# Platelet function testing

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# Are these platelets in working order??

Platelet roles in haemostasis and inflammation

When should we suspect a platelet problem?

Routine platelet assays – aggregometry

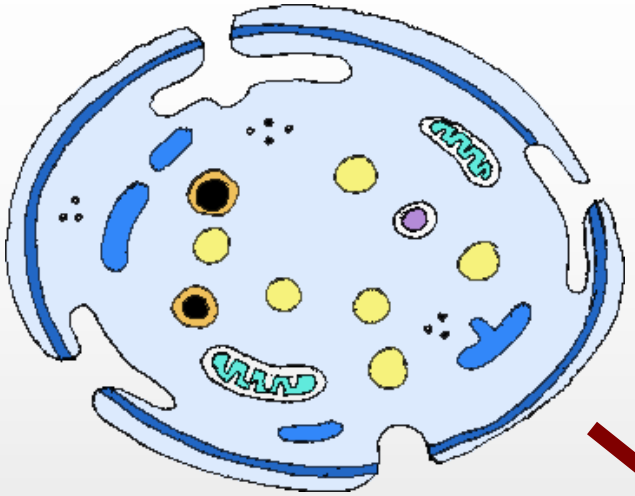
Second-line platelet assays

Point-of-care platelet assays

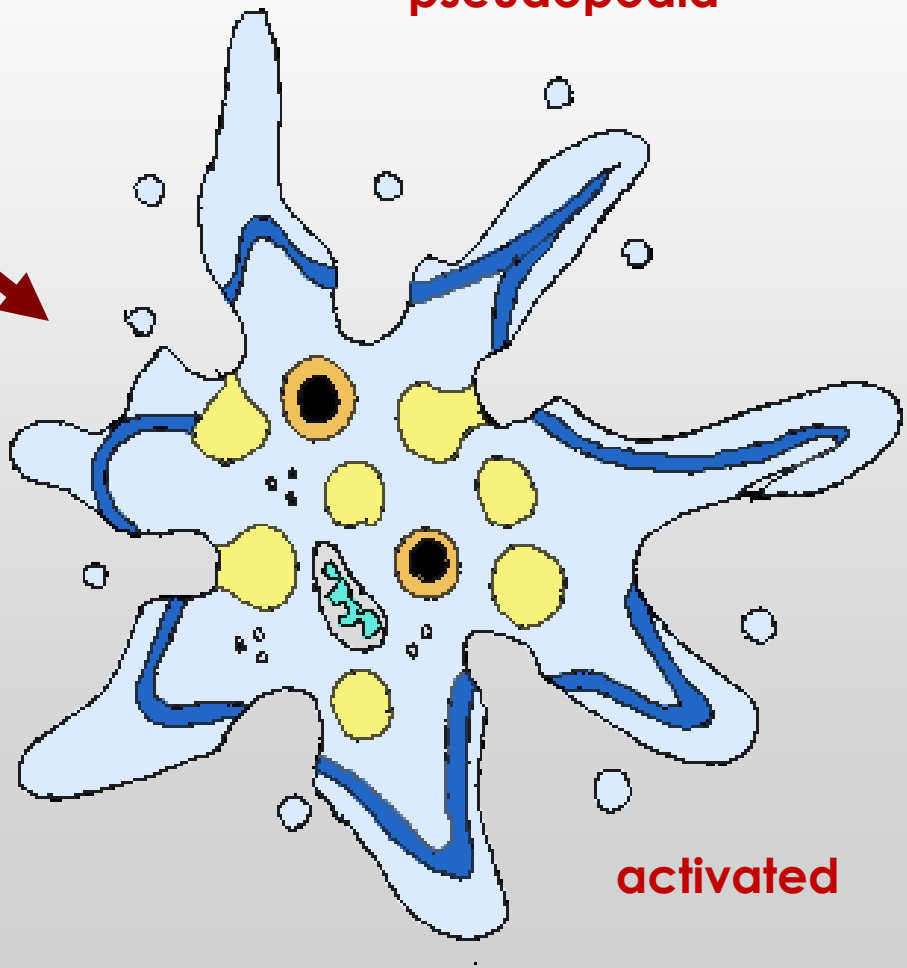
Genotyping - ?the future of diagnostics

# Platelet Activation

resting



Shape change - pseudopodia



activated

Granule release

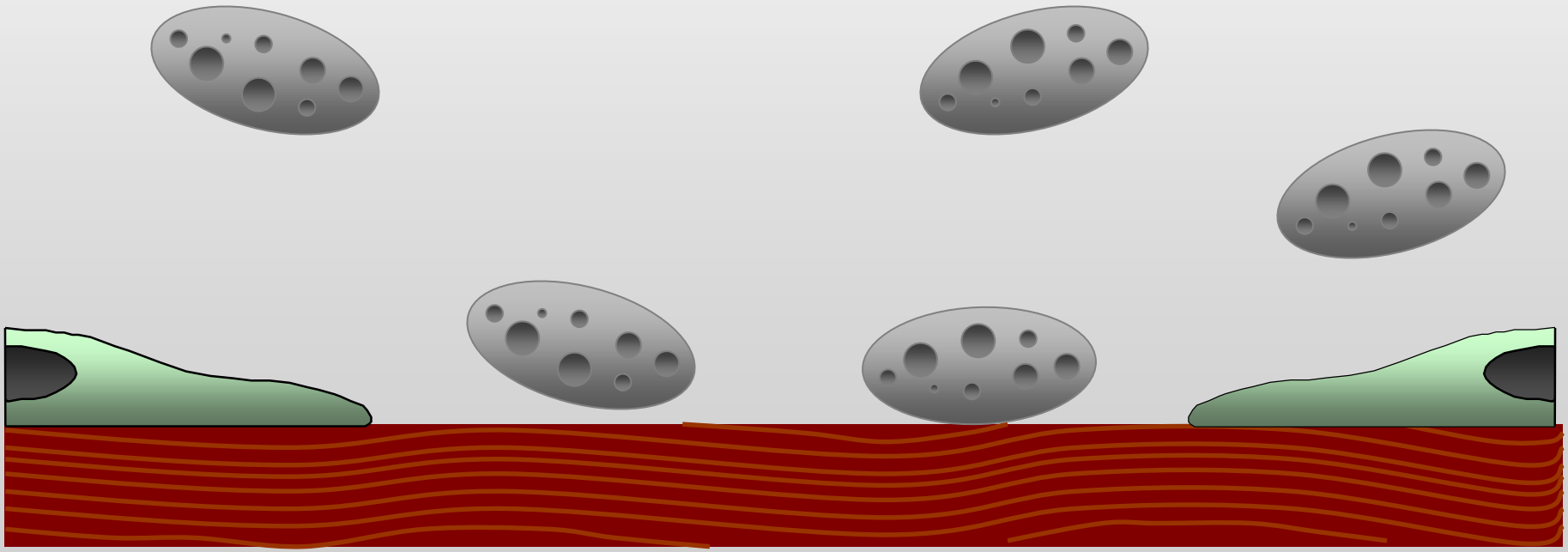
Microparticles shed

Receptor activation

Phospholipid exposure

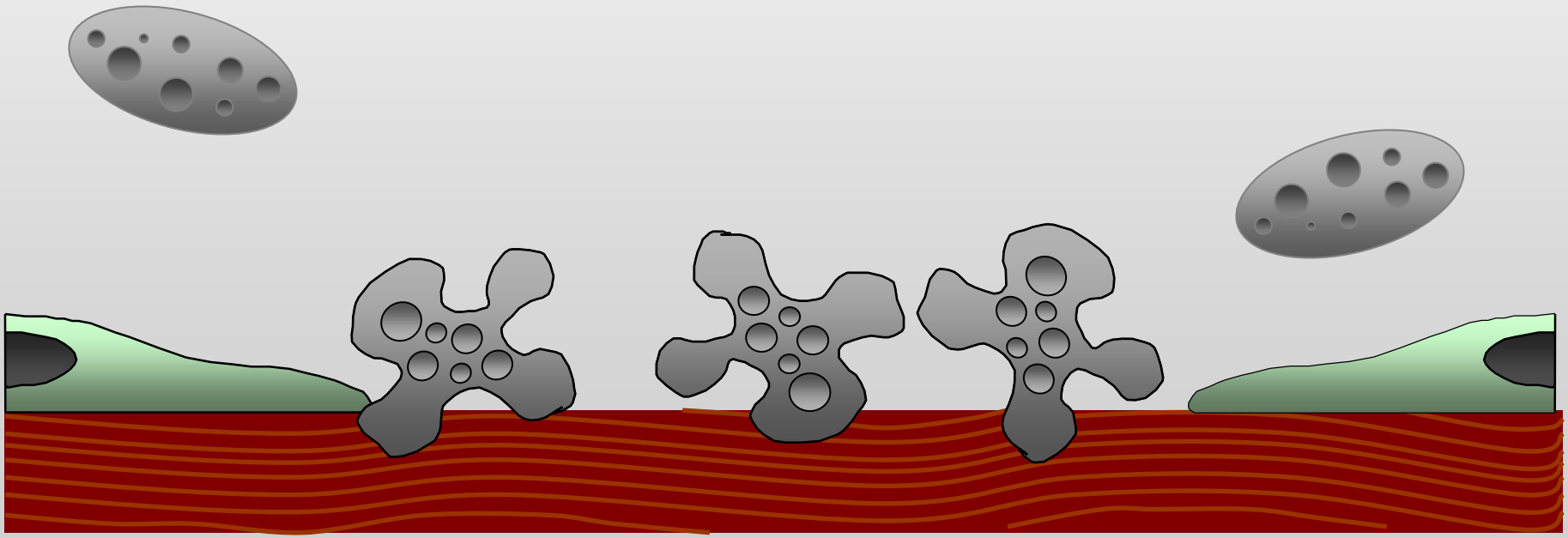
# Adhesion

**Platelet GPIb-V-IX binds matrix vWF at high shear**
**Reversible adhesion to exposed extracellular matrix**



# Activation

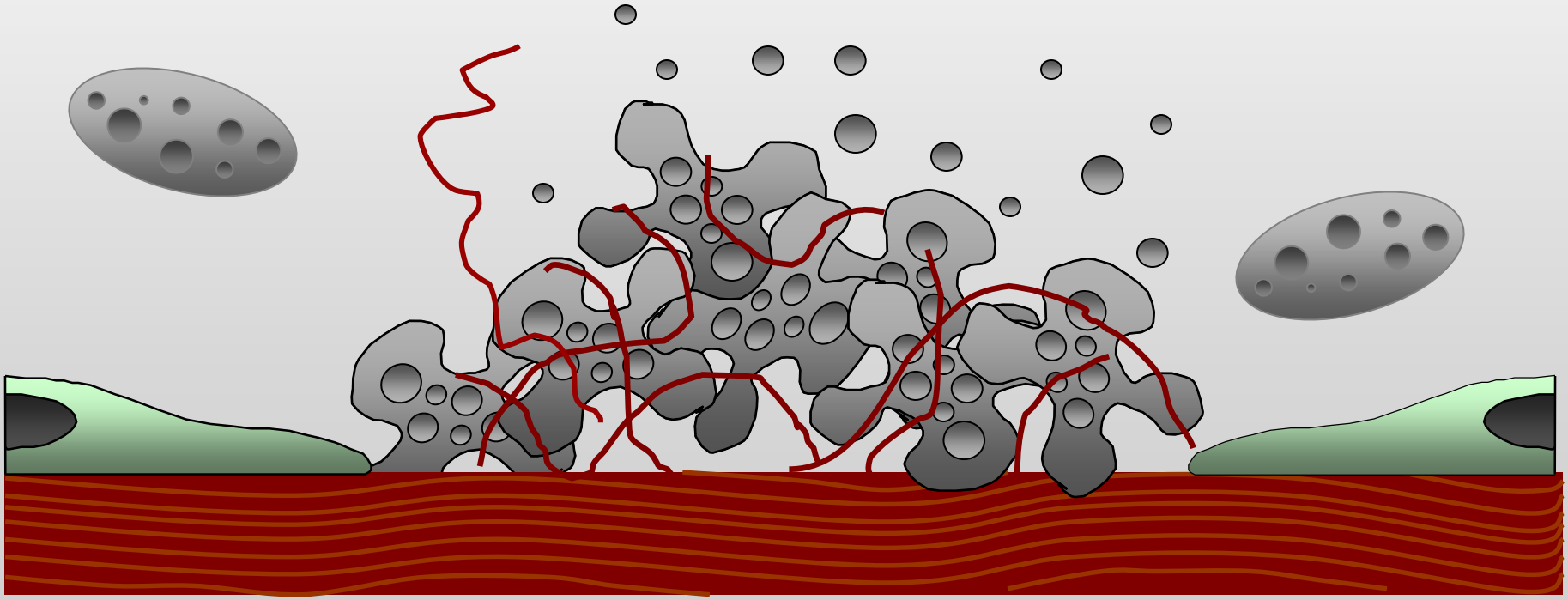
**Adhesion triggers GPIIb-IIIa activation**
**Irreversible binding to matrix ligands, shape change and platelet activation**



# Aggregation

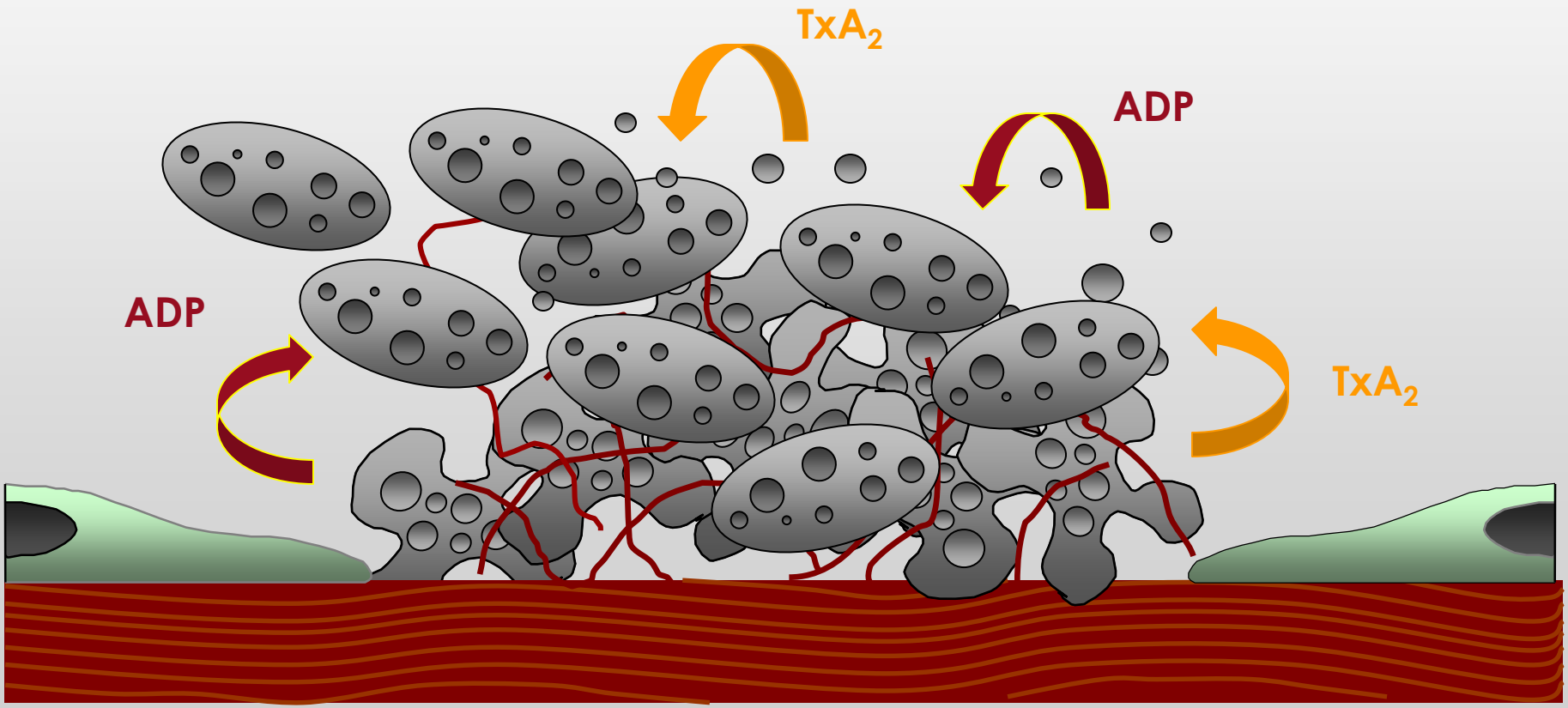
**Activated GPIIb-IIIa mediates aggregation via fibrinogen, vWF**

**Release of granule contents, microparticles recruits additional platelets and triggers coagulation**



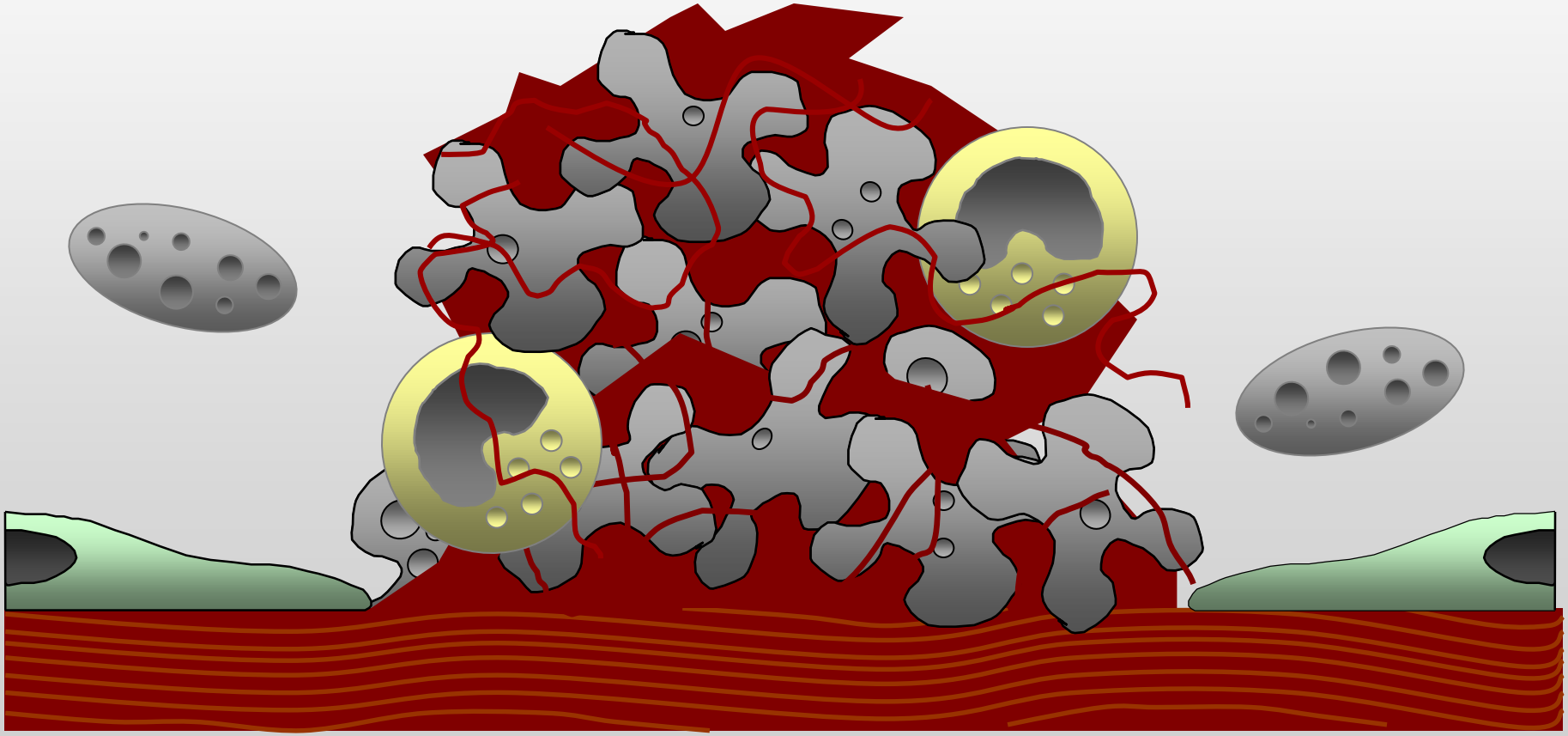
# Stabilisation

**Secreted ADP and thromboxane recruit new platelets to thrombus, and stabilise growing thrombus**



# Haemostatic plug

**A plug of degranulated platelets, fibrin mesh plus leukocytes recruited via P-selectin and fibrinogen receptors, and entrapped RBC**



# Practice point

**When should we suspect platelet function defects?**

Unexplained mucocutaneous bleeding, menorrhagia or postop bleeding

Including patients with a diagnosis of ITP or von Willebrand disease! Inherited platelet defects are not as rare as we are taught…

Bleeding/bruising despite normal coagulation tests

Always start with a blood film examination…

Always suspect drugs (including non-prescription items)

Consider contributing pathologies - renal or hepatic impairment, paraproteins, clonal haematological disorders… these tests are more reliable than platelet function assays

# Acquired platelet function disorders

- **Drugs** affecting platelet function
- **Systemic disorders**
  - Uraemia
  - Antiplatelet antibodies
  - Cardiopulmonary bypass
  - Liver disease
- **Haematological disorders**
  - Myeloproliferative disorders
  - Myelodysplasia, leukaemia
  - Dysproteinaemias
  - Acquired von Willebrand disease
- **Storage defect** (transfused platelets)

# Inherited platelet function defects

| | | |
|---|---|---|
| **Adhesion** | **GPIb-vWF** | von Willebrand disease<br>Bernard-Soulier syndrome |
| **Aggregation** | **GPIIb-IIIa** | Glanzmann thrombasthenia |
| **Activation** | | |
| **AA metabolism** | | Cyclo-oxygenase def (ASA) |
| **Granule release** | | Storage pool deficiency<br>Quebec platelet disorder<br>Release defect |
| **Cytoskeletal reg'n** | | Wiskott-Aldrich |
| **P-lipid exposure** | | Scott syndrome |

# Diagnostic work up

**First step tests**

- Blood smear
- Light transmission aggregometry using Limited number of agonists
- Studies assessing platelet granule release
- Flow cytometry – platelet surface glycoproteins

**Second step tests**

- Light transmission aggregometry using an Expanded agonist panel
- Flow-cytometry
- Clot retraction studies
- Measurement of Serum $TxB_2$
- Transmission electron microscopy

**Third step tests**

- Biochemical studies
- Receptor binding assays
- Genetic studies

*Gresele P, Journal of Thrombosis and Haemostasis, Feb 2015*

# Practice point

**History and examination are important first steps**

Is bleeding recent (suggesting an acquired cause) or lifelong

Ask about specific haemostatic challenges (tooth extraction, surgery, pregnancies)

Establish both prescription and non-prescription medications

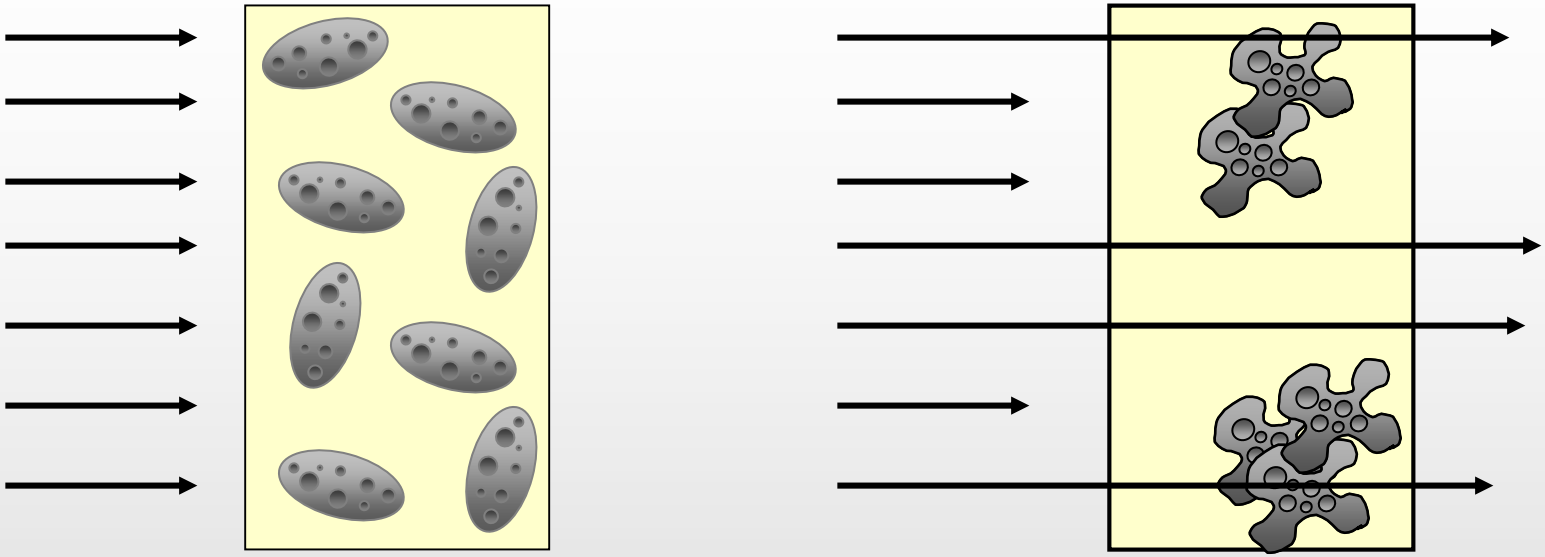
Is there a family history – what pattern?

Syndromic abnormalities can help in the diagnosis

- deafness, cataracts, renal failure in MYH9-RD
- facial and vascular anomalies in Velocardiofacial Syndrome

Formal bleeding assessment tool (ISTH-BAT) can help in comparing patients and family members

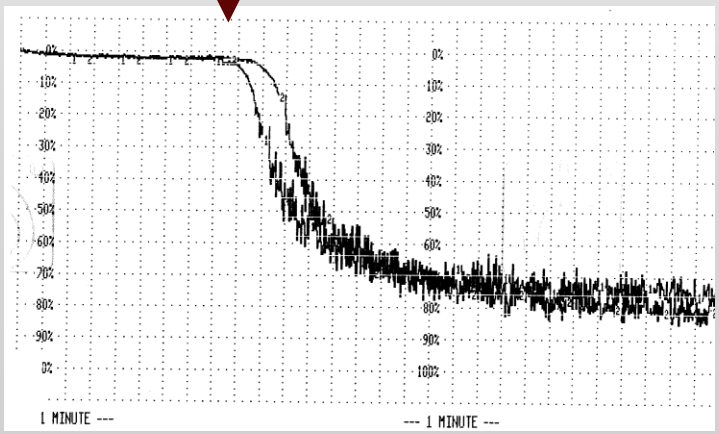
# Optical Aggregometry



**Resting pl - turbid**

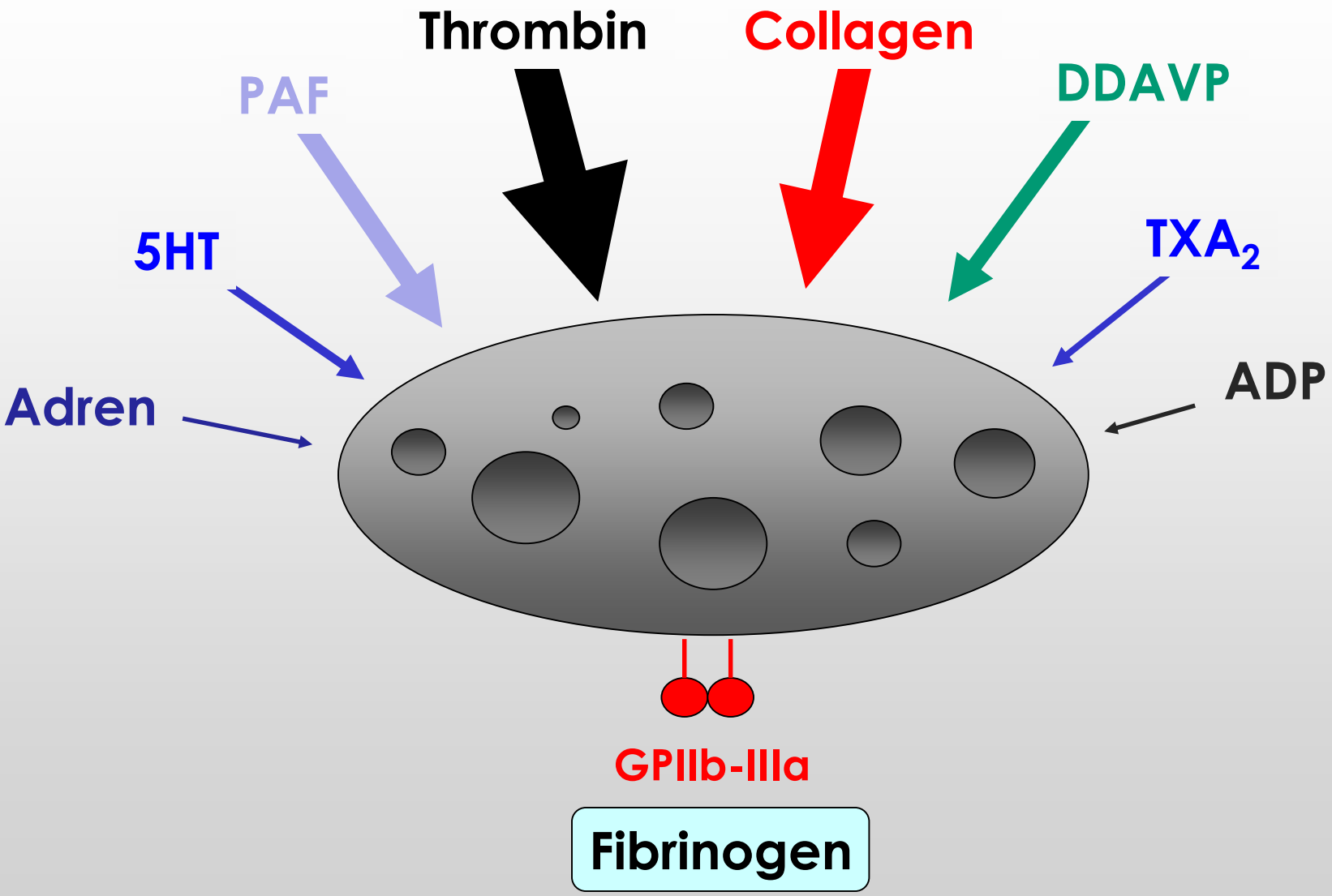
**Clumped pl - clear**

Optical Transmittance




Time

# Platelet Activation : Multiple Pathways to Aggregation



# Practice point

**When should we order aggregometry?**

Not a suitable assay for acutely ill inpatients

More suited for outpatient, elective testing – advise patients to avoid NSAIDs, dietary inhibitors and exercise before testing

Requires an experienced laboratory scientist, remains time-consuming

High degree of variability between individuals, including poor responses to adrenaline and low-dose agonists

NO platelet assays are consistent in their results – critical differences in shear and methodology makes assays difficult to compare

Only rare conditions (BSS, GT) can be confidently diagnosed by optical aggregometry, but it remains the mainstay of testing

# Case 1

14-year old girl presenting with lifelong bleeding
Petechiae at birth
Spontaneous bruising, nosebleeds,
Bleeding with tooth eruption
Severe bleeding with first menstrual period - requiring platelet and red cell transfusion

Thrombocytopenia - large platelets on blood film

No family history

**Congenital platelet function defect:**

**Bernard-Soulier syndrome (deficiency of GPIb-IX)**

# Case 2

3-year old girl with petechiae and bruising at birth
Frequent bruising with minor trauma
Gum bleeding with tooth eruption

Platelet count normal - prolonged bleeding time
Normal coagulation assays (PT, APTT, fibrinogen)

Mother with menorrhagia (von Willebrand disease)
Father normal

**Congenital platelet function defect:**

**Glanzmann thrombasthenia (deficiency of GPIIb-IIIa)**

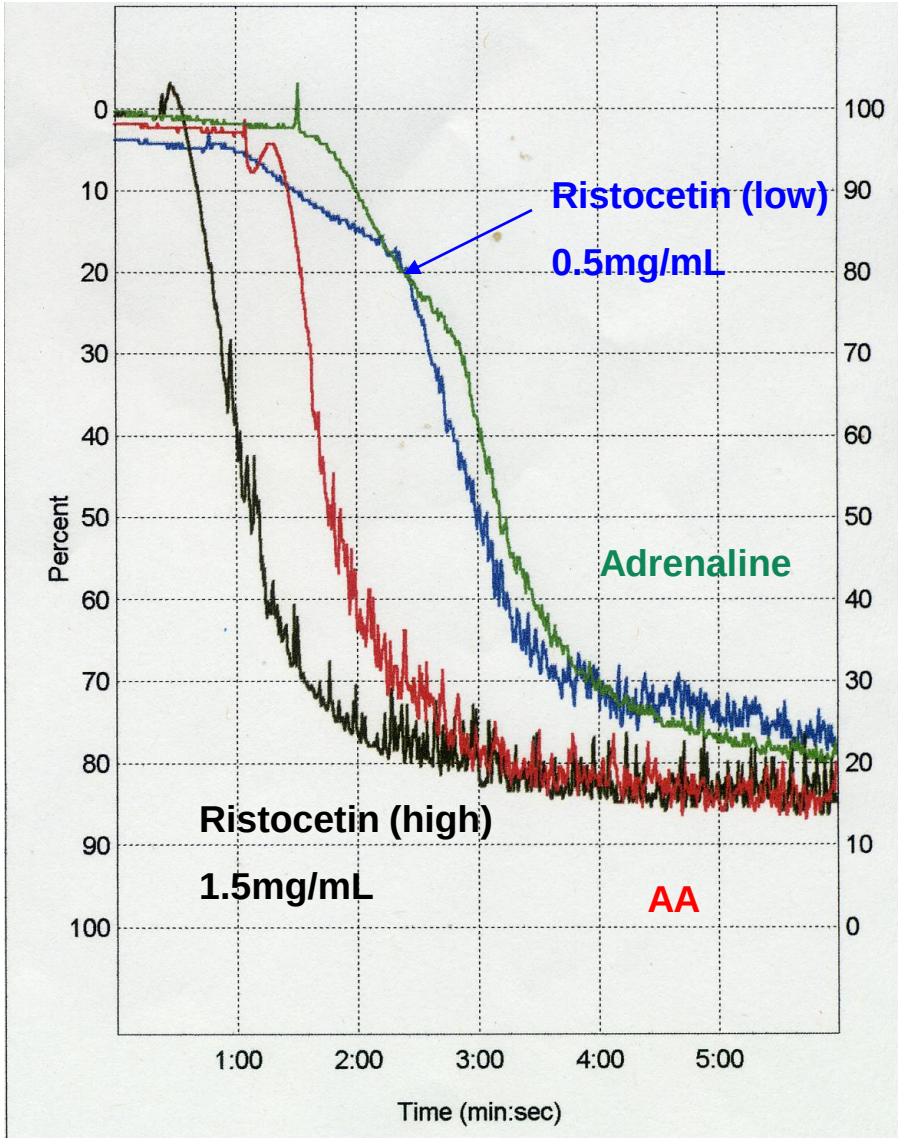
# Case 3

**58-year old woman with significant postoperative bleeding**

**Mild thrombocytopenia -normal platelet size**
**vWD screen normal**

**PFA-100 closure times prolonged**

**Coll/Epi >300 sec**
**Coll/ADP 240 sec**




Aggregation with **all** agonists - **including low-dose ristocetin**

Indicates **enhanced GPIb-vWF binding:**

either **Type 2B vWD**

or **platelet-type vWD**

(activating mutations of GPIb alpha)

# Platelet Aggregation Testing

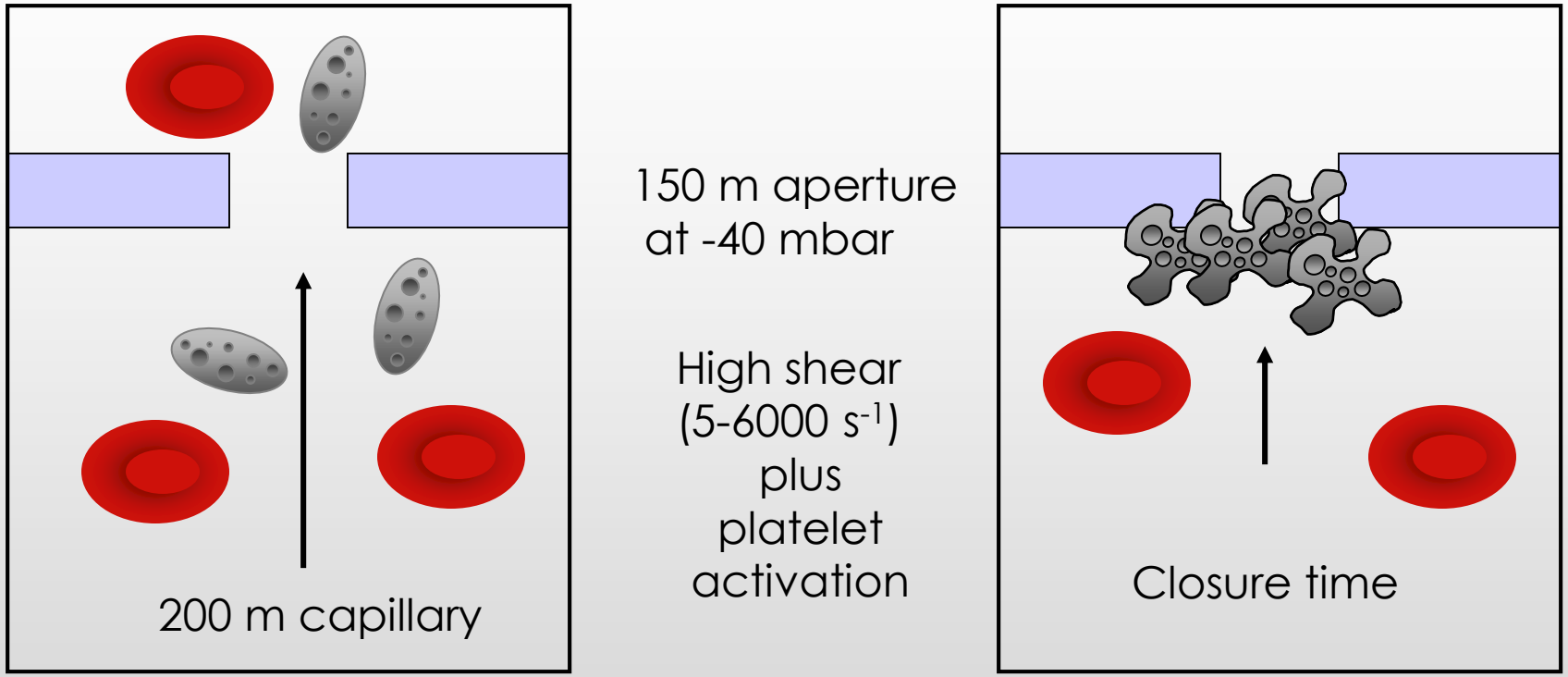
| | ADP<br>5 umol | Coll<br>2 ug/L | AA<br>1 mM | Ristocetin<br>1.5 mg/mL | Risto (low)<br>0.5 mg/mL |
|---|---|---|---|---|---|
| **vWD** | **N** | **N** | **N** | **-** | **-** |
| **2B vWD** | **N** | **N** | **N** | **N** | **Agglutn** |
| **Thrombasthenia** | **-** | **-** | **-** | **N** | **-** |
| **Storage pool** | **1° wave** | **Abn** | **N** | **N** | **-** |
| **Release defect** | **1° wave** | **Abn** | **Abn** | **N** | **-** |
| **Aspirin** | **1° wave** | **Abn** | **Abn** | **N** | **-** |

# Automated (POC) platelet function analysers

| | |
|---|---|
| **PFA-100/200** | vWF-dependent, high shear<br>?sensitivity for mild defects<br>replaced skin bleeding time |
| **Verify NOW** | POC testing |
| **Multiplate** | whole-blood impedance<br>rapid, multiple agonists |

*Paniccia et al Vasc Health Risk Mgt 2015*

# PFA-100™ analyser: a model of primary haemostasis




After Kratzer and Born, developed by von der Goltz
Membrane contains collagen plus adrenaline (Coll/Epi) or collagen plus ADP (Coll/ADP)

Closure time dependent on GPIIb-IIIa, GPIb, vWF **not** fibrinogen

# A fundamental (and unsolved) problem

**Platelet function assays are non-physiological:**

- **e.g. low shear in optical aggregometry**
- **fibrinogen independent in PFA**
- **usually measured in absence of RBC, WBC**
- **neglect contribution of vessel wall (except SBT)**

**Correlations between different assays are very poor**

**Aggregometry methods not well standardised**

**Access to second-line assays is limited**

# Are these platelets in working order??

Platelet roles in haemostasis and inflammation

When should we suspect a platelet problem?

Routine platelet assays – aggregometry

**Second-line platelet assays**

Point-of-care platelet assays

Genotyping - ?the future of diagnostics

# Case 4

**60-year old man - easy bruising, epistaxes and excessive bleeding after tooth extraction**

**Mild thrombocytopenia - platelets appear pale and "grey" on Romanovsky-stained films**

**Mild impairment of aggregation to collagen and ADP**
**Other agonist responses normal**
**Bone marrow shows increase in reticulin**

# Assays for platelet granule function

| | |
|---|---|
| Flow cytometry | CD62P (P-selectin) exposure (alpha granules) |
| | CD63 (lysosomes) |
| | Mepacrine uptake/release (dense granules) |
| Whole mount imaging | Dense granules |
| Electron microscopy | Both |

## Flow cytometry of platelet receptors

| | |
|---|---|
| GPIIb-IIIa (CD41/CD61) | Glanzmann's |
| GPIb-IX (CD42a/CD42b) | Bernard-Soulier |
| GPVI (CD36) | Collagen receptor |
| GPIa-IIa (CD33/CD49b) | Collagen receptor |

## Activation markers

| | |
|---|---|
| PAC-1 (fibrinogen mimetic) | GPIIb-IIIa |
| Annexin V | Anionic phospholipid |

# Platelet function tests predicting clinical outcomes

| Blood flow cessation | ASA | Thienopyr. | GPIIb-IIIa antag. |
|---|---|---|---|
| PFA-100® | Yes | No | NR |
| **Platelet-platelet aggregation** | | | |
| Optical aggregometry (LTA) | Yes | Yes | NR |
| Impedance aggregometry | Yes | Yes | Yes |
| VerifyNow™ | Yes | Yes | Yes |
| Plateletworks® | Yes | Yes | Yes |
| **Shear-induced aggregation (SIPA)** | | | |
| Impact (cone and plate) | Yes | Yes | NR |
| **Clot elasticity** | | | |
| Thromboelastogram | Yes | Yes | Yes |
| **Activation-dependent** | | | |
| Flow cytometry | Yes | Yes | Yes |
| VASP (flow) | No | Yes | No |
| Urinary 11-dehydro-TxB2 | Yes | No | No |

*after Gurbel et al JACC 2007*

# Practice point

**When should we test for clopidogrel resistance?**

Not routinely in patients after stenting

Although poor response to clopidogrel is associated with more vascular events, increasing the dose of clopidogrel does not solve the problem

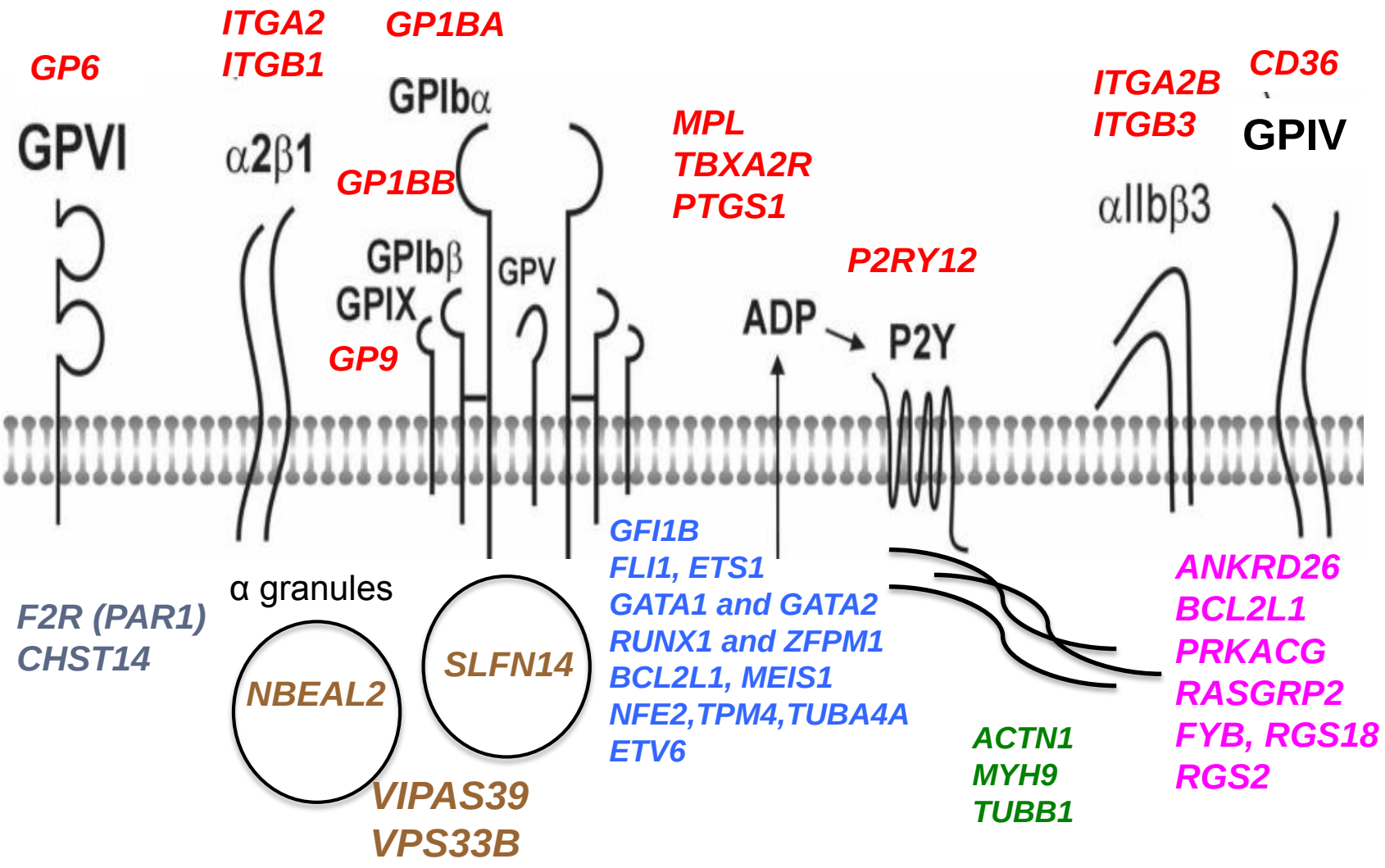
Genotyping (CYP variants) only explains part of the variation in response

Use of newer P2Y12 inhibitors (prasugrel, ticagrelor) overcomes variable metabolism

Consider testing of high-risk patients (interventional neuroradiology stents, coronary stent thrombosis) to guide therapy – if platelet inhibition is poor, switch to a newer agent

# Candidate gene identification and gene panel design

- **Version 3 → 32 genes**




Receptors Cytoskeleton Transcription factors Granule defects/trafficking Undefined Intracellular

# Practice point

**How do we treat platelet function defects?**

Important to define platelet function as a contributor to clinically significant bleeding

Address role of drugs first – can antiplatelet agents be reduced or ceased?

Modify procedures and surgery when patient has a recognised bleeding tendency

General approaches work for platelet function defects, mild vWD and mild haemophilia:

DDAVP infusions preop plus tranexamic acid

Platelet transfusions in high-risk procedures or for severe bleeding, may need to be continued

Consider risk of alloimmunisation in BSS, GT patients

# Platelet function testing

Patient and family history are important

Consider interfering factors (drugs, diet)

Light transmission aggregometry is the major method used, but requires expertise and planning

Potential role for whole-blood impedance aggregometry in testing response to antiplatelet drugs (and HIT)

Consider inherited platelet defects – genotyping may be the most efficient method as phenotyping is often non-diagnostic