Factor Xa versus thrombin response as trigger in actively anticoagulant hydrogel systems

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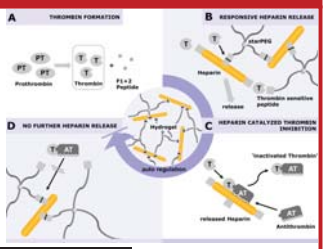
Background and Objectives

Background

- A hydrogel system of heparin and four-armed poly(ethylen glycole) (starPEG) has been developed as anticoagulant coating
  - Porous meshwork provides accessibility and preserves affinity to thrombin and antithrombin
- Feedback loop system with enhanced anticoagulation by use of thrombin-cleavable peptide linkers between starPEG and heparin
- Coagulation factor Xa may act as an alternative trigger of the feedback loop.
- Despite structural relation, there are a number of significant differences between the two coagulation factors, which could influence the performance of the hydrogel (table)



	Thrombin	FXa	Consequence of difference
Function	Activates fibrinogen	Activates thrombin	Earlier position in coagulation cascade ⇨Faster response ⇨Lower concentration
Plasma-concentration (zymogen)	1-2 µmol/l	0.16 µmol/l	Lower concentration of FX ⇨ slower peptide cleavage in blood ⇨ slower heparin release
Heparin binding site	Yes	(Yes) Ca^{2+} dependent in prothrombinase complex occupied	Occupied heparin binding site may constrain accumulation of FXa at the hydrogel
Heparin binding required for ATIII sensitivity	Yes	No	
Sensitive sequence in the hydrogel	(D)-Phe-Pip-Arg-Ser	Glu-Gly-Arg-Met	Only natural amino acids in FXa sensitive sequence ⇨Faster turnover

Objectives

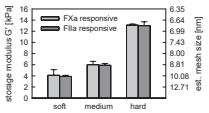
- Set-up of a feedback controlled anticoagulant hydrogel system with responsiveness to factor Xa
- Comparison of the FXa responsive gel system with the existing thrombin-responsive gel

Study

Gel Characterization

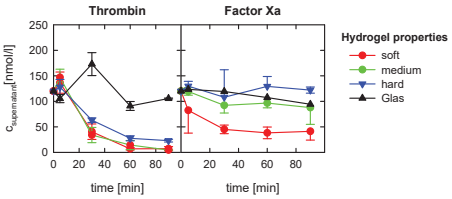
Mechanical gel properties
Method
- Storage modulus G' of hydrogels determined by rheometry
- Estimation of mesh size ξ according to the rubber elasticity theory: $\xi = \left(\frac{G'N_A}{RT}\right)^{-\frac{1}{3}}$

Result
- Corresponding properties of FXa and thrombin-responsive gels
- Mesh size estimated as 10 – 9 – 6.8 nm



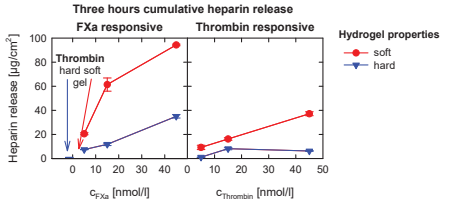
Affinity of thrombin or FXa to heparin gels
Method
- Immersion of non-cleavable hydro-gels in 120nM solution of thrombin or FXa in presence of Ca^{2+}.
- Kinetics of enzyme activity in super-natant by chromogenic assays.

Result
- Thrombin: Rapid absorption to all gels
- FXa: Adsorption only to soft gels
  ⇨ Mesh size tends to be too low for FXa (9.5 × 9.5 × 12 nm³)



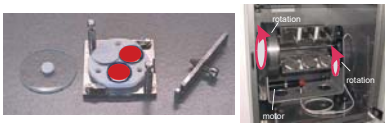
Degradation of hydrogels
Method
- Immersion of FXa- or thrombin-responsive gels in respective enzyme solutions
- Determination of released heparin after 3 hours
- Control of specifity: FXa responsive gels in 45 nM thrombin solution

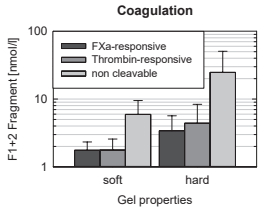
Result
- Gel degradation is a function of crosslinking degree and of enzyme concentration
- Faster degradation of FXa responsive gels than thrombin responsive gels at corresponding enzyme concentrations
- No degradation of FXa responsive gels by thrombin



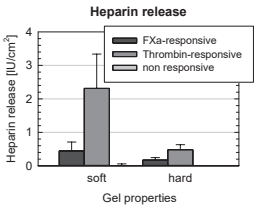
Blood Incubation

Whole blood incubation assay
Method
- Freshly drawn whole blood with heparin 1U/ml
- Hydrogel coatings as top and bottom of incubation chambers (2 ml blood, 6 cm² sample surface)
- Incubation with slow rotation for 3h at 37°C





Coagulation activation
F1+2 fragment as measure of thrombin activation after 3h incubation of the hydrogels with whole blood.
- Anticoagulant effect scales with crosslinking degree
- Responsive gels perform better than non-responsive gels
- No difference between FXa- and thrombin responsiveness



Heparin release
Heparin release (anti FXa activity) after 3h incubation of the hydrogels with whole blood.
- Release decreases with crosslinking degree
- Substantially lower heparin-release from FXa-responsive gels than from thrombin responsive ones.

Faster turnover of FXa responsive sequence compared to thrombin-responsive sequence
- High turnover over-compensates the low affinity of Factor Xa to the hydrogel
- In whole blood: no compensation of lower enzyme concentration

FXa has earlier position in coagulation cascade than thrombin
- Lower heparin release with better timing has equal anticoagulant effect as the more delayed and high-dose release from thrombin-responsive gels.

Conclusions

FXa-responsive starPEG-heparin hydrogels achieve similar thromboprotective effect as thrombin-responsive hydrogels
- Higher turn-over speed of peptide sequence compensates for lower affinity to heparin and (partly) for lower concentration
- Earlier position in coagulation cascade allows more efficient timing of heparin release

Advantages
- Slower degradation / longer stability
- Less release of active substances