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Natural and synthetic coagulation inhibitors for hemocompatible coatings

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Natural and synthetic coagulation inhibitors for hemocompatible coatings

Outline

- Biomedical devices in blood
- Blood compatibility by surface passivation
  - Inorganic
  - Organic, biological
- Immobilization of biomolecules
- Immobilization of synthetic inhibitors
- Overview and outlook

Focus on Blood Contacting Materials

Extracorporeal devices

- Hemodialysis membranes
- Oxygenator membranes
- Hemoperfusion columns
- Catheters, tubings, disposables

Implants

- Vascular stents, stent grafts
- Vascular prostheses, shunts
- Heart valves, artificial hearts and cardiac assist devices

Common problems

- Activation of hemostatic pathways
- Inflammatory reactions
- Hemolysis

Need for a more rational design of blood contacting surfaces

Undesired Side Effects of Medical Devices

- **Activation of blood coagulation**
  - Thrombosis ⇨ Embolism, infarction
- **Activation of blood platelets**
  - Thrombosis ⇨ Embolism, infarction
  - Release of growth factors ⇨ Restenosis
  - Leukocyte activation ⇨ Inflammation
- **Complement activation** ⇨ Inflammation, cell death
- **Activation of leukocytes**
  - Reactive oxygen species ⇨ Cell damage
  - Destructive enzymes ⇨ Matrix degradation, lung fibrosis
  - Cytokines and growth factors ⇨ Intima proliferation
  - Feedback loops to coagulation and platelets
- **Hemolysis**
  - Loss of red blood cells
  - Kidney damage by free hemoglobin
  - Platelet activation

Hemocompatible Coatings
Background and Principles

Passive Surface Modifications

- Modification of basic physical and chemical surface properties
  - Surface energy
  - Surface charge
  - Surface topography
  - Chemical groups

⇩ Influencing protein adsorption

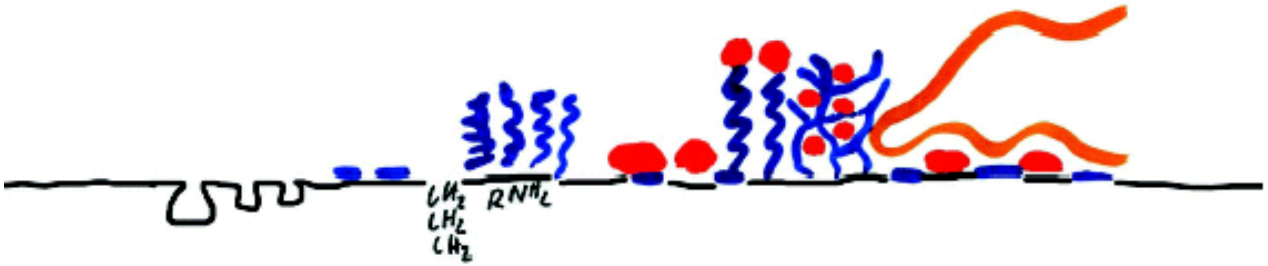
⇩ Conformation changes

⇩ Enzyme activation/ inactivation

⇩ Cellular reactions

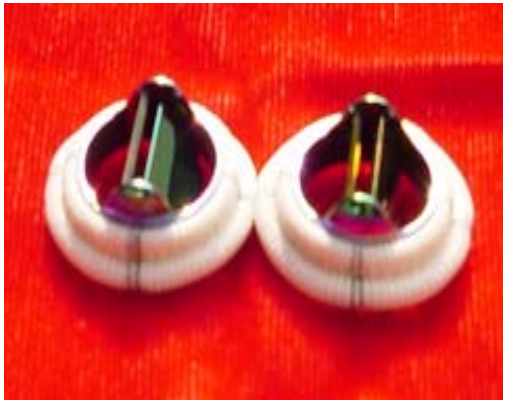
Bioactive Surface Modifications

- Directly targeting specific biomolecules
  - Coagulation factors
  - Cell adhesion molecules
  - Cellular receptors

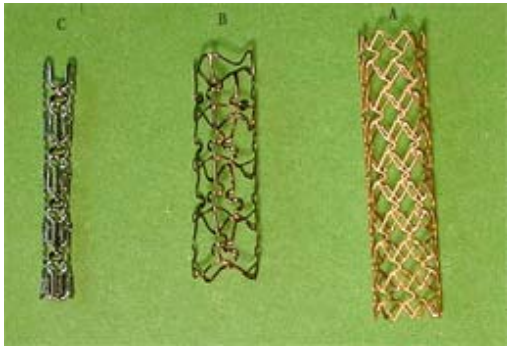


Passive Surface Modifications
Inorganic Coatings

- Mainly applied coatings
  - Metal oxides or nitrides (Ti, Ir, Ta, Hf)
  - Carbon based materials
    - Diamond-like carbon (a-C:H)
    - Amorphous silicon carbide (a-SiC:H)
- High biological stability
- High chemical stability
- High mechanical stability
  - High smoothness
  - Low friction
  - Low wear
- Applications
  - Heart valves
  - Vascular stents



Heart valves with DLC or TiN coatings



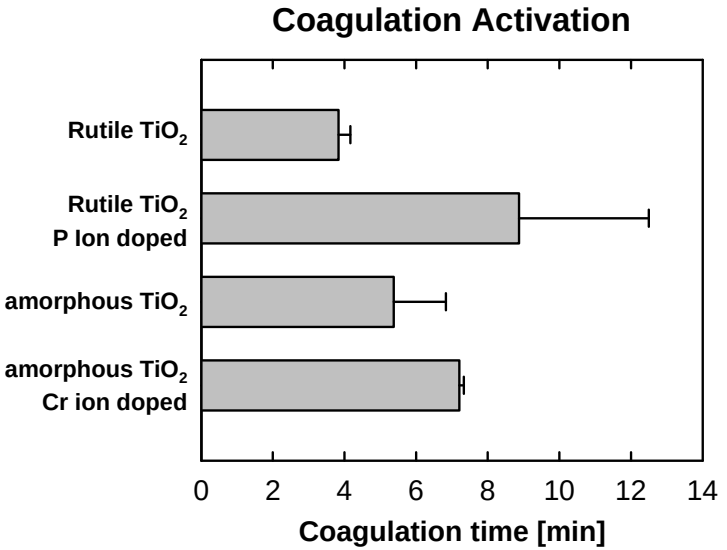
Vascular stents with coatings

Images by N. Huang, SWJTU Chengdu

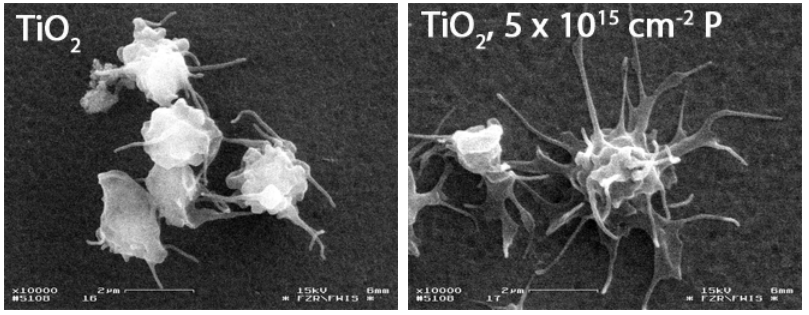
Underlying Principles

- Surface wettability
  - Protein adsorption
  - ↳ Coagulation activation
  - ↳ Blood platelet adherence

- Further influences
  - Crystallinity and crystal structure
  - Radicals degrading effect of IrO_x
  - Photochemical effect of TiO_2
  - Semiconductor properties
  - Titanium oxides and nitrides
    - Lower fibrinogen adsorption
    - Lower blood platelet adhesion
    - Lower coagulation activation



Decreased coagulation activation by ion doping of TiO_2



P ion doping enhances blood platelet adhesion

Organic Materials – Passivation

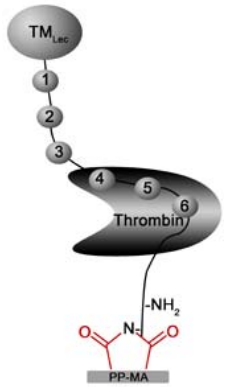
- Brushes of long-chain hydrophilic molecules
  - Polyethylene Oxide (PEO)
  - Polyethylene Glycol (PEG)
  - Tetraethylene glycol dimethyl ether (tetraglyme)

  - Inert, biocompatible modification
  - Prevents protein- and cell adhesion
  - Only limited biological stability

- Surface decoration with inert biomolecules (albumin)
  - Masking from the immune system
  - Problems
    - Conformation changes
    - Limited possibility for sterilization
    - Limited shelf- and biostability

Bioactive Modifications

Biological Molecules



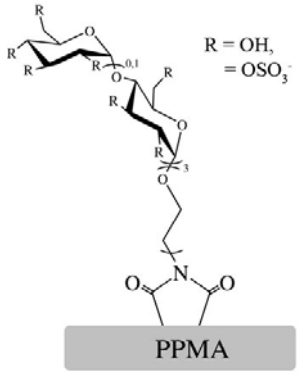
Thrombomodulin

Applied Molecules
- Heparin
- Thrombomodulin
- Hirudin
- Urokinase

Advantage
- High activity

Disadvantage
- Low (bio-)stability
- High expenses

Biomimetic Molecules

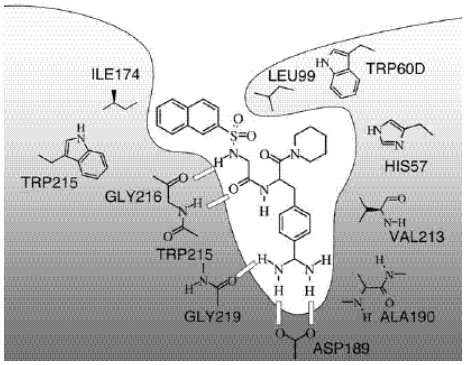


Sulfated saccharides

Applied Molecules
- Heparin-analogues
- Sulfated polymers

Advantage
- Higher resistance against biodegradation
- Lower expenses

Synthetic Molecules



Molecular designed inhibitors (direct thrombin inhibitors)

Applied Molecules
- Benzamidine derivatives
- Dipyridamol
- Tirofiban

Advantage
- Higher stability
- High activity
- Lower expenses

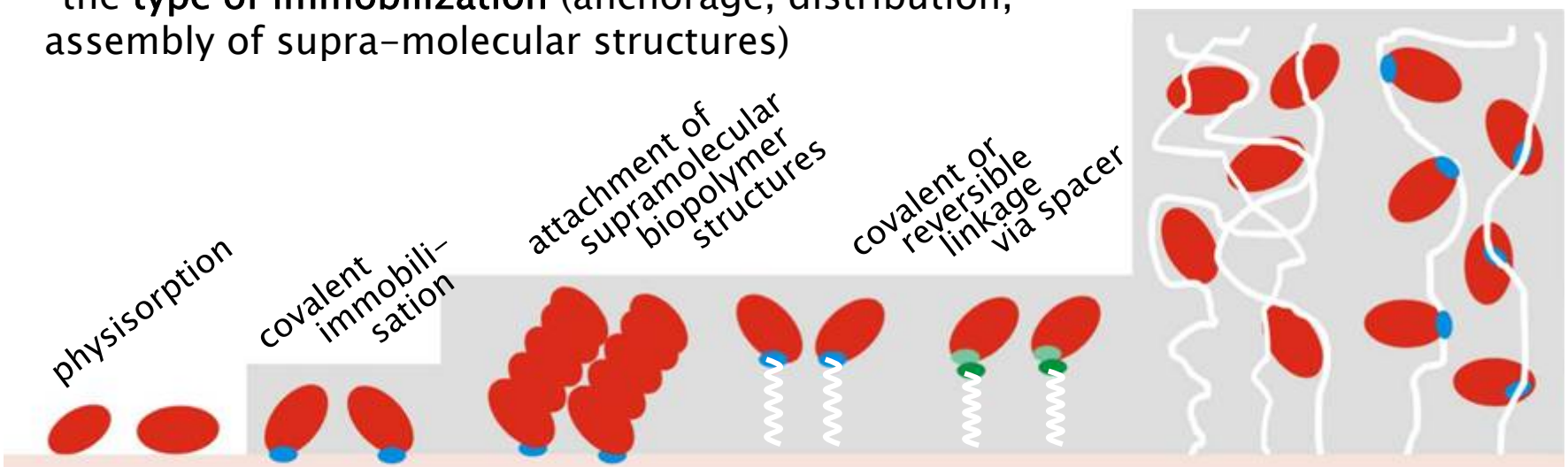
Functionalization Background and Principles

The performance of bioactive coatings is often limited

- **due to inefficient orientation/linkage, degradation** or **structural changes** of the bioactive elements
  and/or
- **unspecific** (secondary) **adsorption** of biomolecules

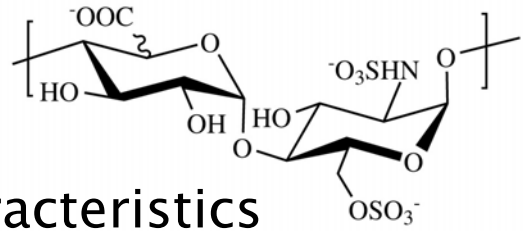
Options to address this problem include

- the choice of the **bioactive molecules** (biopolymers, biomimetic molecules)
- the **type of immobilization** (anchorage, distribution, assembly of supra-molecular structures)



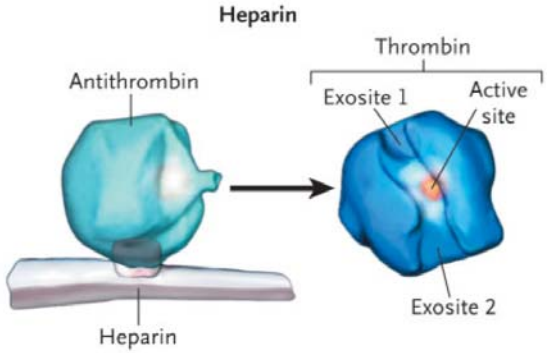
Adjusting the interfacial mode of bioactive molecules for maximum performance

Heparin



Characteristics

- Polysulfated polysaccharide
- Inhibits several coagulation factors
- Activity depends on Antithrombin III (indirect inhibitor)
- Thrombin-Antithrombin complex is released from heparin



N. DiNisio, N Engl J Med 353:1028 (2005)

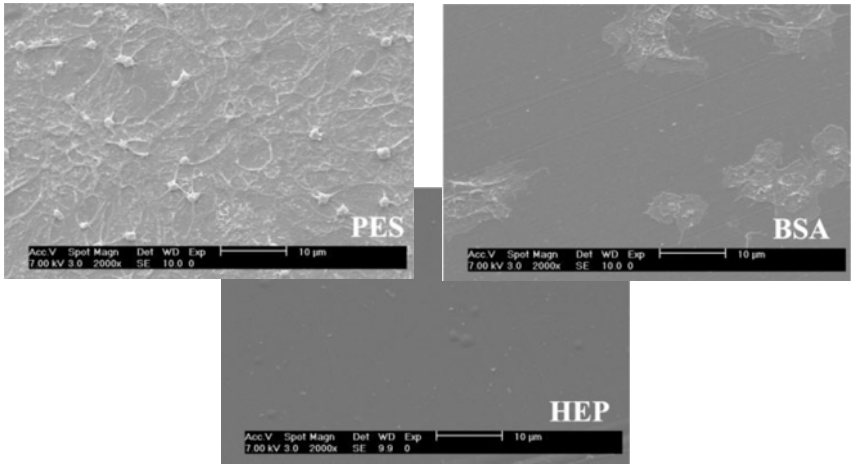
Heparin catalyzes the thrombin-antithrombin binding

Immobilization methods

- Physisorption
- Covalent immobilization
- Layer-by-layer deposition with albumin

Drawbacks

- Reduced activity when immobilized
- Subject to degradation

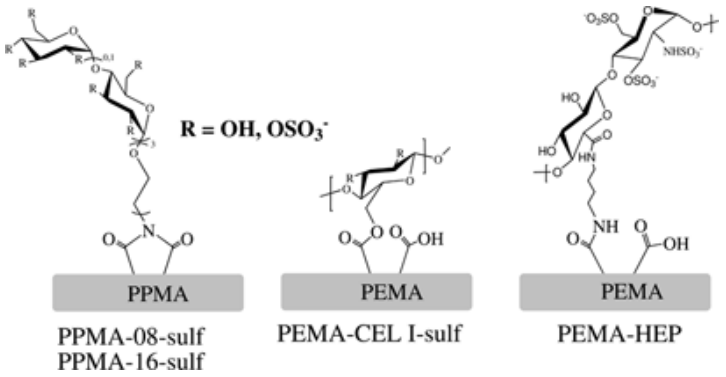


C. Sperling, J Biomed Mater Res 76A:681 (2006)

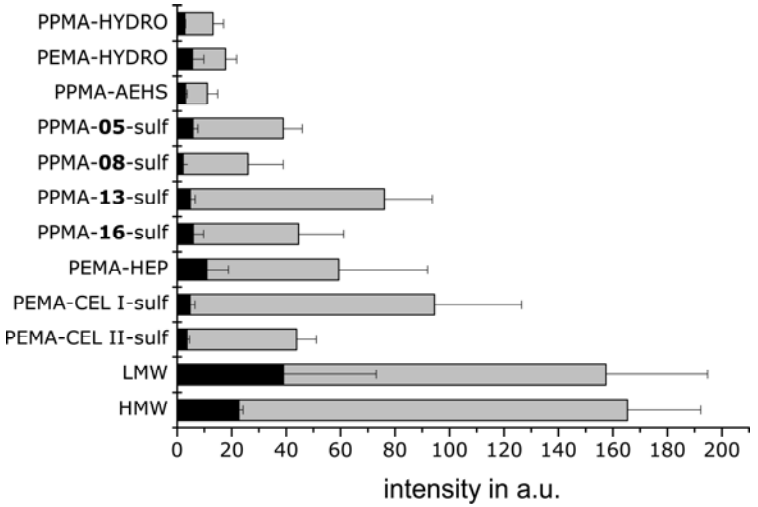
Anticoagulant properties of albumin-heparin multilayers

Heparin Analogues

- Heparin functionalities on surfaces
  - Polysulfated polysaccharide
  - Sulfate groups
- Desired properties
  - Higher bio-stability
  - One-step production with the bulk
- Results
  - Increased affinity for antithrombin III
  - Low effect in complex system of blood



Sulfated di- and polysaccharides as heparinoids

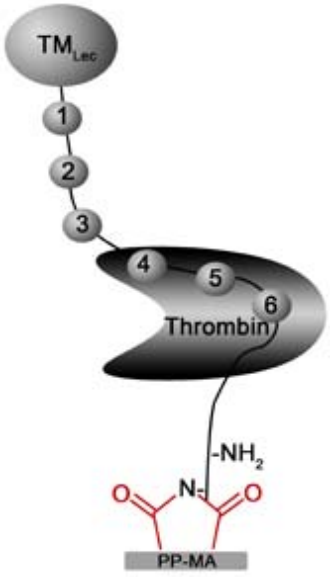


Affinity for antithrombin III increases with the complexity of the modification

R. Grombe, Macrobiol Biosci 7: 195 (2007)

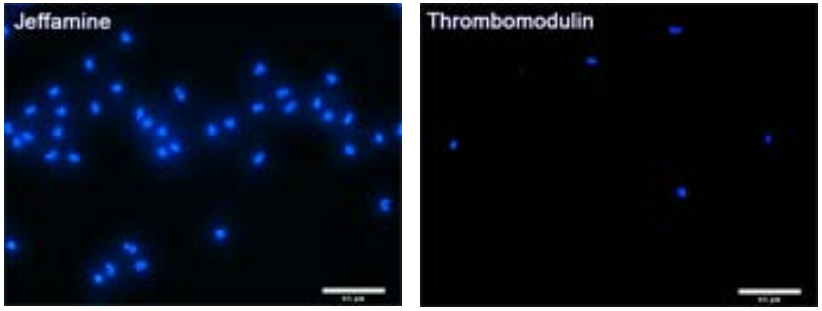
Thrombomodulin

Different functions within one molecule:

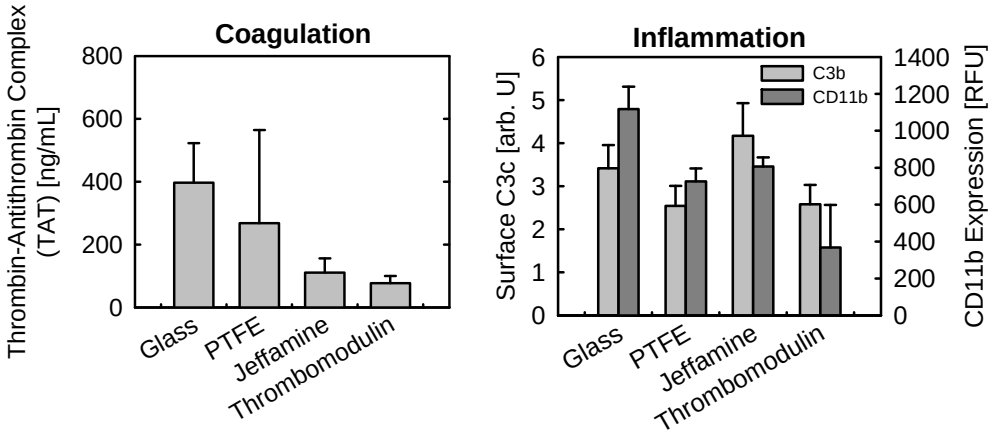


- Leading anticoagulant and antiinflammatory molecule in the endothelium cell membrane.
- Inhibits coagulant thrombin activity
- Activates anticoagulant Protein C pathway
- Antiinflammatory properties in lectin-like top-domain

Immobilized in physiological orientation on model substrates using lysine residues in the basal region of the molecule.



Reduced leukocyte adhesion on thrombomodulin



Low coagulation and inflammatory properties on thrombomodulin modified substrates

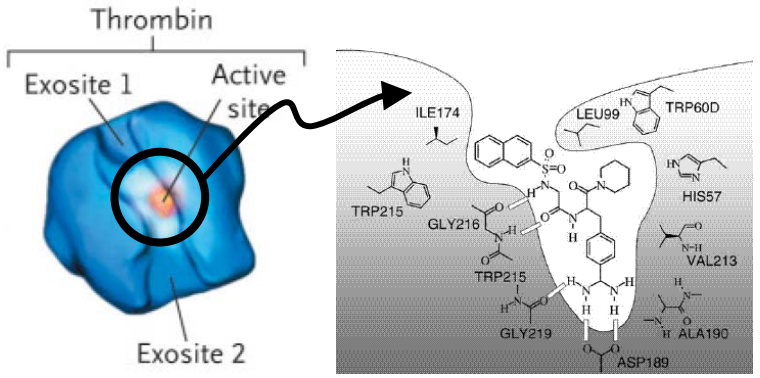
Synthetic Inhibitors of Coagulation Factors

Background

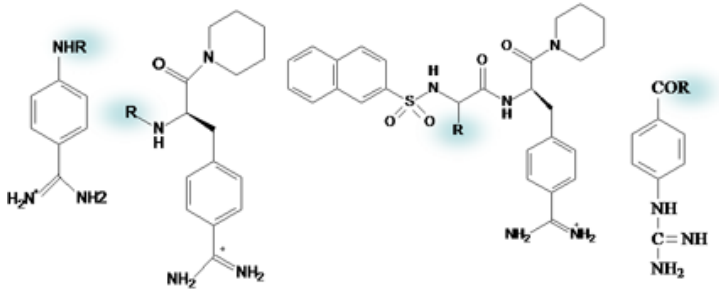
- Imitation of thrombin-inactivating effect of thrombomodulin
- Immobilization of molecular weight inhibitors
  - Resistivity against degradation
  - No natural antagonists
  - Lower expenses

Principle

- Benzamidine as general inhibitor for serin-proteases
- Molecular design for more complex inhibitors
  - Higher specifity
  - Higher affinity

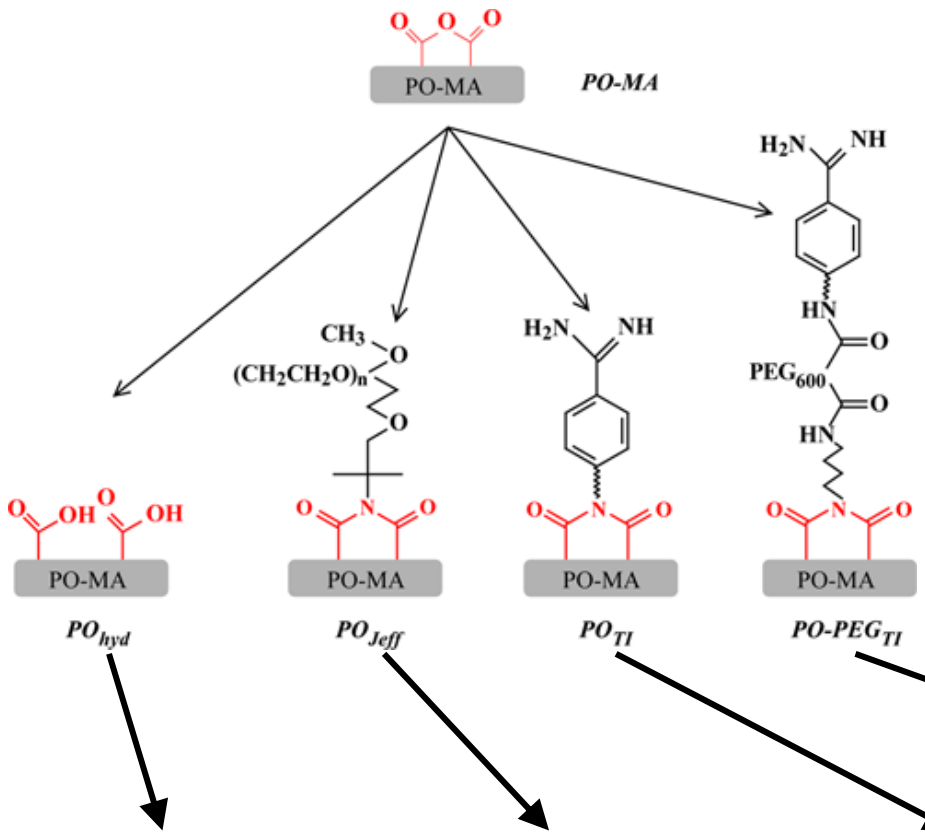


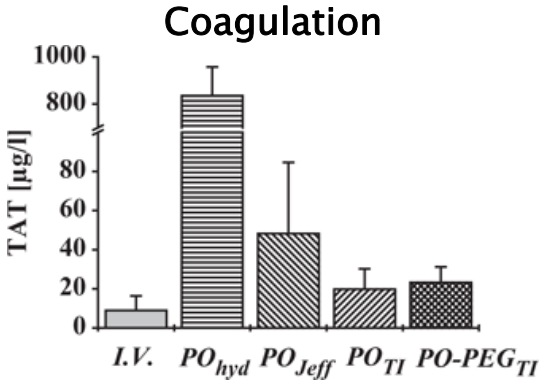
Benzamidine NAPAP Guanidine

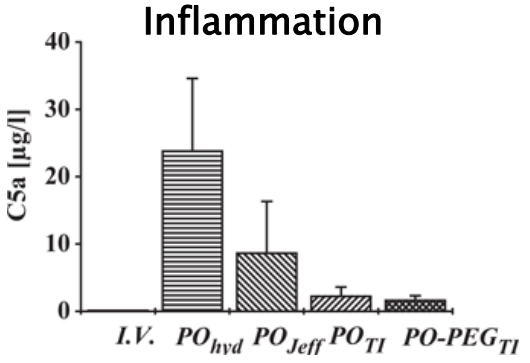


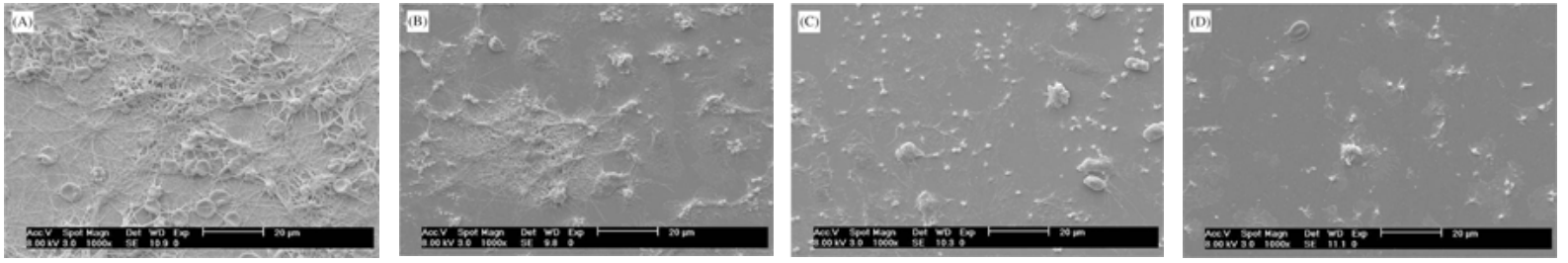
H K_i=422µM	H K_i=1778µM	H K_i=0.011µM*	
$CO(CH_2)_{11}NH_3^+$ K_i=101.5µM	$CO(CH_2)_{11}NH_3^+$ K_i=12.4µM	$CO(CH_2)_4NH_3^+$ K_i=0.08µM	$NH(CH_2)_4NH_3^+$ K_i>10.000 µM
PEG11-NH_3^+		$(CH_2)_4NHCO(CH_2)_{11}NH_3^+$ K_i=0.021µM	

Immobilized Benzamidine based Inhibitors in Whole Blood Conditions









M.-F. Gouzy, Biomaterials 25: 3493 (2004)

Anticoagulant Surfaces with Benzamidine based Inhibitors

Hemocompatibility effects

- Reduced coagulation
- Reduced thrombocyte adhesion and activation
- Reduced complement activation
- Reduced leukocyte adhesion

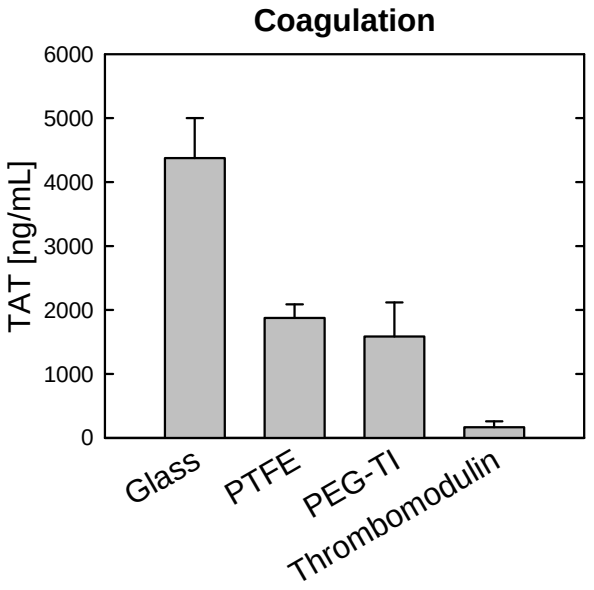
- Slightly better effects by flexible immobilization with spacer molecule
- Effects mainly inferior to immobilized thrombomodulin

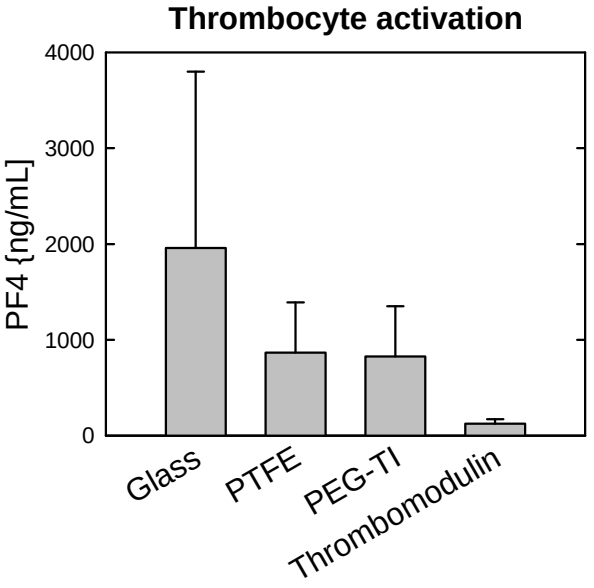
Limitations

- Benzamidines act as a sink for thrombin

⇨ Saturation effects possible

⇨ For short term application and/or combination with other principles





Summary and Perspectives

Modification	Main Characteristic
"Passive" inorganic surfaces ⇨ also apply smart principles	• Sterilization and shelf stability • Biostability • Desirable mechanical properties
Organic passivation ⇨ prevent protein adsorption	• Higher efficiency • Shorter stability
Active biomolecules	• Highly effective • Low shelf and sterilization stability • Subject to physiological regulation and degradation • Frequently high expenses
Synthetic inhibitors ⇨ imitation of principles of the vessel wall	• Improved stability • Frequently lower efficiency

Future directions

- Combination of various principles (passivation and inhibitor)
- Addressing the anticoagulant Protein C pathway

Biomaterials Department:
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Outside
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