Different hemocompatibility of silk nanoparticles under static and flow conditions

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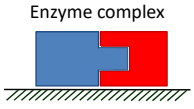
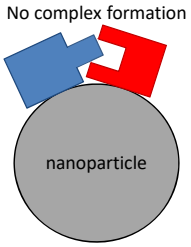
Background and Objectives

Background

- Nanoparticles are administered to the blood stream in nanomedicine
- Silk nanoparticles are attractive as drug delivery agent for anti-cancer therapy
- High surface rate and curvature provide them different properties than the bulk materials
  - Assembly of multi-enzyme complexes may be hindered
- Nanoparticle aggregation to bigger clusters may alter their properties





Objectives

- Test of hemocompatibility of silk nanoparticles without and with aggregation

Concept

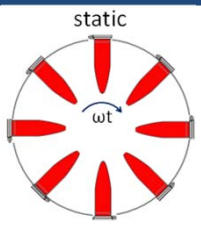
Nanoparticles:

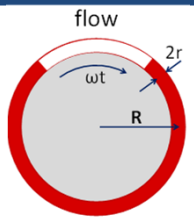
- Silica 102 ± 9 nm silica particles
- Silica-NH_2 102 ± 7 nm NH_2 functionalized silica nanoparticles
- Silk 106 ± 0.8 nm silk nanoparticles
- Silk-PEG 116 ± 0.2 nm silk nanoparticles PEGylated with 5kDa PEG

Incubation with 1.5U/ml heparinized human whole blood for 2 hours

Particle concentration in blood: 250 µg/ml

- Quasi-static incubation, preventing only sedimentation
- Flow incubation: Chandler-Loop system, flow 12 cm s^{-1}

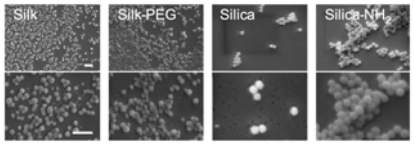


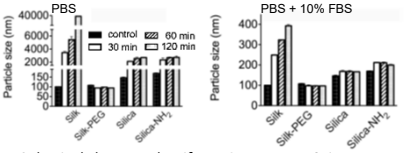


Results

Nanoparticle characterization

Particle size and morphology





- Spherical shape and uniform dimension of the nanoparticles
- Time-dependent aggregation of silk-nanoparticles
- Reduced aggregation in presence of proteins (FBS)
- No aggregation of PEGylated particles

Endotoxin contamination

Endotoxin is a frequent contamination of nanoparticles affecting blood reactions.

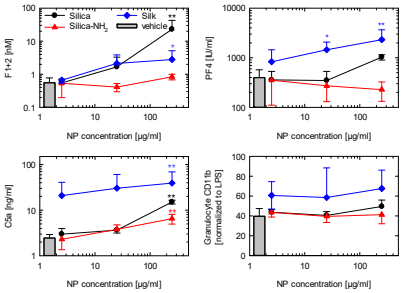
Endotoxin release from 250 µg/ml nanoparticle suspension with 0.2% Tween-20

- All release was well below the FDA-limit of 0.5 EU/ml
- 0.5 EU/ml spike was recovered by silk-nanoparticles but quenched by other particles

Static blood incubation

Dose dependent blood activation

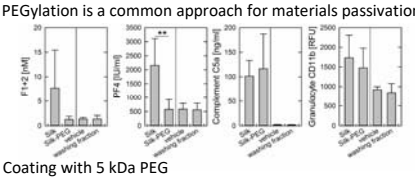
Two hours static whole blood incubation with different nanoparticle concentrations



- Low coagulation activation (F1+2) by silk nanoparticles but elevated platelet activation (PF4)
- Excessive complement activation (C5a) by silk nanoparticles with little dose dependence but only moderate granulocyte activation (CD11b)

PEGylation of silk nanoparticles

PEGylation is a common approach for materials passivation.



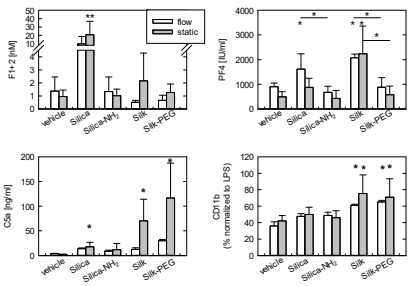
Coating with 5 kDa PEG

- decreased coagulation and platelet activation
- had no effect on inflammatory reactions (C5a and CD11b expression)
- Effects cannot be attributed to soluble factors, as the washing medium was not activating.

Flow incubation

Static incubation *versus* shear forces

Shear forces of flow may disperse aggregated nanoparticles. Whole blood incubation in a Chandler loop system was compared with quasi-static incubation, preventing only sedimentation.



Flow incubation induced …

- … highly reduced the complement activation with silk and silk-PEG nanoparticles (C5a), but only little effect on … leukocyte activation (CD11b)
- slightly reduced coagulation activation (F1+2) for silica-, silk- and silk-PEG nanoparticles.
- … generally elevated blood platelet activation (PF4).

Discussion and Conclusion

- The high curvature of the dispersed nanoparticles under flow can prevent matrix dependent assembly of enzyme complexes and thus the propagation of the enzyme cascades (complement or coagulation).
- Complement is more sensitive to the aggregate formation than the coagulation system.
- Static hemocompatibility evaluation of nanoparticles may be misleading in the levels of complement- and coagulation activation that occur under flow condition *in vivo*.

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Background: Nanomedicine often administers nanoparticles into the blood stream for diagnostic or therapeutic purposes. The high surface rate and high curvature of these nanosized particles provides them different properties than the corresponding bulk materials. Depending on the type, pro- or anticoagulant properties for nanoparticles are described. Nanoparticles frequently tend to aggregate from nano- to microsized clusters, what may alter their interaction with the blood cascade systems and ultimately their hemocompatibility.

Aim: Test the effect of nanoparticle clustering on their hemocompatibility.

Method: Silk nanoparticles of about 100 nm and reference silica particles were incubated in human whole blood for two hours. The incubation was either under static conditions under flow conditions in a Chandler Loop set-up. The shear condition in the Chandler loop incubation keeps the nanoparticles better suspended than the static incubation. After the incubation, parameters of hemostasis and inflammation were determined.

Results and discussion: Coagulation activation, measured as prothrombin F1+2 fragment at the silk nanoparticles was generally low when compared to silica. For both materials, there was a trend for higher activation during static condition (Fig. 1A). The silk nanoparticles induced very high complement activation (C5a) under static condition, but it was almost completely suppressed under flow, where particles are dispersed (Fig. 1B). PEGylation of the particles did not sufficiently suppress aggregation and complement activation (Fig. 1B, C). The quantitative difference between complement and coagulation activation may be attributed to the bigger dimension of the complement activation complex.

Conclusion: Nanoparticle aggregation, as it happens under static test conditions, may cause higher activation of enzyme cascade systems than under physiological flow conditions.

Figure 1: Incubation of nanoparticles with whole blood under static or under flow conditions.