF
    - Might enhance platelet adhesion as well as increase fibrinogen
- Administration of fibrinogen concentrate
  - (note that this is an off-label use)
  - Infectious disease risk probably less than cryo
  - Can give a known dose of fibrinogen

Higher levels of fibrinogen produce more tightly packed and stable fibrin clots *in vitro*
and
increase fibrin content of clots and resistance to lysis *in vivo*

Machlus et al. Blood 2011, 117:4953-63

Take-home messages in hemostasis

- The cascade model helps us interpret the PT and aPTT tests
- A cell-based model gives us insight into hemostatic mechanisms *in vivo*
- Common lab tests can help identify a cause of bleeding and guide transfusion therapy, but don't tell us whether a given patient will bleed or not


Questions?