Investigation of blood clotting mechanism in contact with nanofibers

Paweł Nakielski, IPPT PAN

Substantive support: Lt. Col., MD Robert Brzozowski, Military Medical Institute

Department internal seminar, 19.10.2016

Motivation

Despite many innovations in medical device field, hemorrhage still remains the primary cause of preventable death in combat and civilian trauma situations.

Among the major causes of death from trauma, massive bleeding is responsible for 30-40% of mortality. In the hospital, massive bleeding are the second most common cause of death (22%) just after cardiac factors (33%).

There is an ongoing debate about the impact of specific materials and their surface chemistry to trigger intristic clotting cascade.

Source of data: Impact of Hemorrhage on Trauma Outcome: An Overview of Epidemiology, Clinical Presentations, and Therapeutic Considerations, Kauvar D et al. J of Trauma-Injury Inf & Crit Care, 60(6):3-11, 2006; Causes of sudden unexpected death of adult hospital patients, Nichols L i Chew B, J Hosp Med, 7(9):706-8, 2012

What is hemostasis?

Hemostasis or haeomostasis (Greek: *aimóstasis*, from *aíma* "blood" + *stásis* "stagnation") is the physiological process that maintains a closed circulatory system after vascular damage, preventing excessive blood loss.

Function of hemostasis:

- Arrests bleeding
- Keeps blood in fluid state
- Repair and reestablish the blood flow through the injured vessels
- Remove haemostatic plug

If any of the above functions is exaggerated or impaired it will cause either thrombosis or hemorrhage respectively; so hemostasis is a balance between thrombosis and hemorrhage.

Blood elements participating in coagulation

Platelets

Important mediators in the coagulation cascade, involved in surface induced blood coagulation.

Clotting factors

Several zymogens activated in coagulation cascade

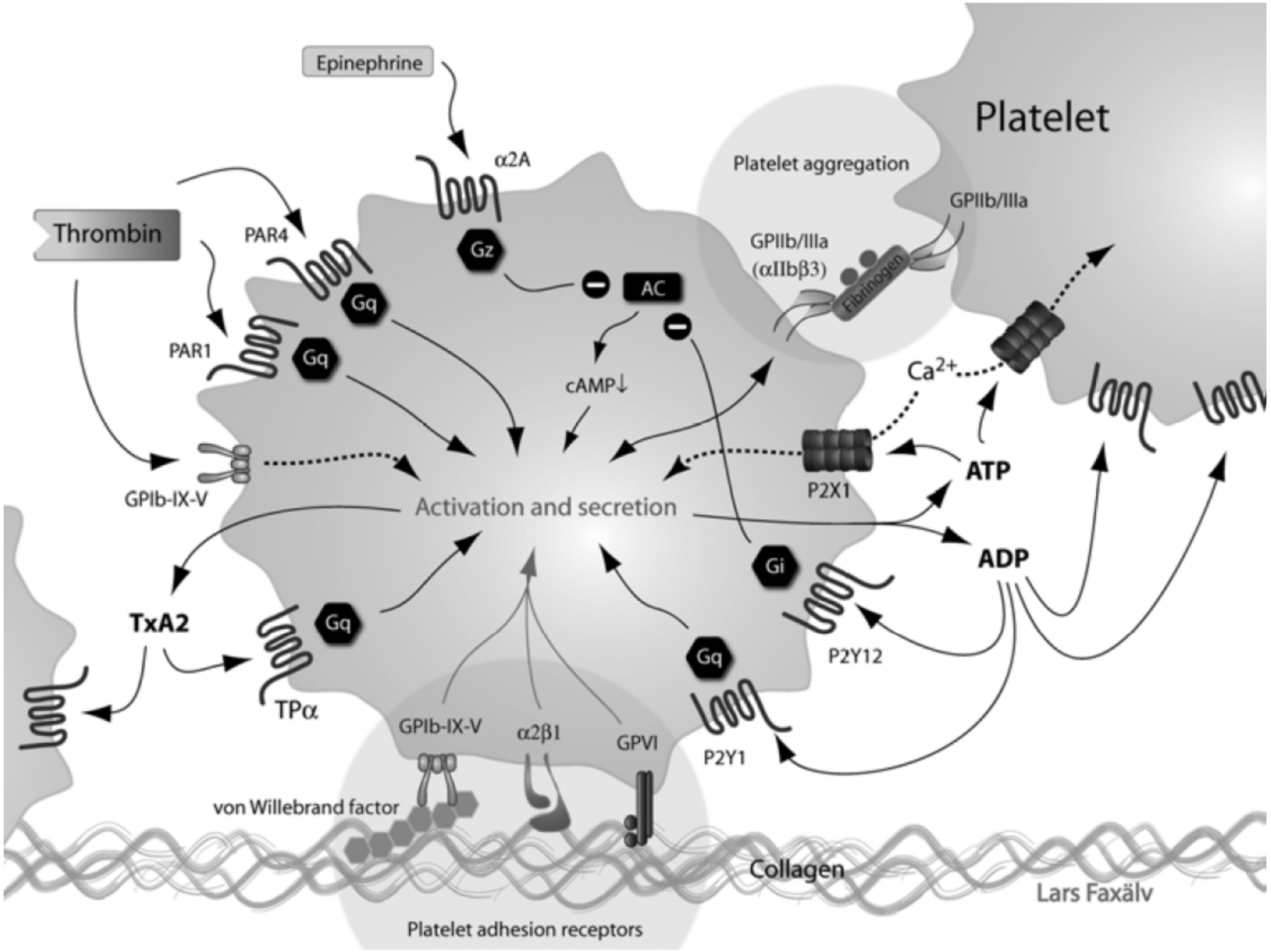
Fibrnogen

Soluble in water, converted to insoluble fibrin during coagulation

Other cells and proteins

Red Blood Cells
von Willebrand factor
HMWK
Prekallikrein

Platelets



Source: Imaging methods for haemostasis research, Lars Faxälv, Sweden, 2009

Clotting factors

Table 17.3 Blood Clotting Factors (Procoagulants)

FACTOR NUMBER	FACTOR NAME	NATURE	SOURCE	PATHWAY; FUNCTION
I	Fibrinogen	Plasma protein	Liver	Common pathway; converted to fibrin (insoluble weblike substance of clot)
II	Prothrombin	Plasma protein	Liver*	Common pathway; converted to thrombin (converts fibrinogen to fibrin)
III	Tissue factor (TF)	Plasma membrane glycoprotein	Tissue cells	Activates extrinsic pathway
IV	Calcium ions (Ca^{2+})	Inorganic ion	Plasma	Needed for essentially all stages of coagulation process; always present
V	Proaccelerin	Plasma protein	Liver, platelets	Common pathway
VI†				
VII	Proconvertin	Plasma protein	Liver*	Both extrinsic and intrinsic pathways
VIII	Antihemophilic factor (AHF)	Plasma protein	Liver, lung capillaries	Intrinsic pathway; deficiency results in hemophilia A
IX	Plasma thromboplastin component (PTC)	Plasma protein	Liver*	Intrinsic pathway; deficiency results in hemophilia B
X	Stuart factor	Plasma protein	Liver*	Common pathway
XI	Plasma thromboplastin antecedent (PTA)	Plasma protein	Liver	Intrinsic pathway; deficiency results in hemophilia C
XII	Hageman factor	Plasma protein; activated by negatively charged surfaces (e.g., glass)	Liver	Intrinsic pathway; activates plasmin; initiates clotting in vitro; activation initiates inflammation
XIII	Fibrin stabilizing factor (FSF)	Plasma protein	Liver, bone marrow	Cross-links fibrin, forming a strong, stable clot

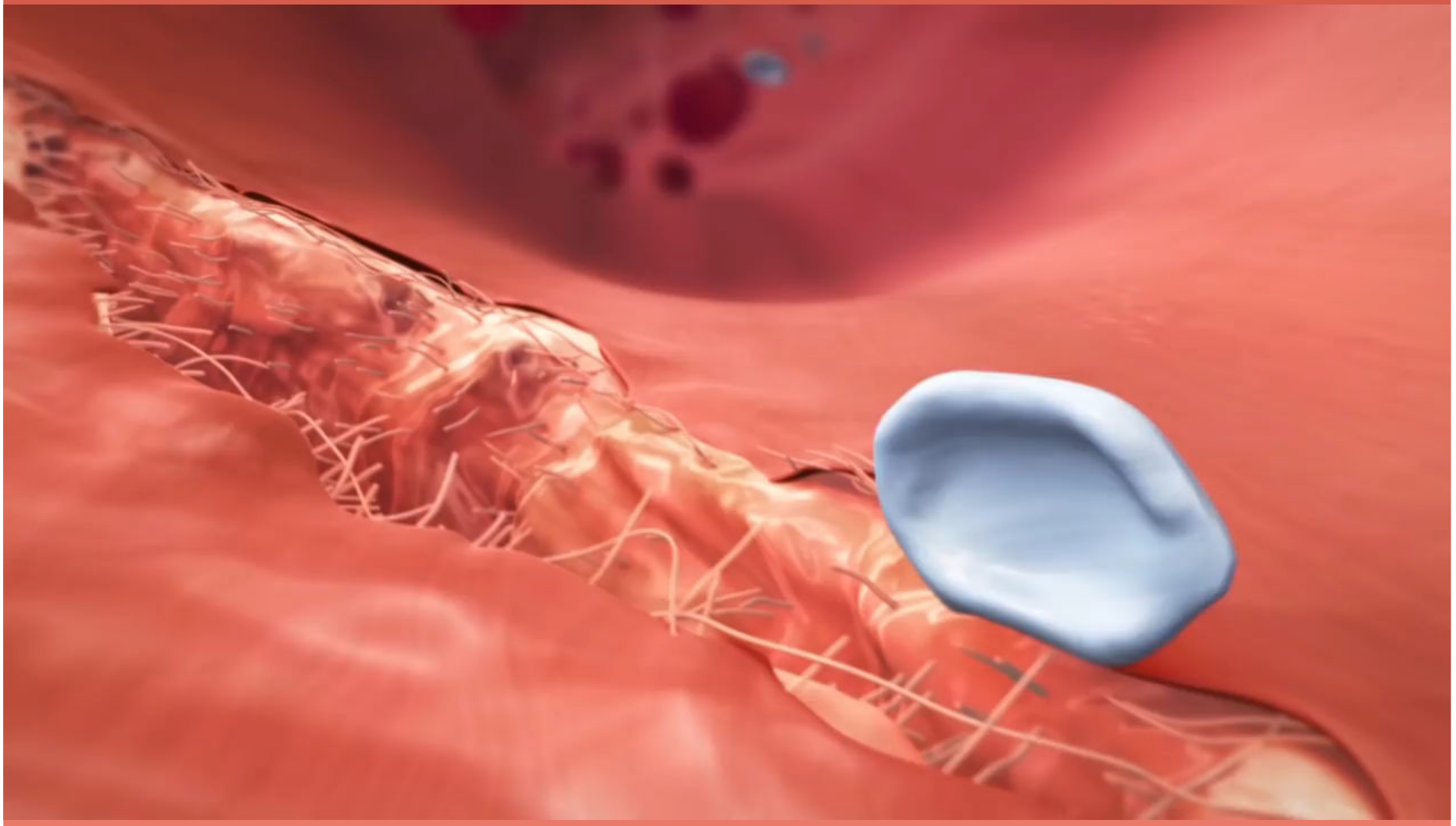
*Synthesis requires vitamin K
†Number no longer used; substance now believed to be same as factor V

Source: http://www.thrombocyte.com/clotting-factors/

Hemostasis phases

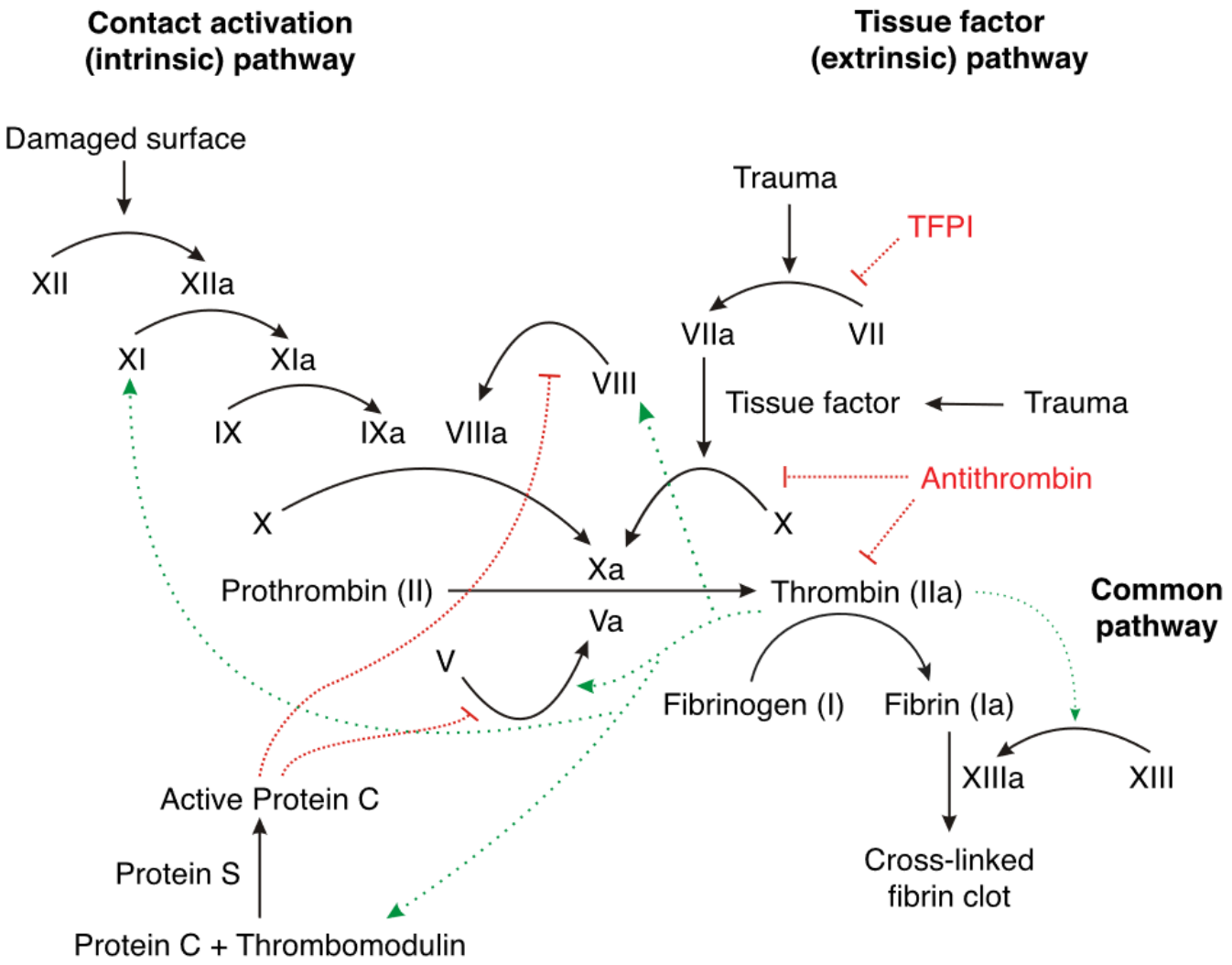
1) primary hemostasis, initiated by platelet adhesion to the underlying extracellular matrices (ECM) of damaged tissue,
   - blood vessel constriction
   - release of tissue or exogenous factors
2) secondary hemostasis that is subsequent activation of the clotting cascade,
3) fibrinolysis.

Platelet activation



Source: Thrombosis Adviser: https://www.youtube.com/watch?v=R8JMfbYW2p4

Coagulation cascade



Source: https://upload.wikimedia.org/wikipedia/commons/b/b6/Coagulation_full.svg

Hemostatic agents and methods used in military and civilian medicine

- Direct pressure (tourniquets)
- Mucoadhesive agents, e.g. positively charged chitosan glueing RBC's and platelets (strong adherence to the tissues)
- Water absorption, increasing concentration of coagulation factors (superabsorbent sponge, kaolin)
- Addition of procoagulation factors (materials releasing thrombin and fibrinogen)
- Other (cauterization, injection with thrombin, adrenaline)

State of the art.
Nanofibers application in hemostasis

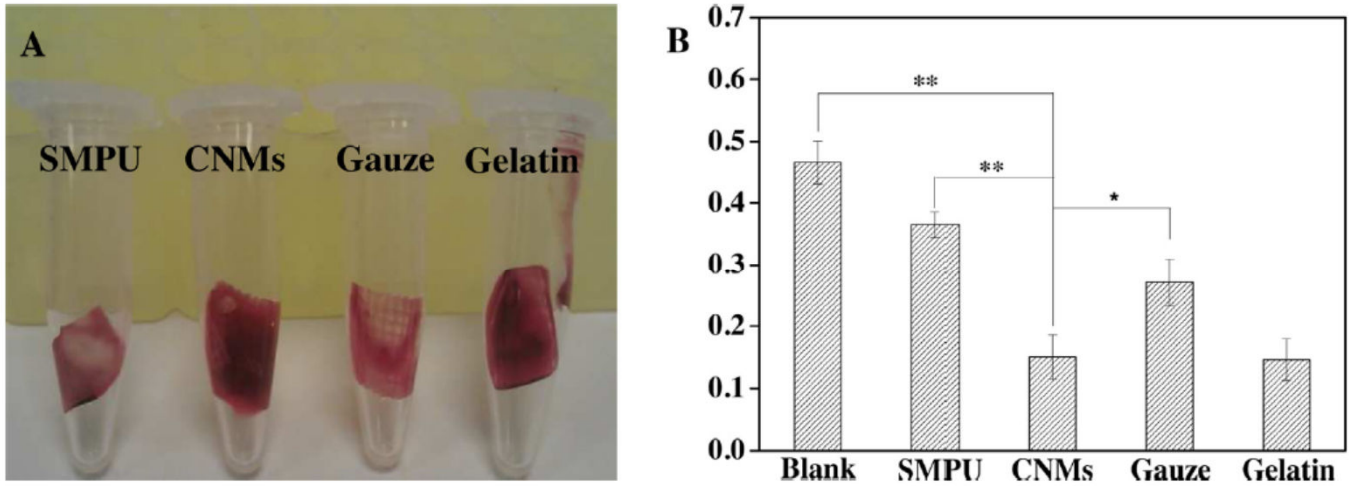
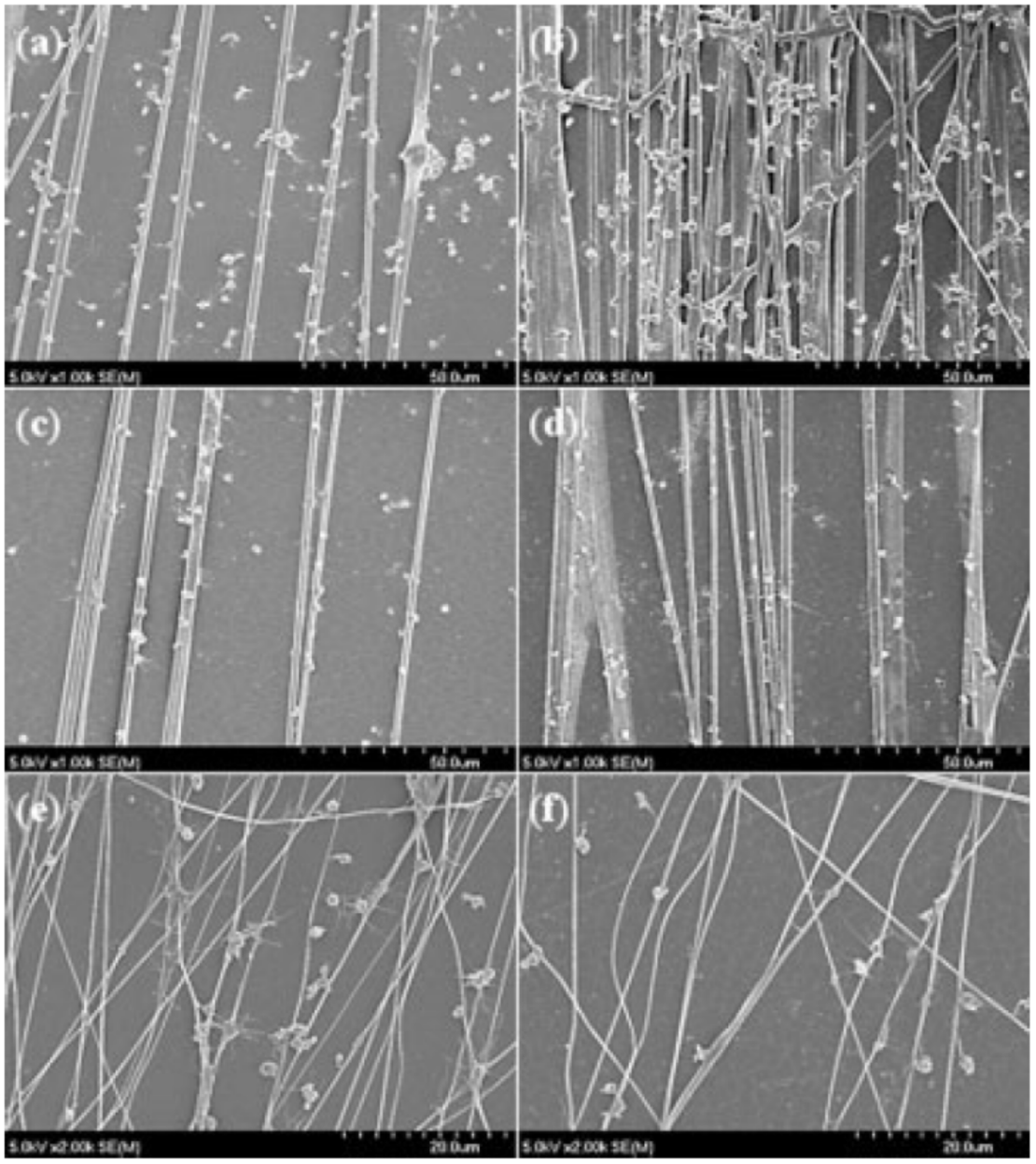


Fig. 9. Photographs of the whole blood clotting (A) and corresponding absorbance of hemoglobin from hemolyzed uncoagulated RBCs (B). (Notes: * ($p < 0.05$) and ** ($p < 0.01$) indicates a significant difference from other groups.).

Source: Study of multi-functional electrospun composite nanofibrous mats forsmart wound healing



Source: Polymer surfaces structured with random or aligned electrospun nanofibers to promote the adhesion of blood platelets

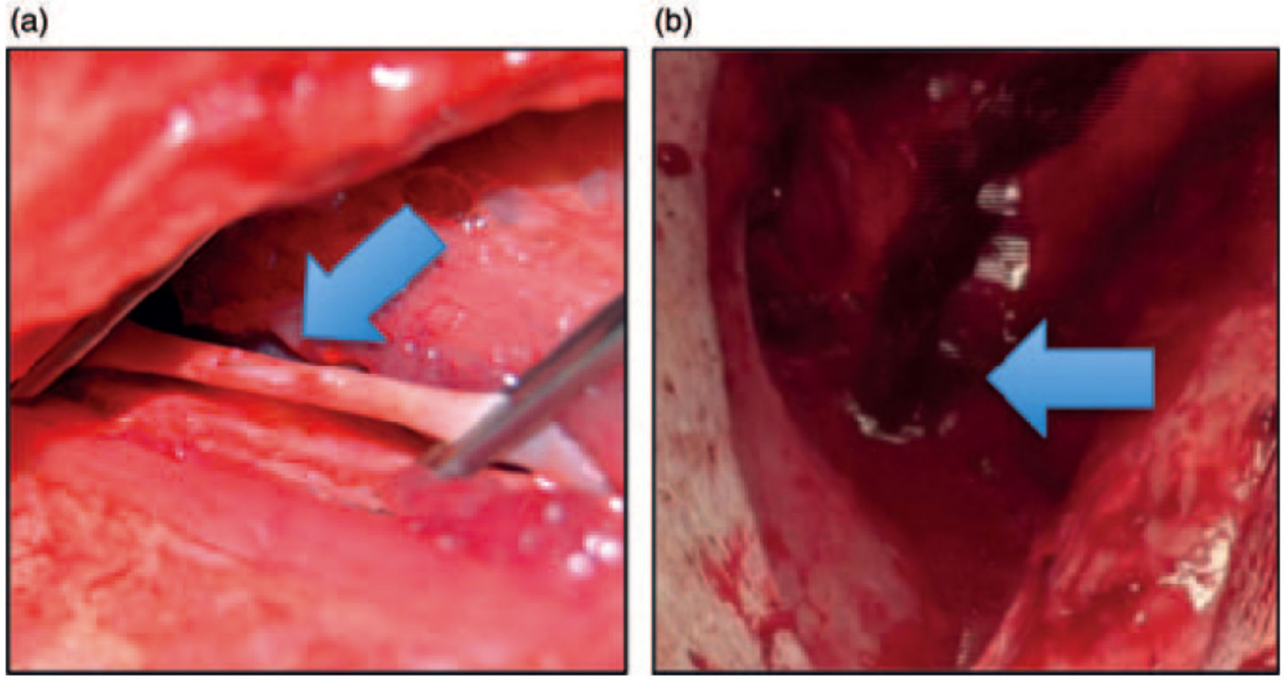


Figure 2. Shown are images of the injury. In (a) a $6 \times 2\,mm^2$ arterial punch is used to form a consistent injury (blue arrow shows the puncture wound on the clamped artery). In (b), blue arrow shows an example of the high flow bleeding injury before treatment.

Source: Novel keratin (KeraStat) and polyurethane (Nanosan-Sorb) biomaterials are hemostatic in a porcine lethal extremity hemorrhage model

How nanofibers can affect blood coagulation?

- Release of pro-coagulation drugs
- Phyisical modifications:
  - Surface nanostructure (porous nanofibers, surface roughness)
  - Fibers' diameter, porosity of the material (higher water absorption)
  - Nanofiber composition with collagen or gelatin affecting platelets adhesion
  - Fibers' morphology
- Surface modifications:
  - Surface functionalization for selective adsorption of fibronectin, fibrynogen and other integrin connecting proteins
  - Natural polymers layer deposition on the surface of nanofiber (chitosan, RADA peptide – self assembling peptide)

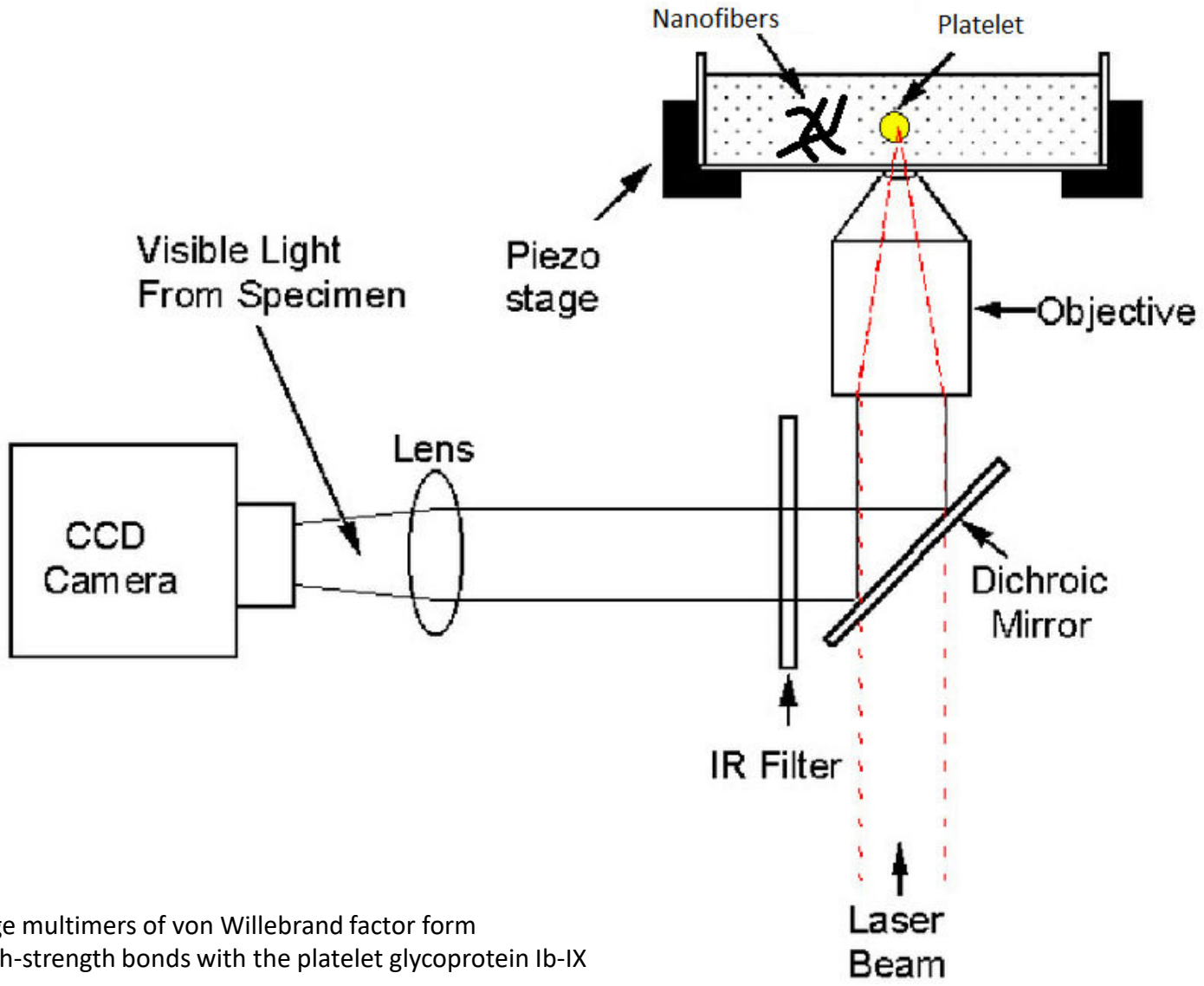
Research hypotheis

- Nanofibers have the capacity to enhance coagulation by contributing to both primary and secondary hemostasis,
- Chemical composition of the nanofibres, surface modification and porosity of the material has significant impact on the process of blood coagulation and time of plug and clot formation,
- Release of active pharmaceutical ingredients from the nano- and microfibers will decrease blood clotting time,
- Bleeding control occurs both in the case of blood containing heparin and in a temperature reduced to 32°C,
- Introduced antibacterial drug does not cause an adverse effect on clotting.

Project stages

1. **Formation of electrospun materials and physical, chemical and mechanical analysis of nonwoven material**
   - Design of electrospun nanofibers decreasing coagulation time
   - Pore size and porosity determination
   - Surface wettability
   - Drug release
   - Blood proteins adsorption
2. ***In vitro* studies of the materials in the contact with human blood**
   - Single platelet adhesion (optical tweezers)
   - Platelets adhesion and activation (SEM, DIC microscopy)
   - Plasma clotting assays
   - Whole blood coagulation kinetics on (in presence of heparin and reduced temperature)
3. ***In vitro* studies in designed experimental system**
   - Blood plasma/whole blood coagulation kinetics

Optical tweezers



Source: Ultralarge multimers of von Willebrand factor form spontaneous high-strength bonds with the platelet glycoprotein Ib-IX