Situation-adjusted anticoagulant release can simulate feedback responsive behavior of the blood vessel wall

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Background and Objectives

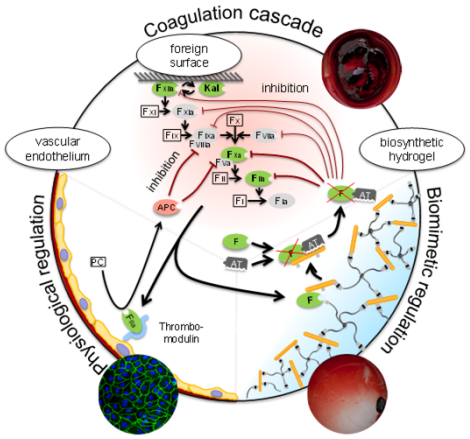
Background

- The vessel wall protein thrombomodulin has inherent feedback characteristic:
  - → It generates and releases the anticoagulant *Activated Protein C* (APC) only in presence of active coagulation (thrombin)
  - → This way, thrombin antagonizes its own formation
- Hydrogels, which release an anticoagulant (heparin) only in presence of activated coagulation factors can mimic this behavior (biomimetic regulation)
  - → Transfer of anticoagulant activity from surface to liquid phase
  - → Blocking of more upstream coagulation factors

Objectives

Influence of degrading enzyme characteristics on the hydrogel performance needs to be explored

- Affinity of the degrading enzyme to the hydrogel matrix
- Onset of activation during coagulation process
- Plasma concentration of active enzyme
- Size of the molecule/enzyme complex
- Sensitivity to heparin-mediated inhibition



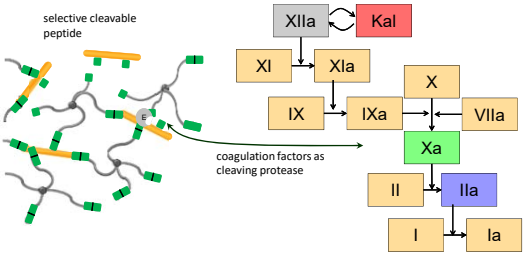
Sketch of physiological anticoagulant feedback control by endothelium and biomimetic regulation by responsive starPEG-heparin hydrogels

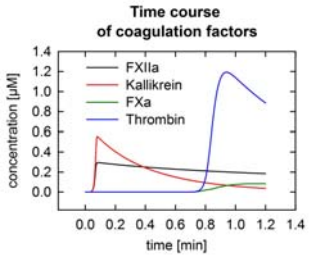
Methods

Hydrogels containing cleavable linker peptides with sensitivity to different steps of the coagulation cascade:

Thr-slow: slow turn-over by thrombin
Thr-fast: fast turn-over by thrombin
Thr-scr: scrambled sequence, no degradation
FXa-resp: degradation by FXa
Kal-resp: degradation by kallikrein/FXIIa

Analysis of interaction with isolated enzymes and with whole blood

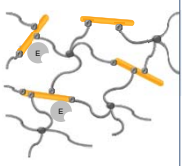


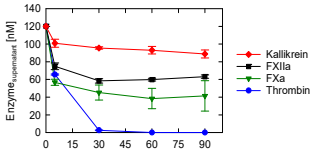


Results

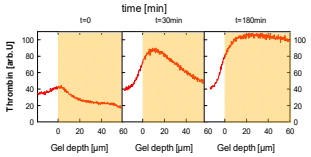
Enzyme-Matrix-Interactions

- Stable hydrogels immersed in solutions of coagulation enzymes (E)
- Measurement of enzyme concentration in supernatant and observation of penetration into the hydrogel





Remaining enzyme concentration in supernatant



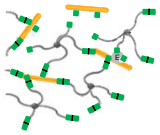
Penetration of thrombin into the hydrogel

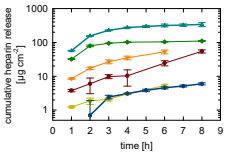
Results

- Thrombin: rapid adsorption and penetration
- Kallikrein: low/no adsorption
- FXa, FXIIa: moderate adsorption

Hydrogel Degradation

- Immersion of cleavable hydrogels in clotting factor solutions (c = 45 nM)
- Measurement of released fluorescent labeled heparin





Heparin-release from degradable hydrogels by various enzymes

	3h release [µg cm^{-2}]	k_{cat} [s^{-1}]	release (norm)	k_{cat} (norm)
Thr-slow	9.8	60	1	1
FXa-resp	94.4	200	9.6	3.3
Kal-resp	227	160	23	2.7

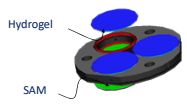
Comparison of observed release rates with documented turn-over rates of the peptides

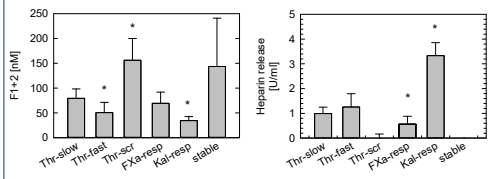
Results

- Heparin release is tuned by the degradable peptides
- FXa- and kallikrein-responsive gels release more heparin than predicted from the turn-over rate of the peptide

Whole Blood Incubation

- Incubation with freshly drawn human whole blood, 1 U/ml heparin, in in-house developed chambers
- Co-incubation with pro-coagulant surfaces (SAM, 83% COOH)





Left: Blood coagulation (measured as prothrombin F1+2 fragment) in presence of responsive hydrogels.
Right: Associated heparin release.

Results

- Kallikrein responsive hydrogel: highest anticoagulant effect based on high heparin release
- FXa responsive hydrogel: equal anticoagulant effect as Thr-slow at 35% lower heparin release

Conclusions

- Affinity of degrading coagulation factor to the hydrogel does not enhance the gel degradation
- Response to upstream coagulation factors improves the activity profile
- High anticoagulation by contact phase (FXIIa/Kallikrein) responsive hydrogels: Lack of feedback control
- FXa responsive gels show equal anticoagulant effect as thrombin-responsive gel at lower heparin release: better timing of heparin release

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Objective: Healthy vascular endothelium is considered the most effective anti-thrombogenic surface, which can adjust its anticoagulant properties to the actual requirements. Its transmembrane protein thrombomodulin has inherently responsive anticoagulant properties: Only in complex with the activated coagulation factor thrombin it converts Protein C to the anticoagulant Activated Protein C (APC). Activated coagulation thus triggers the formation and release of this anticoagulant. Once formed, the activity of APC is not restricted to the endothelial surface, but interrupts the coagulation process in the fluid phase.

A biohybrid hydrogel of starPEG crosslinked with heparin using thrombin-cleavable linker peptides presents a coagulation responsive anticoagulant system with similar behavior: Thrombin cleaves the linker peptides and releases heparin; this inhibits the thrombin activity and interrupts further degradation of the hydrogel. Since thrombin is a late enzyme in the coagulation cascade, the timing of the heparin release by this trigger may be suboptimal for thromboprotection. Faster degradation by thrombin or by more upstream coagulation factors may provide more efficient anticoagulant hydrogels.

Method: starPEG-Heparin hydrogels were prepared using the previously described thrombin-cleavable peptide, a peptide with faster turn-over, a non-cleavable scrambled sequence of the peptide, a factor FXa cleavable, and a kallikrein/FXIIa cleavable peptide as responsive linkers. They were characterized for their general response behavior and for the performance in whole blood.

Results: At equal concentration of the respective enzymes, the kallikrein responsive hydrogel was degraded fastest, followed by the FXa responsive gel and then the different thrombin responsive gels. In whole blood, the kallikrein-responsive gel also showed best anticoagulation, associated with highest heparin release, what is attributed to the low feedback effect of heparin on the contact phase system. The FXa- and the standard thrombin-responsive hydrogel had equal anticoagulant properties, but the FXa responsive system achieved this with lower heparin release due to the improved timing of the degradation.

Conclusion: Responsive anticoagulant hydrogels responding on FXa as trigger show a favorable profile of thromboresistance and inhibitor release.

Caption

Feedback control of the coagulation cascade: Physiologically, thrombin (FIIa) activates in complex with the vessel wall protein thrombomodulin the Protein C (PC) to the anticoagulant activated PC (aPC) which interrupts the coagulation cascade in the blood. A biosynthetic hydrogel built of the anticoagulant heparin, four-armed poly(ethylene glycol) (starPEG) crosslinked with linker peptides, which can be selectively cleaved by activated coagulation factors can mimic this behavior: FIIa cleaves the clinker peptides in the hydrogel and with this releases the anticoagulant heparin to the blood, where it inhibits a several steps of the coagulation cascade in a antithrombin (AT) dependent manner and also terminates further degradation of the hydrogel.