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# Multilayer hydrogel coatings to combine hemocompatibility and antimicrobial activity

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ABSTRACT

While silver-loaded catheters are widely used to prevent early-onset catheter-related infections [1], long term antimicrobial protection of indwelling catheters remains to be achieved [2] and antiseptic functionalization of coatings often impairs their hemocompatibility characteristics. Therefore, this work aimed to capitalize on the antimicrobial properties of silver nanoparticles, incorporated in anticoagulant poly(ethylene glycol) (PEG)-heparin hydrogel coatings [3] on thermoplastic polyurethane materials. For prolonged antimicrobial activity, the silver-containing starPEG-heparin hydrogel layers were shielded with silver-free hydrogel layers of otherwise similar composition. The resulting multi-layered gel coatings showed long term antiseptic efficacy against *Escherichia coli* and *Staphylococcus epidermidis* strains *in vitro*, and similarly performed well when incubated with freshly drawn human whole blood with respect to hemolysis, platelet activation and plasmatic coagulation. The introduced hydrogel multilayer system thus offers a promising combination of hemocompatibility and long-term antiseptic capacity to meet an important clinical need.



## 1. Introduction

Bacterial infections related to indwelling medical devices rank as the fifth-leading cause of hospital patients death in the US [4]. Central venous catheter-associated bloodstream infections occur with a frequency of 5 per 1000 catheter days and result in a lethality rate of 20% [5]. Therefore, in addition to sterile handling and systemic administration of anticoagulants, antimicrobial prevention is of paramount importance for the safety of central venous catheter applications [6]. Current strategies to locally prevent infections include bactericidal and bacteriostatic approaches using antiseptically impregnated coatings or antibiotic lock therapies. For that purpose, both the catheter bulk material and hydrophilic hydrogel coatings, providing catheters with a “slippery-when-wet” lubricious surface, were customized to embed and release bioactives with silver being the most frequently applied bioactive component. In these approaches, silver is applied as silver bulk material, in ionic form or as silver nanoparticles (AgNPs) referring to both oxidation states ($Ag^0/Ag^+$). Although the antimicrobial mechanism of silver is still controversially discussed [7–13], there both oxidation states are considered to be active against bacteria, most probably through a conversion of bacterial sulfhydryl (R-SH) groups [9]. For silver ions, the biological activity is multifold including the inhibition of cell respiration and the inactivation of enzymes to effects on DNA replication and cell division [7,8].

A common strategy for sustained $Ag^+$ release is the reduction of ionic silver in solution to form AgNPs with a large surface-to-volume ratio [14]. Previous studies have shown that AgNPs can either penetrate bacterial cells [10] or become deposited on the bacterial cell wall, affecting membrane permeability as well as electrolyte and metabolite transport [11]. The cytotoxicity of silver nanoparticles results from interactions with membrane proteins, the activation of signaling pathways and the inhibition of cell proliferation [12,13]. The toxicity of silver for bacterial and eukaryotic cells has been extensively tested and compared in earlier studies, showing a significantly lower toxicity of silver for the eukaryotic cells [15,16].

The use of silver-loaded hydrogel coatings for central venous catheters dates back to 1998 [2] and was found suitable to prevent early-onset catheter-related infections but concluded to be limited

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for long lasting protection [1,2]. Recently reported strategies have exploited the in situ formation of AgNPs within swollen hydrogel networks of catheter coatings [17–19] which were developed to minimize protein adsorption and platelet adhesion [18]. However, the antiseptic functionalization of biomaterials with silver-based compounds often is associated with a loss of the beneficial hemocompatibility characteristics since silver not only affects microorganisms, but also can exert undesired side effects on mammalian cells [20,21]. Thus, biomaterial coatings combining sustained, long-term delivery of antimicrobial active silver and non-thrombogenic properties are particularly important targets of current research [22].

Herein, we present a new concept to address these challenges. A recently introduced star-shaped poly(ethylene glycol) (PEG)-heparin hydrogel [23] was grafted as a thin film coating onto thermoplastic polyurethane bulk materials and further modified with silver by incubation in $AgNO_3$ solution (see Scheme 1). The building blocks, star-shaped PEG and heparin, widely applied in the surface modification of biomaterials due to their protein-resisting and anticoagulant characteristics, were covalently linked to form a hydrogel network. In an effort to further modulate this system, a multilayer gel coating was developed, where a silver loaded hydrogel is coated onto the surface of the polyurethane bulk material with a second silver-free hydrogel layer on top to act as a diffusion barrier for prolonged silver release and for avoiding direct cellular interactions with AgNPs at the coating-blood interface. These multilayer hydrogel coatings were thoroughly evaluated in whole blood hemocompatibility assays for coagulation and inflammatory activation. The antiseptic capacity of the coating was verified with different relevant bacterial strains.

## 2. Materials and methods

### 2.1. Preparation of starPEG-heparin hydrogel coatings

Biohybrid starPEG-heparin gels were prepared using amino functionalized four-armed starPEG and EDC-s-NHS activated heparin as previously described [3]. Briefly, a solution of heparin (14 000 g/mol; Calbiochem, Darmstadt, Germany) was activated using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysulfosuccinimide (sulfo-NHS) (ratio 2:1) at a stoichiometric balanced concentration of sulfo-NHS to the starPEG–$NH_2$–groups. Subsequently, a starPEG solution (10 000 g/mol Polymer Source Inc., Dorval, Canada) was added to the activated heparin to a final concentration of 133 mg/ml. The starPEG-heparin mixture was spread on thermoplastic polyurethane discs (Elastollan® 1190A, provided by BASF Polyurethanes GmbH, Lemförde, Germany) and was subsequently covered with a hydrophobized glass coverslip (by dipping the coverslip into a Sigmacote® [Sigma Aldrich, St. Louis, USA]). The coverslip facilitates the formation of a defined gel disc and can be easily removed after gel formation. Alternatively, the hydrogel was applied to thermoplastic polyurethane (TPU) tubes by dip coating. After overnight polymerization and removal of the glass coverslip, gels were swollen for 12 h in acetate buffer (160 mmol/l; 314 mOsm). During swelling the acetate buffer was exchanged several times to ensured complete removal of EDC/s-NHS and unreacted hydrogel building blocks. If needed, unmodified heparin was mixed with 1% Alexa-488 labeled heparin (Invitrogen, Darmstadt, Germany) to allow for fluorescence microscopic analysis.

Different TPU surface modification techniques were evaluated to ensure stable grafting of starPEG-heparin hydrogels to TPU substrates. TPU was either treated with ammonia plasma (MicroSys apparatus by Roth&Rau, Wüstenbrand, Germany; treatment time: 10 s, power: 600 W, ammonia gas flow: 15 standard cubic centimeter per minute, pressure: $7 \times 10^{-3}$ mbar) or air plasma using a plasma cleaner (Harrick, Ithaca, NY, USA; treatment time: 10 min pressure: approx. 0.2 mbar). Alternatively samples were treated with a low energy electron beam (ADU, Advanced Electron Beams, Wilmington, USA 100 keV) with an absorbed dose of 258 kGy under inert $N_2$ atmosphere or in air conditions to induce reactive groups on the TPU surface. Fluorescently labeled hydrogels grafted to TPU were analyzed by confocal laser scanning microscopy (cLSM, SP-5, Leica Microsystems, Wetzlar, Germany) in xy- and z-scan for coating thickness and coating homogeneity, respectively.

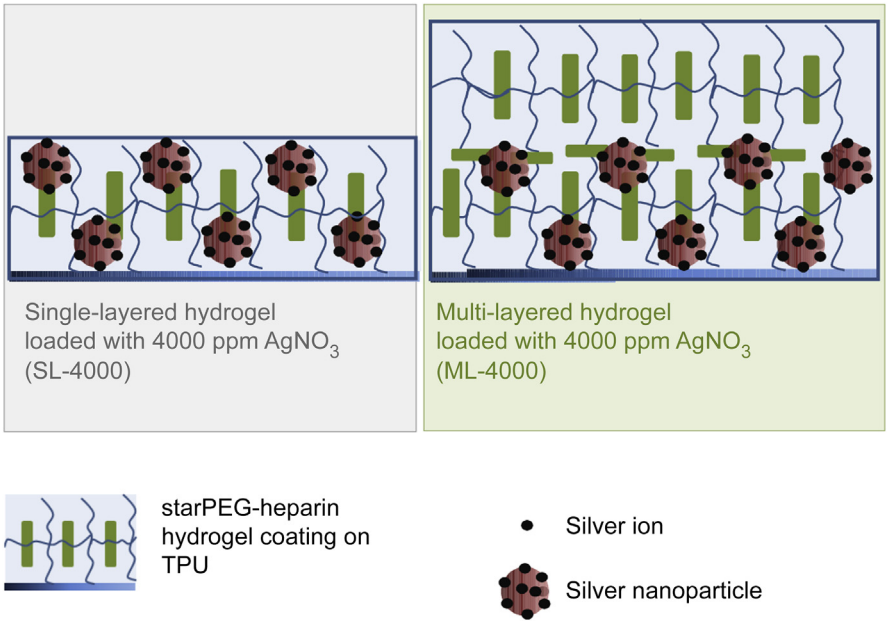
The thickness of the swollen starPEG-heparin hydrogels on planar TPU substrates was approx. 50–100 μm and the heparin content was approx. 0.3 mg heparin per $cm^2$ surface area. StarPEG-heparin gels coated to TPU tubes revealed a thickness of 16–34 μm. For rheometry and AgNP analysis, freestanding gel discs were prepared by applying the gel mixture between two Sigmacote®-coated coverslips.

### 2.2. Antiseptic modification of hydrogels

Hydrogels were modified with AgNPs by immersion into acetate buffer containing $AgNO_3$ (Sigma Aldrich, Munich, Germany) for 1 h at room temperature. Silver nitrate solutions were freshly prepared prior to use. Solutions and samples were stored in the dark. The applied $AgNO_3$ concentrations were chosen according to the minimal inhibitory concentration (MIC) given by Copra et al. and Ip et al. [24,25] and ranged from 80 to 4000 ppm (corresponding to 1.5 μg- 75 μg $AgNO_3/mm^3$ gel volume). A volume of 5.0 ml $AgNO_3$ solution was applied per gel scaffold of 265 $mm^3$.

### 2.3. Preparation of multi-layered hydrogels

Multi-layered hydrogels were prepared by vacuum drying TPU-bound AgNP-loaded hydrogels at room temperature. Subsequently, a reaction mixture of starPEG and heparin was applied to the dried gel layer and covered with Sigmacote® coverslips as described above. After polymerization, coverslips were removed and gels




**Scheme 1.** Schematic depiction of the explored variants of multifunctional hydrogel coatings.