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PROCOAGULANT AND CELL DERIVED MICROPARTICLES: « Measurement in plasma and clinical significance »

Jean AMIRAL, Anne Marie VISSAC
HYPHEN BioMed

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7768 Service Center Drive • West Chester OH 45069

Phone: 513.770.1991

Toll Free: 866.783.3797

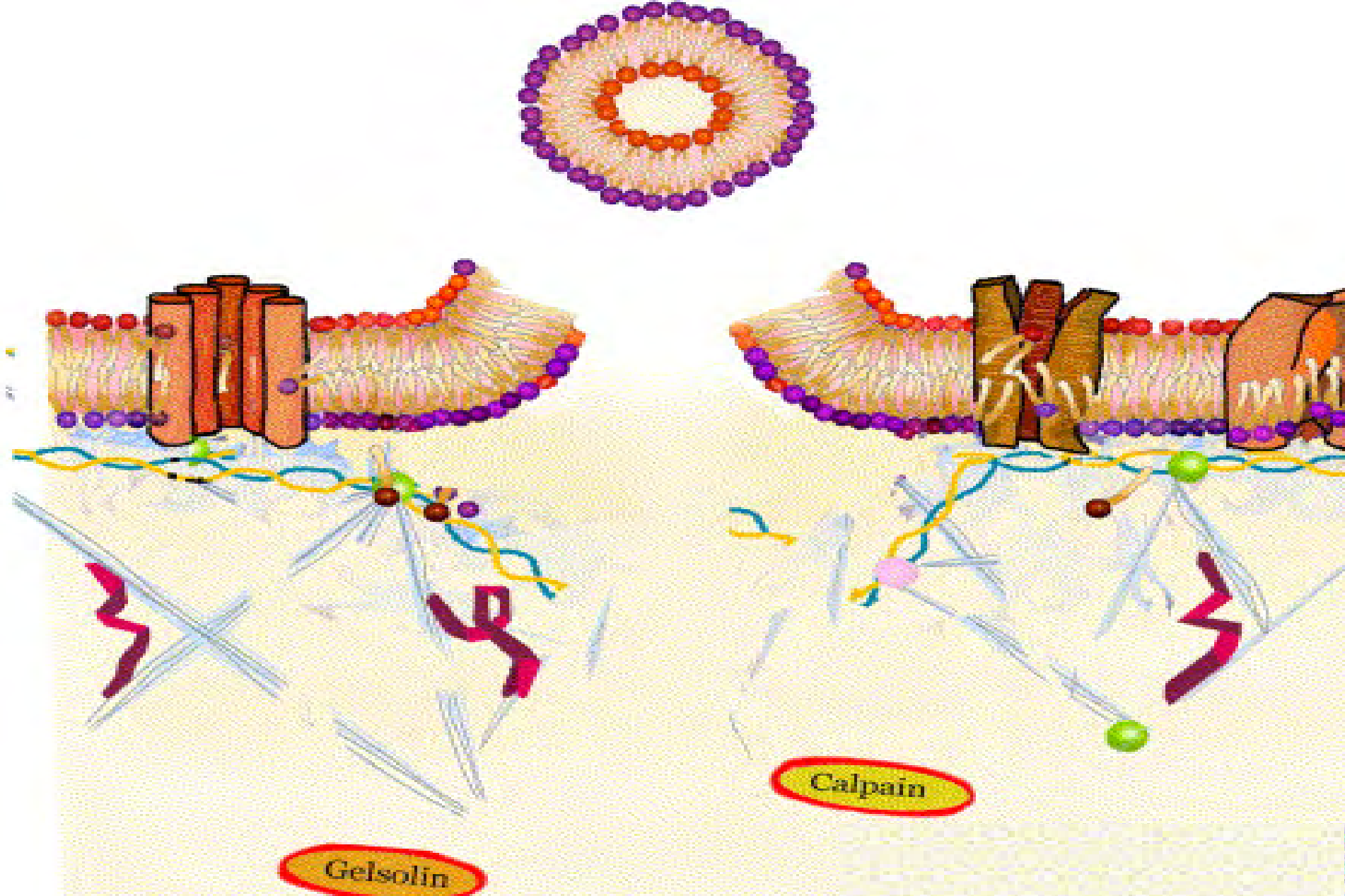
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Email: info@aniara.com

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What are microparticles

- Circulating procoagulant Microparticles (MPs) are pathogenic markers of enhanced coagulability associated to a variety of disorders and released from stimulated blood and vascular cells.
- Cellular MPs are submicrometric fragments resulting from the remodeling of the plasma membrane in response to numerous conditions, including activation and apoptosis.



Piccin A, *et al.* Blood Rew 2007



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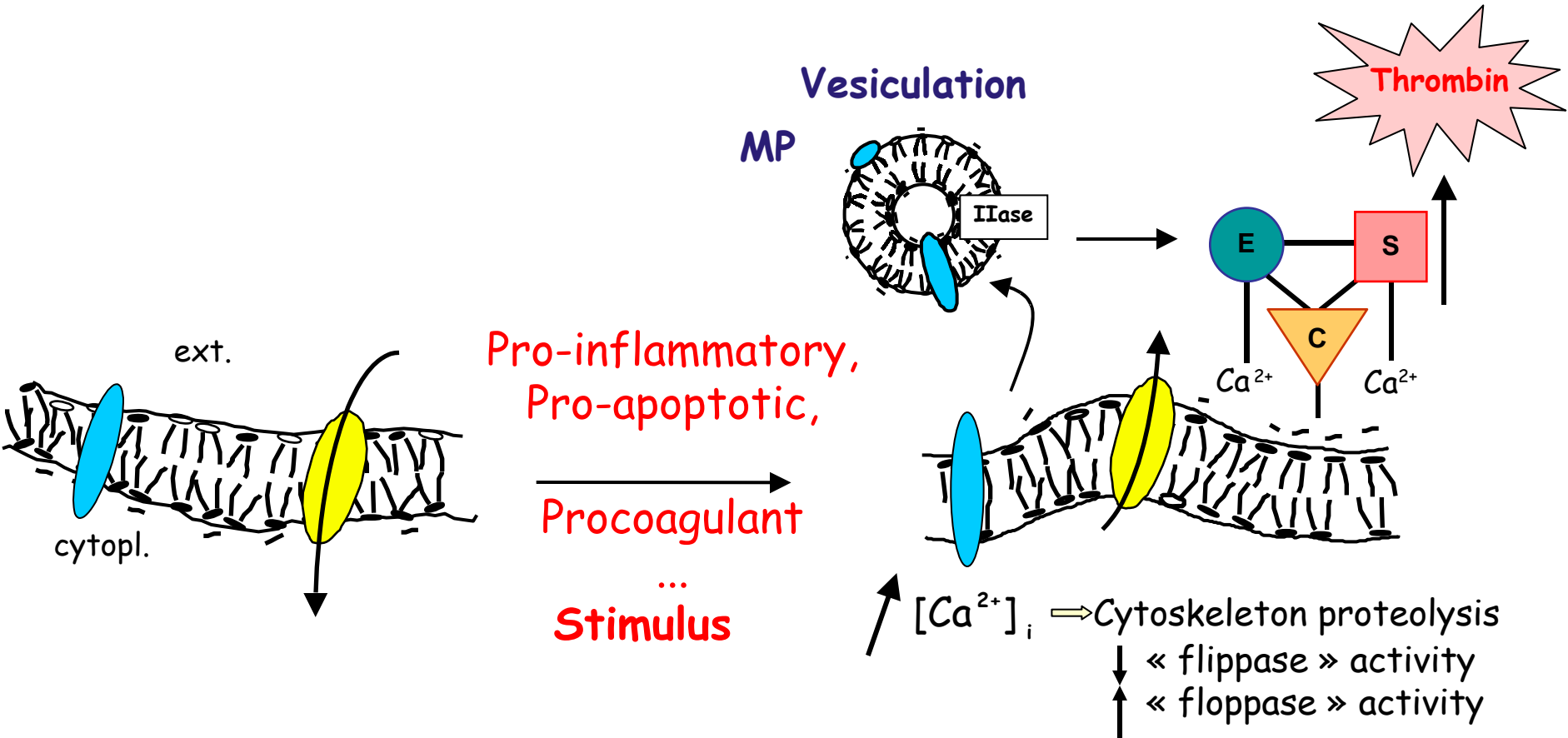
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Characteristics of MPs

- The general consensus is that MPs are small $<1\mu\text{m}$.
- Microparticle membranes consist mainly of lipids and proteins.
- Expose the anionic phospholipid phosphatidylserine.
- Express membrane antigens that reflect their cellular origin and the cellular processes triggering their formation.

Haemostasis and cell membrane remodeling



Microparticles = in vivo cell activation markers

Microparticles

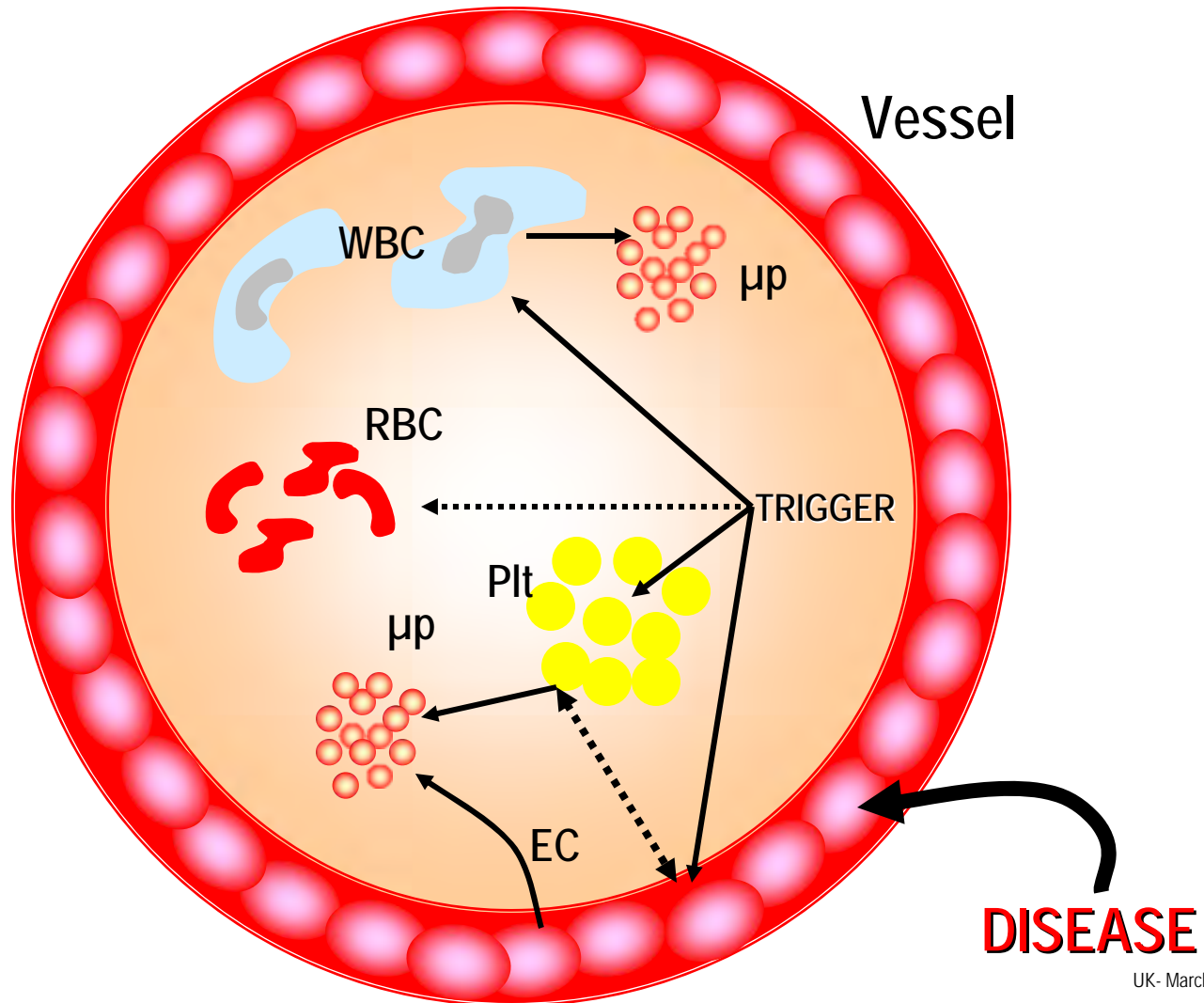
- Long shelf life (\approx 6 days)
- Bind to Annexin V
- Released from many blood cells
- Bear CDs, TF, TM, GP IIb-IIIa, ...

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Clinical usefulness of MPs study

- Modulate the Hemostatic balance and can cause its disruption.
- Procoagulant MPs in Immune-mediated Thrombosis.
- Procoagulant MPs in Atherothrombosis.
- Angiogenesis and MPs.
- Circulating MPs : Effectors in the Tuning of Thrombotic Propensity Associated with Cardiovascular Risk.
- Pharmacological Modulation of Circulating MPs.

Generation of microparticles



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Cellular origin of microparticles

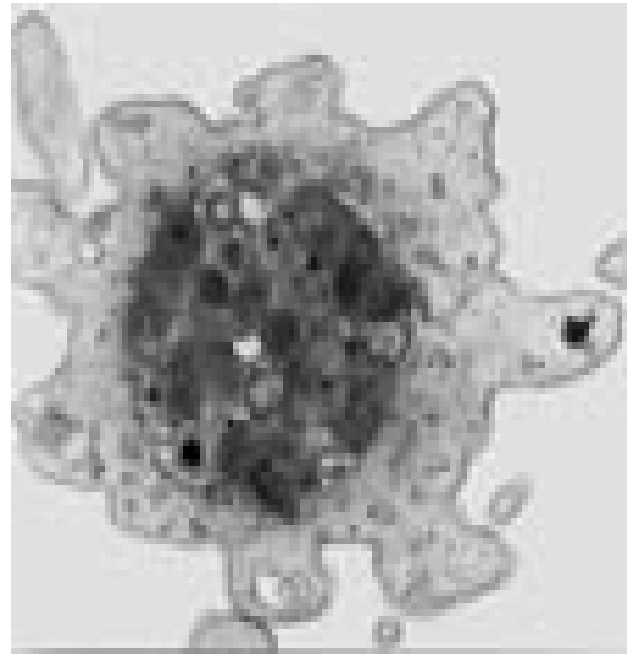
- Platelet (activation of coagulation)
- Endothelial cells (auto-immune diseases, TTP, activation of coagulation)
- Monocytes (inflammation, infection, ...)
- Leucocytes (inflammation, ...)
- Lymphocytes (diabetes mellitus, ...)

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Platelet activation



Resting

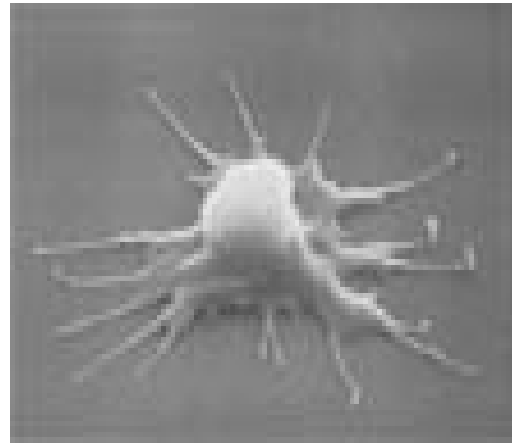


Activated

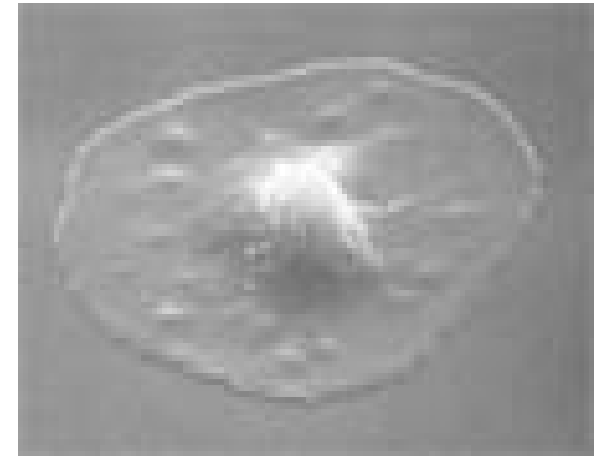
Platelet activation



Resting Platelet



Activation



Adhesion and spreading

Endothelial MPs



2 μ m
HUVEC + FCS

Taraboletti et al. *Am J Pathol* 2002



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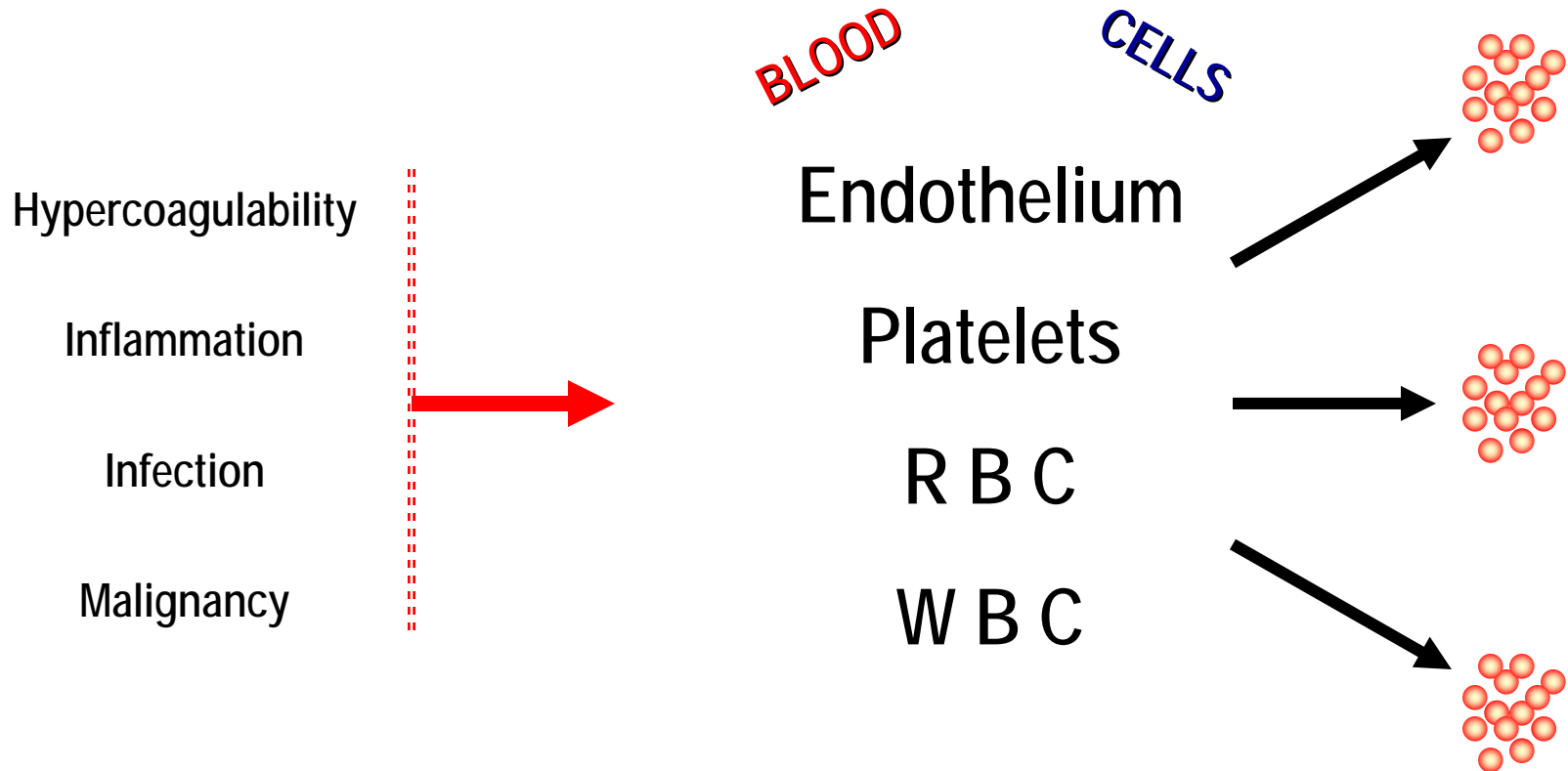
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Biological effects of microparticles

- **Procoagulant** (phospholipids)
- **Prothrombotic** (TF bearing μP , TF upregulation, platelet-leucocytes aggregates)
- **Enhancement of monocyte-endothelium adhesiveness**
- **Pro-inflammatory** (TNF- α ,...)

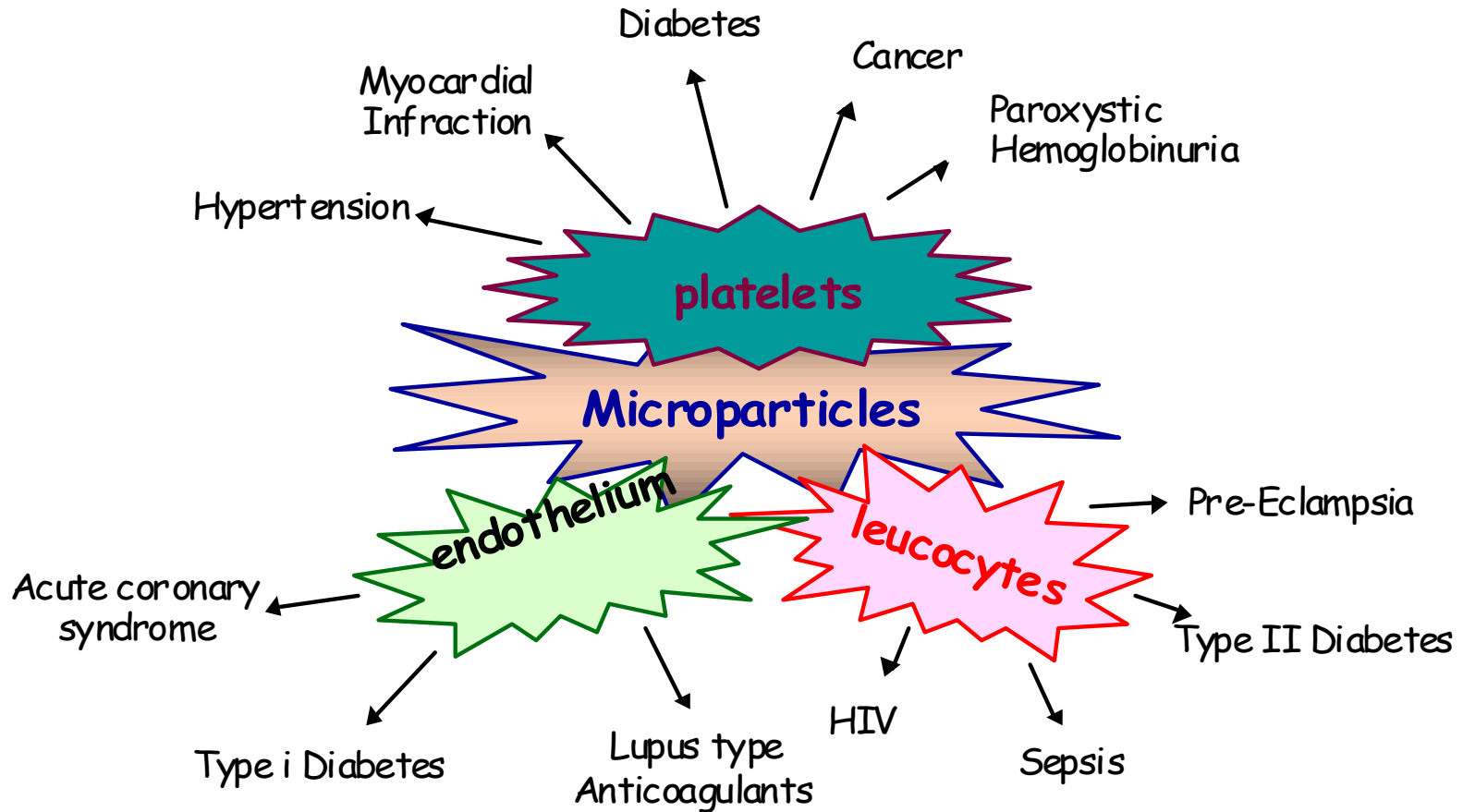
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Microparticles as a diagnostic marker



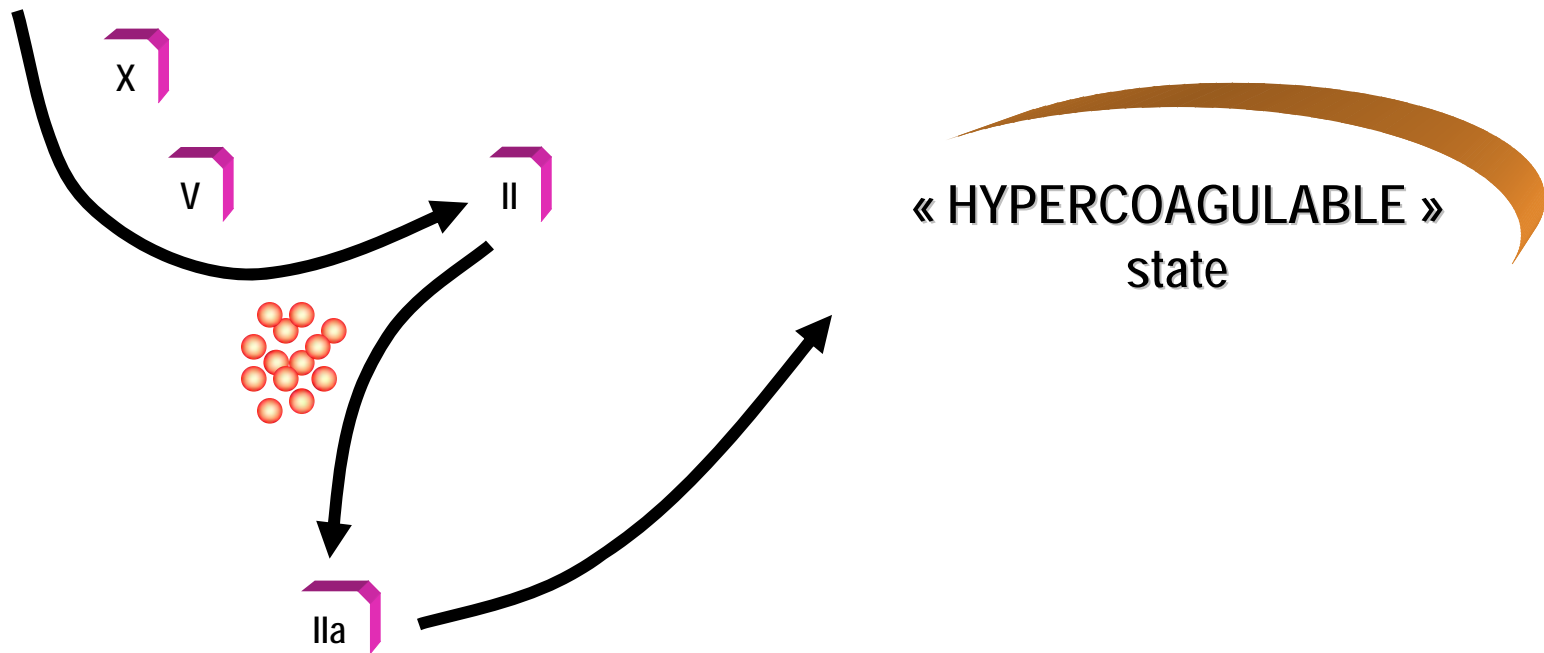
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Association of Microparticles with Disease



Martinez *et al.*, Am. J. Physiol (2005)

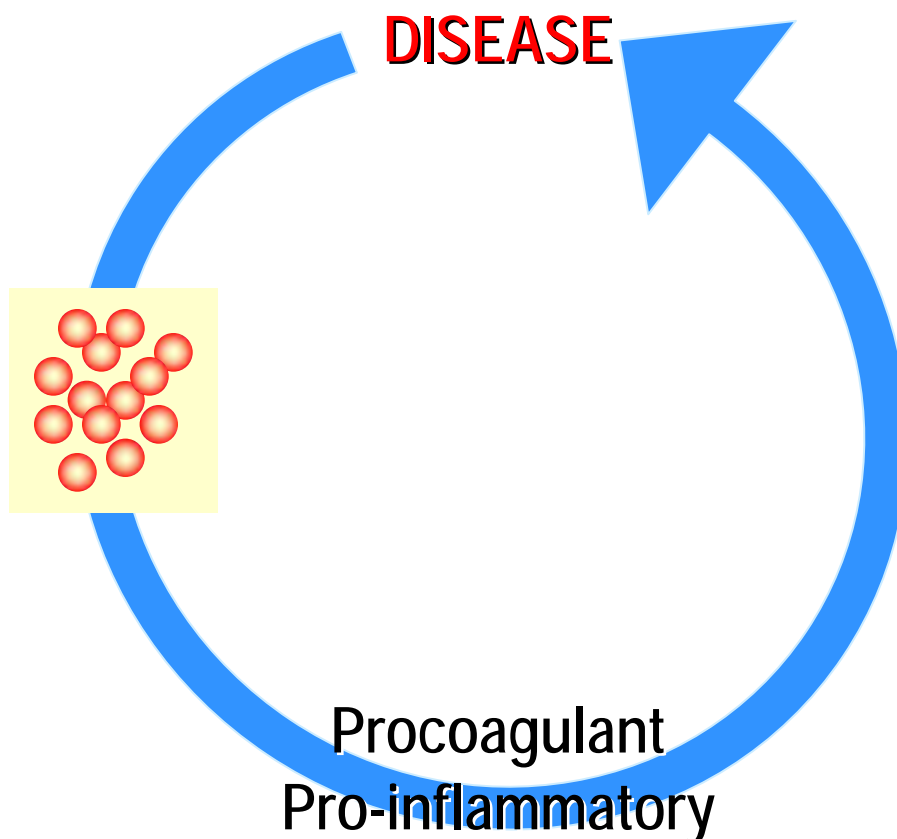
Microparticles as a risk for disease



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Microparticles

Cause and consequence of disease states



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Clinical applications

- **Prognosis of myocardial infarction.**
- **Follow-up and therapy monitoring of patients with myocardial infarction.**
- **Prognosis of recurrence risk.**
- **Diabetes, Malignancy, Pregnancy.**

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Pathological variations of microparticles

- Elevated in M.I. (x2 to x10)
- Elevated in cancer (predictor of metastasis?)
- Increased Predicts vascular complications in diabetes
- Elevated in haemophilia (x10)
- During Novoseven (Vlla) therapy
- Correlates with severity of hypertension

Note: responds to therapy efficacy

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Procoagulant Microparticles

- Many studies ongoing in Europe, Japan, USA.
- Many clinical data presented.
- Patented technology available for measuring MPs coagulant activity.
- Methods in development: Elisa, Latex (?), Others (?)

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Microparticle measurement

Different methodologies are available for MPs determination:

- Flow cytometry rely on the antigenic composition of MPs and allow them to be enumerated according to their cellular origin.
- ELISA capture on Annexin V or antibody and determination of procoagulant activity of MPs.

Measurement, Characterisation of Microparticles

■ **Flow Cytometry:**

- Only « large microparticles ($> 0.4 \mu$) are measured (size, content).
- Characterised by antibody/label used.

■ **Activity/Immuno-Assay:**

- All MPs are measured (including < 0.4 or 0.1μ)
- Measurement of associated procoagulant activity (PS equivalent).
- Identification of cell origin with MoAbs.

Cellular origin of microparticles

- Platelet (activation of coagulation)
- Endothelial cells (auto-immune diseases, TTP, activation of coagulation)
- Monocytes (inflammation, infection, ...)
- Leucocytes (inflammation, ...)
- Lymphocytes (diabetes mellitus, ...)

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Conclusions

- **New Assay for a New Parameter in Full « Concert » with Present Scientific Approaches.**
- **Many New Clinical Applications.**
- **Can be useful for disease recurrence, prognosis, and monitoring of therapy efficacy.**
- **High potential, in clinical practice, provided the pre-analytical variables are well controlled.**

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