Thrombin-responsive hydrogels with varied cleavage kinetics

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Statement of Purpose: Heparin based coatings are frequently applied to improve the hemocompatibility of biomedical devices, yet their anticoagulant efficiency is frequently lower than expected due to a low accessibility of heparin for the target molecules thrombin and antithrombin as well as a low concentration, desorption and pre-term consumption of heparin.
To overcome these draw-backs, heparin was integrated into a hydrogel covalently linked with a bio-inert molecule four-branched poly(ethylene glycol) (starPEG) via thrombin-cleavable peptide-linkers. Such hydrogel provides heparin in high amounts as well as with good accessibility. As an additional benefit, it releases heparin only in response to thrombin formation, i.e. clotting activation. The hydrogel system thus forms a feedback loop system as indicated in figure 1.

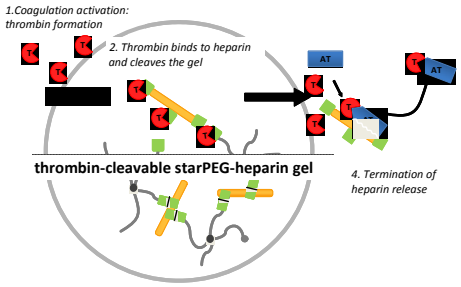

Figure 1. Sketch of the mechanism of action of the thrombin-cleavable heparin-containing hydrogel.

A first thrombin-responsive linker peptide (dFXR, table 1) was based on the sequence of chromogenic thrombin substrates, which contains two non-proteogenic amino acids. It is assumed, that these amino acidy may increase specifity and affinity but delay the cleavage and also result in a delayed heparin release of the hydrogel.
We synthesized 4 additional peptides derived from the initial peptide with altered thrombin cleavability by exchange of the non-natural amino-acids to their natural homologues and by change of the amino acid sequence.

Methods: Peptides as shown in table 1 were synthesized using Solid Phase Peptide Synthesis. The peptides were probed for their degradation by thrombin.

Table 1. Abbreviation and sequence of synthesized peptides showing cleavage region in green, and variables in yellow.

FPR	NH_2-Gly-Gly-Phe-Pro-Arg-Ser-Trp-Gly-Cys-Gly-$CONH_2$
dFPR	NH_2-Gly-Gly-(D)Phe-Pro-Arg-Ser-Trp-Gly-Cys-Gly-$CONH_2$
FXR	NH_2-Gly-Gly-Phe-Pip-Arg-Ser-Trp-Gly-Cys-Gly-$CONH_2$
dFXR	NH_2-Gly-Gly-(D)Phe-Pip-Arg-Ser-Trp-Gly-Cys-Gly-$CONH_2$
dFSRX	NH_2-Gly-Gly-(D)Phe-Ser-Arg-Pip-Trp-Gly-Cys-Gly-$CONH_2$

The peptides were coupled at their C-terms to the four arms of maleimide terminated starPEG (Mr = 4 × 2.5 kDa). The hydrogel was formed by crosslinking carbodiimide activated carboxylic groups of heparin with the free N-terms of the peptides. The meshsize can be tuned by stoichiometry of PEG and heparin and is in the range of proteins like thrombin and antithrombin.

Results and discussion: The rheologic measurement confirmed that stiffness, and therefore also the pore size was comparable for the hydrogels with the various linker-peptides.
In thrombin solution, the cleavage of all hydrogels was a function of the thrombin concentration, confirming the predominance of enzymatic degradation over spontaneous hydrolysis. The exchange of Pip and Ser in the scrambled peptide sequence (dFSRX) completely prevented cleavage by thrombin (figure 2). The cleavability with thrombin was highest for dFPR, slightly slower for FPR and considerably retarded for FXR. This indicates that the non-proteogenic aminoacid D-Phe in the sequence enhances cleavage compared to the physiological L-Phe. The six-ring pipecolic acid retarded the degradation compared to the physiological five-ring proline (Fig. 2).

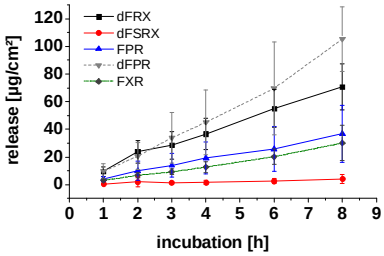

Figure 2. Heparin release for different hydrogels for a thrombin concentration of 45 nM.

Whole blood incubation of the hydrogels with the various thrombin-cleavable peptides did not show major differences in the coagulation activity, measured as prothrombin F1+2 fragment. This may be attributed to the general high heparin content of the hydrogels and low pro-coagulant situation. A more demanding incubation situation is required to demonstrate the benefit of the different cleavability of the hydrogels.

Conclusion: A feedback controlled bioresponsive material has been created by conjugation of four-armed PEG with heparin using thrombin-cleavable linker peptides based on the sequence (D)Phe-Pip-Arg-Ser. In this sequence, (D)Phe enhances thrombin-cleavage, while Pip inhibits the cleavage compared to the natural counterparts. The positions of Pip and Ser are mandatory. Due to the general high hemocompatibility of the gels, stressful testing conditions are required to demonstrate an influence of the different cleavability in whole blood.

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References: Freudenberg U et al. Biomaterials 2009; 30: 5049-5060

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