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Supporting Information

Analyzing the antiseptic capacity of silver-functionalized poly(ethylene glycol)-heparin hydrogels after human whole blood exposure

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Figure S1. Count of vital adherent bacteria on poly(styrene) surfaces (A) and ratio of dead bacteria portion in the supernatant (B) for control as well as plasma, heat-inactivated (HI) plasma and albumin solution pre-incubated surfaces upon 3 h of bacterial incubation. Bacterial suspensions of *P. aeruginosa* and *S. epidermidis* were used. Live dead staining by PI/Syto9 was done to determine the vital and dead density of bacteria. Statistical significances: * is significantly different from the respective control (p<0.05).



Figure S2. Activation of coagulation and inflammation. Prothrombin fragment F1+2 (A), platelet factor PF4 (B) and complement fragment C5a (C) measured in plasma after whole blood incubation assay by ELISA. Poly(styrene) (PS), non-responsive (PHG) and thrombin-responsive (tcPHG) hydrogels as well as their silver-functionalized equivalents (+ Ag) were incubated in whole blood. Afterwards the blood was analyzed by means of ELISA. Statistical significances: * is significantly different from the poly(styrene) surface (p<0.05).



Figure S3. Relative activation level of granulocytes and platelet-granulocyte conjugates. Poly(styrene) (PS), non-responsive (PHG) and thrombin-responsive hydrogels (tcPHG) as well as their silver-functionalized equivalents (+ Ag) were incubated in whole blood. Activated granulocytes were measured by their expression of CD11b cell surface marker (A). Amounts were calculated relative to an activation by 100 EU/mL LPS. Platelet activation (B) was measured by means of granulocyte-platelet conjugates. The measurement was performed by FACS analysis. Statistical significances: * is significantly different from the poly(styrene) surface (p<0.05).



Figure S4. Granulocyte (A) and platelet (B) decrease in blood upon surface incubation whole blood. Poly(styrene) (PS), non-responsive (PHG) and thrombin-responsive hydrogels (tcPHG) as well as their silverfunctionalized equivalents (+ Ag) were incubated in whole blood. Then, blood was analyzed by means of coulter analysis for the decrease of blood cells and with this their adherence on the surfaces. Statistical significances: * is significantly different from the poly(styrene) surface (p<0.05).



Figure S5. Bacterial vitality on control surface samples after 10 min (A) and 3 h (B) bacterial incubation [%]. Poly(styrene) (PS), non-responsive (PHG) and thrombin-responsive (tcPHG) hydrogels as well as their silver- functionalized equivalent (+Ag) were incubated in acetate buffer only and subsequently exposed to bacterial solution of *E. coli*, *P. aeruginosa*, *S. epidermidis* and *S. aureus* for 10 min and 3 h. Surfaces were rinsed and bacteria were stained by PI/Syto9 staining and visualized by fluorescence microscopy. Statistical significances: * is significantly different from the corresponding non-loaded hydrogel type (p<0.05).



Figure S6. Bacterial vitality on blood pre-incubated surface samples after 10 min (A) and 3 h (B) bacterial incubation [%]. Poly(styrene) (PS), non-responsive (PHG) and thrombin-responsive (tcPHG) hydrogels as well as their silver-functionalized equivalent (+Ag) were incubated in whole blood (2 hours at 37 °C, 1.5 IU/heparin) and subsequently exposed to bacterial solution of *E. coli, P. aeruginosa, S. epidermidis* and *S. aureus* for 10 min and 3 h. Surfaces were rinsed and bacteria were stained by PI/Syto9 and visualized by fluorescence microscopy. Statistical significances: * is significantly different from the corresponding non-loaded hydrogel type (p<0.05).



Figure S7. Scanning electron microscopy images for control samples incubated for 10 min in bacterial suspension. Poly(styrene) (PS), non-responsive (PHG) and thrombin-responsive (tcPHG) hydrogels as well as their silver-functionalized equivalent (+ Ag) were incubated in buffer only for control conditions. Afterwards they were incubated in bacterial suspension of *E. coli*, *P. aeruginosa*, *S. epidermidis* and *S. aureus* for 10 min and visualized by scanning electron microscopy. Scale bar = $10 \mu m$.



Figure S8. Scanning electron microscopy images for control samples incubated for 3 h in bacterial suspension. Poly(styrene) (PS), non-responsive (PHG) and thrombin-responsive (tcPHG) hydrogels as well as their silver-functionalized equivalent (+ Ag) were incubated in buffer only for control conditions. Afterwards they were incubated in bacterial suspension of *E. coli*, *P. aeruginosa*, *S. epidermidis* and *S. aureus* for 3 h and visualized by scanning electron microscopy. Scale bar = $10 \mu m$.



Figure S9. Scanning electron microscopy images for blood pre-incubated samples subsequently incubated in bacterial suspension for 10 min. Poly(styrene) (PS), non-responsive (PHG) and thrombin-responsive (tcPHG) hydrogels as well as their silver-functionalized equivalent (+ Ag) were incubated in whole blood (2 hours at 37 °C, 1.5 IU/heparin). Afterwards they were incubated in bacterial suspension of *E. coli*, *P. aeruginosa*, *S. epidermidis* and *S. aureus* for 10 min and visualized by scanning electron microscopy. Scale bar = 10 μ m.



Figure S10. Scanning electron microscopy images for blood pre-incubated samples subsequently incubated in bacterial suspension for 3 h. Poly(styrene) (PS), non-responsive (PHG) and thrombin-responsive (tcPHG) hydrogels as well as their silver-functionalized equivalent (+ Ag) were incubated in whole blood (2 hours at 37 °C, 1.5 IU/heparin). Afterwards they were incubated in bacterial suspension of *E. coli*, *P. aeruginosa*, *S. epidermidis* and *S. aureus* for 3 h and visualized by scanning electron microscopy. Scale bar = 10 μ m.



Figure S11. Fraction of vital bacteria in the supernatant after 3 h of bacterial incubation on control and blood pre-incubated surface samples. Non-responsive (PHG) and thrombin-responsive (tcPHG) hydrogel surfaces under silver-functionalized and non-loaded conditions were incubated in buffer only and in whole blood for control and blood pre-incubated samples respectively. Exposition to bacterial solution of *P. aeruginosa* (A) and *S. epidermidis* (B) for 3 h and staining of the supernatant by PI/Syto9 was done subsequently. Fluorescence activating cell sorting was used to determine the vital cell fraction. Statistical significances: * is significantly different from the corresponding control surface.