Impact of processing parameters of *Bombyx mori* silk on inflammatory and coagulation response

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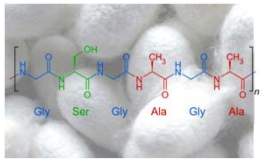
Background and Objectives

- Silk has tradition in surgery as suture material
- Purified silk proteins find interest as scaffold material for vascular tissue engineering
- Preparation parameters can influence composition and structure
  - Amount of sticky protein sericin versus structure protein fibroin
  - Degree of β-sheet structures in the protein

⇨ High load of silk protein and special application require better analysis of biocompatibility



Silk cocoons and structure of the silk protein fibroin

Materials and Methods

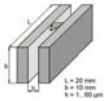
Silk preparation

- Pieces of silk cocoons were washed. Sericin was removed in boiling 25 mM $NaCO_3$. The proteins then were dissolved in 9.3 M LiBr solution and desalted by dialysis against water.
- Films were cast and air dried (Silk, Silk+Ser).
- Treatment with 100% methanol (Silk-MeOH)
- Glass and polytetrafluoroethylene (PTFE) as controls

Whole blood incubation chamber with optimized surface-blood volume ratio

Analyses

- FTIR: β-sheet content
- Microslit cell: surface potential, isoelectric point
- Whole blood incubation
  - 2 U/ml heparin anticoagulated blood
  - 2 hours incubation under rotation, 37°C
  - Analysis of cellular and plasmatic hemostasis
  - Analysis of cellular and plasmatic inflammation

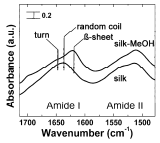


Microslit cell for streaming potential measurement. Zeta potential titration in 1 mM KCl from alkaline to acidic pH

Silk Properties

Secondary structure

Deconvolution of the FTIR amide I band for secondary structue analysis. Methanol treatment strongly enhanced the β-sheet content.

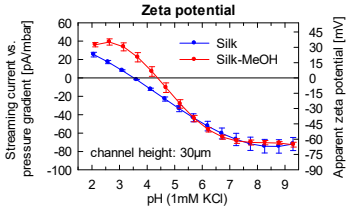


FTIR spectrum of silk (bottom) and silk-MeOH. Deformation of amide I band by MeOH treatment is obvious.

Secondary structure (%)	Silk	Silk-MeOH
β-sheet	35.1	53.8
Random coil, α-helix	24.1	19.2
Side chains	2.9	4.9
β-turns	37.9	22.1

Zeta potential

Methanol treatment decreased the acidity (isoelectric point) of the silk preparation

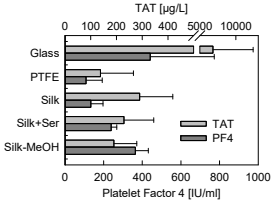


Streaming current vs. pressure gradient and apparent zeta potential. MeOH treatment caused almost 1 pH higher isoelectric point.

Hemocompatibility

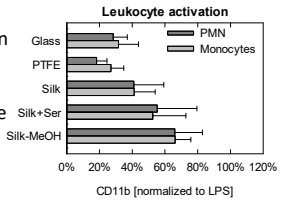
Hemostasis

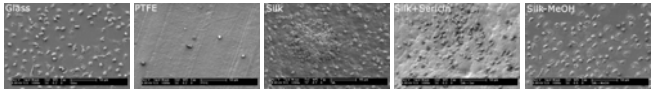
- High plasmatic coagulation activation (thrombin-antithrombin complex TAT) by silk compared to Silk+Ser and Silk-MeOH
  - Coagulation activation by negative surface potential of silk
- Independent trend of platelet activation (platelet factor 4 PF4 release)



Inflammation

- High granulocyte and monocyte activation by silk films.
- Serinin-containing and methanol-treated silk induce higher activation than the pure silk.
- Inflammatory reaction also confirmed by high cell density of adherent leukocytes.





SEM images of the specimen after two hours incuabtion with whole blood. High leukocyte density.

Conclusions

⇨ Silk structure and physical-chemical properties can be controlled by processing techniques

⇨ Processing techniques also influence the blood bompatibility

⇨ Pure silk has advantage concerning inflammation, but induces high coagulation

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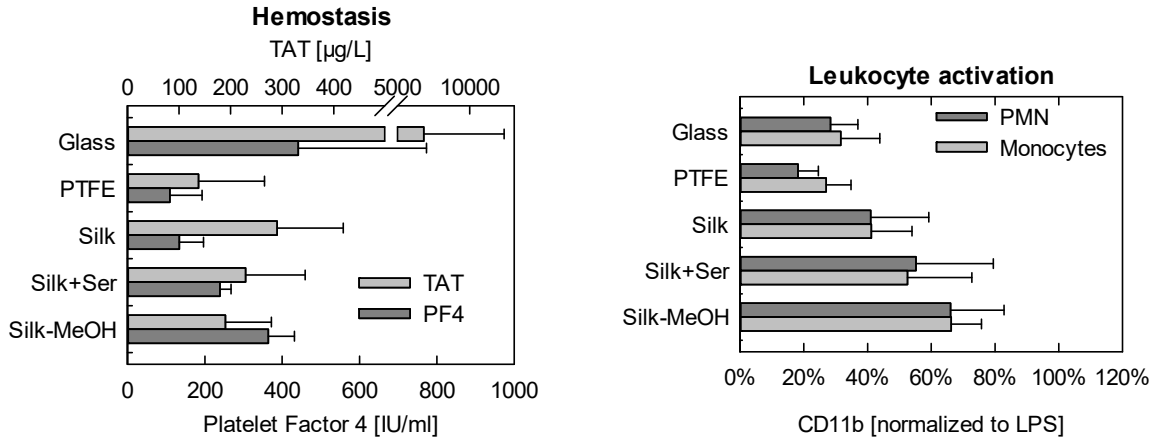
Background: Silk has a long standing tradition for its application in surgical sutures and is generally accepted to be biocompatible. However, the use of purified silk proteins as a scaffold material for vascular tissue engineering goes beyond its traditional use and thus demands application orientated biocompatibility testing. Using different processing parameters of the silk, the sticky protein sericin and also the secondary structure of fibroin can be controlled. The impact of these parameters on the biocompatibility has not been clarified yet.

Method: Silk proteins of *Bombyx mori* were isolated either containing the sericin protein or removing it by boiling in $NaCO_3$ solution. The proteins were dissolved in LiBr solution, films were cast and air dried ("Silk+Ser" and "Silk"). A subset of sercin-free silk fibroin samples was subsequently treated with 100% methanol ("Silk-MeOH"). With this treatment the β-sheet content increased from ~13% to ~54%.

These samples in comparison to the positive control glass and the inert material PTFE were incubated with human whole blood for two hours and surface and liquid phase parameters of inflammation and coagulation were assessed.

Results: Pure silk-fibroin samples induced an elevated plasma coagulation (measured as thrombin-antithrombin complex) than the sericin or the beta-sheet rich methanol treated samples. This could be attributed to the high acidity of fibroin, which decreased after the methanol treatment. Platelet activation but also inflammatory reactions, such as complement and leukocyte activation favored more the pure silk fibroin than sericin containing or the β-sheet rich methanol treated preparation.



Hemostasis (left) and inflammation markers (right) in blood after two hours incubation with silk samples (glass and PTFE as reference).

Conclusion: Silk processing had a significant impact on haemostasis and inflammatory response *in vitro*. Inflammation and blood platelet activation showed an advantage of the pure silk with ~13% β-sheet content. The negative isoelectric point of silk fibroin however, activates plasmatic coagulation, what would need further control for blood contacting applications.