Thrombin-responsive feedback system as thromboresistant material

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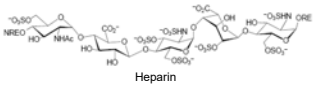
Background and Objectives

Background

- Heparin based coatings are frequently applied to improve the hemocompatibility of biomedical devices
- The anticoagulant efficiency is lower than expected. Reasons are limited accessibility of heparin for the target molecules, small amount of heparin, desorption and pre-term consumption.

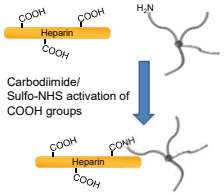
Objective

- Immobilization of high amounts of heparin at good accessibility
- Heparin release in response to the actual coagulation situation


Heparin

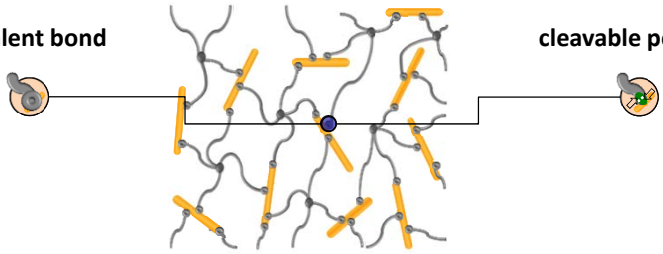

Clinical applications of heparin coatings

Concept

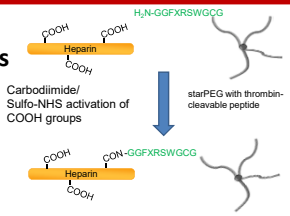


Non-responsive hydrogels as carriers of heparin

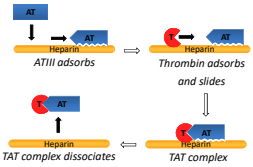
covalent bond



cleavable peptides

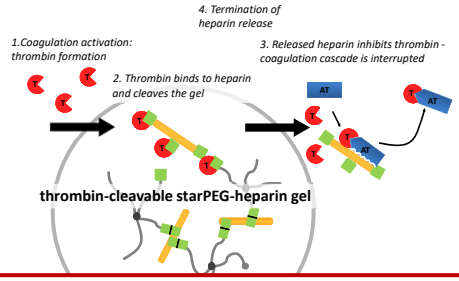


Thrombin-cleavable peptide linkers between starPEG and heparin forms a coagulation-responsive heparin-release system as a feedback loop.



Hydrogel with heparin as structure forming element covalently linked with four-branched poly(ethylene glycol) (starPEG).

- starPEG (M_r = 4 × 2500 Da) with amino-terminated arms linked to COOH-groups of heparin by carbodiimide chemistry
- Heparin concentration in the gel in physiological media was found to be constant independent of the crosslinking degree
- Regulation of heparin amount via gel thickness
- Meshsize can be tuned by stoichiometry of PEG and heparin
- Meshsize in dimensions of proteins (~10 nm) provides access for proteins
- Affinity for coagulation factors with heparin binding site



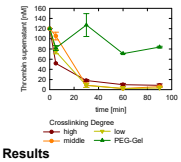
Results

Non-responsive Gels

Affinity of thrombin to heparin in gel

Method

- Immersion of starPEG heparin-gels in thrombin solution in buffer.
- Determination of thrombin activity in supernatant (chromogenic assay)



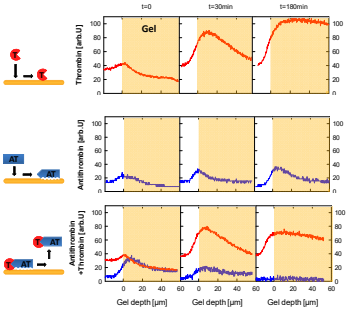
Results

- Rapid adsorption of thrombin
- Porous gel structure allows > 1 monolayer adsorption

Protein permeability and TAT complex formation

Method

- Immersion of gel in solution of fluorescent labelled thrombin (top) antithrombin (middle) and thrombin after antithrombin loading (bottom)
- Analysis of depth distribution by confocal laser scanning microscopy



Thrombin: Rapid absorption and deep distribution

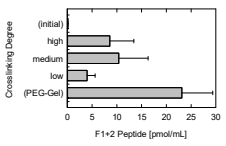
Antithrombin: Mainly superficial adsorption

Thrombin in AT loaded gel: AT depletion Reduced thrombin absorption

Whole blood incubation

Method

- Freshly drawn blood
- 1 U/ml heparin
- 3h incubation at 37°C preventing sedimentation
- Hydrogels form top and bottom of incubation chamber
- Measurement of F1+2 fragment as coagulation marker



Result

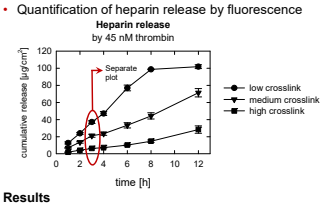
- Heparin-containing hydrogels better than pure PEG gel
- Best thrombo-resistance with low crosslinking degree

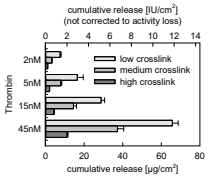
Thrombin-responsive Gels

Thrombin-induced gel-degradation

Method

- Gels prepared with fluorescence labelled heparin
- Incubation in thrombin-solution of various concentrations at 37°C
- Quantification of heparin release by fluorescence





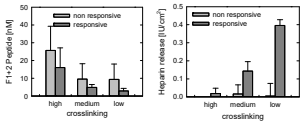
Results

- Linear heparin release kinetics with time
- Release scales with crosslinking degree and time

Whole blood incubation

Performance compared to non-responsive gels

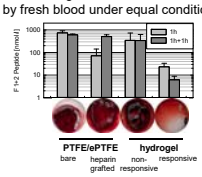
- Blood anticoagulation with heparin 1 U/ml
- 3h incubation at 37°C



- Outperformance of responsive gels over non-responsive ones
- Heparin release and anticoagulant effect scale with crosslinking-degree

Test under near physiological conditions

- Comparison with clinically applied materials
- Test of exhaustion effects (blood flow): Incubation with non-anticoagulated blood; after 1 h replacement by fresh blood under equal conditions.



- Responsive gel also in repeated exposition effectively suppresses thrombin generation

Conclusions

starPEG-heparin gel coatings

provide high amounts of heparin in a 3D meshwork

⇨ conserved affinity to thrombin and antithrombin

Incorporation of thrombin-cleavable peptide linkers

results in a feedback loop system with enhanced anticoagulation

⇨ Anticoagulant effect is tunable by gel characteristics

⇨ Gels stabilize non-anticoagulated blood for 3+ hours

⇨ Anticoagulant effect preserved at blood exchange

Thrombin responsive biofeedback system as thromboresistant material

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Aim: Prevention of surface mediated blood clotting activation so far is based on the control of physical-chemical surface properties, immobilization or release of heparin or other anticoagulants. These systems exhibit their thromboresistant properties independent of the actual coagulation situation with possible problems of overdosage and preterm consumption. The aim is to create a thromboresistant material, which releases an anticoagulant in response to the coagulation activity in plasma as feedback system.

Method: A hydrogel system of four-branched poly(ethylene glycol) (starPEG) cross-linked with heparin has been set up using thrombin-cleavable peptides as linker molecules between PEG and heparin at various degrees of cross-linking. Permeability of the gel for thrombin and antithrombin (AT) was probed with fluorescent labeled proteins, degradability of the gel was tested in a thrombin solution and the anticoagulant effect of thrombin-cleavable vs. corresponding non-cleavable gels was tested in a static *in vitro* whole blood incubation assay.

Result: Due to the heparin content, the gels accumulate thrombin and antithrombin from the medium, however, the uptake decreased with the degree of cross-linking. Degradation of the gels in pure thrombin solutions also was controlled by the degree of cross-linking; the gels could be adjusted that the heparin release reaches pharmacologically relevant levels. In whole blood incubation the thrombin cleavable gels all showed lower coagulation activation than their corresponding non-cleavable counterparts. Only the thrombin responsive gels could prevent clotting of otherwise non-anticoagulated blood for three hours and more or suppress coagulation activation from other sources.

Conclusion: A hydrogel system composed of starPEG and heparin with thrombin cleavable peptides as linker can act as tunable thromboresistant material, in which the release of the anticoagulant heparin occurs in response to an activated coagulation cascade.