Antibacterial and Anti-biofouling Nanofibrous Membranes

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Statement of Purpose: Nanofibrous membranes have been investigated in the area of tissue adhesion prevention, wound dressing and scaffolds for tissue engineering recently.[1] To develop nanofibrous membranes providing anti-biofouling and anti-bacterial properties may further extend their applications.[2] Layer-by-layer (LBL) assembly technique is a simple and powerful method to generate multifunctional surfaces created by consecutive alternate deposition of positively and negatively charged species.[3] In the present report, layer-by-layer assembly technique was used to develop antibacterial polyacrylonitrile (PAN) nanofibrous membranes by combining the fouling-release property of heparin (HP) with the antibacterial property of polyhexamethylene guanidine hydrochloride (PHGH).

Methods: PAN(MW150000, Jiangsu Haide Co., CHINA). PHGH (MW800, Huashenghuanneng Bio & Chem Co. CHINA). HP (Sigma-Aldrich). PAN nanofibrous membranes were obtained by electrospinning polymer solution at concentration of 11% (g ml^{-1}) in DMF. LBL-functionalized nanofibrous membranes were fabricated by hydrolyzing PAN nanofibrous membranes (PAN-COO^-)followed by exposing to PHGH and HP stock solutions having concentrations of 1 mg ml^{-1} alternatively. Repeating LBL deposition cycle, PAN nanofibrous membranes coated with multilayer thin films of PHGH/HP with 5, 5.5,10, 10.5 bilayers were obtained. The membranes were investigated by scanning electronic microscope (JEOL JSM-6360, 10kV, Japan) equipped with an energy-dispersive X-ray spectroscopy (EDX, Genesis-60S) and attenuated total reflectance Fourier transform infrared spectroscopy (ATR/FT-IR)(Nicolet 5700, Thermo) . Gram-positive *Staphylococcus aureus* (*S. aureus*, ATCC 25923) and Gram-negative *Escherichia coli* (*E. coli*, ATCC DH5 α) were used to evaluate the bactericidal efficacy of PAN nanofibrous membranes having multilayer coatings. Anti-fouling property was determined as follows: Nanofibrous membranes (1 cm^2) were placed in a 24-well cell culture plate. Then, 1.0 ml of S. aureus cells (10^9 cells ml^{-1}) was added to each well and incubated at 37°C. After 24 h incubation and washing, adhered bacterial cells were investigated under SEM.

Results: Layer-by-layer deposition of PHGH and HP alternatively on PAN nanofibrous membranes was confirmed by ATR/FT-IR and EDX. The peak centered at 1030 $cm^{-1}$and 1226 cm^{-1} due to C-O and S=O groups (FTIR) and the characteristic peak of S element (EDX) indicates the presence of HP after the LBL assembly process. The morphology of the PAN nanofibrous membranes was observed by SEM images. It was revealed that the layer-by-layer process successfully placed PHGH/HP coatings onto nanofibrous membranes, leading to increased diameter of nanofibers.

The antibacterial activities of PHGH/HP deposited nanofibrous membranes were analyzed against *S. aureus* and *E. coli* cells (Figure 1). 99.999% of *S. aureus* was killed within 2 h contact time when the PHGH/HP bilayers were deposited on nanofibrous membranes, whereas those membranes with higher bilayers of PHGH/HP exhibited higher antibacterial activities. PAN nanofibrous membranes terminated with PHGH have better antibacterial activity than HP-ended nanofibrous membranes. The anti-biofouling efficacy of the multilayer coatings was evaluated against *S. aureus* by quantitative determination of the number of viable cells on the LBL-functionalized surfaces. It was showed that the number of viable bacterial cells in all the LBL-functionalized nanofibrous membranes was greatly reduced by 98% compared with that of pristine PAN nanofibrous membrane, while PAN-COO--$(PHGH/HP)_{10.5}$ surface exhibiting the highest anti-biofouling efficacy of 99.62%. Apparently, the formation of thicker and denser LBL layers helps to account for higher anti-biofouling abilities.

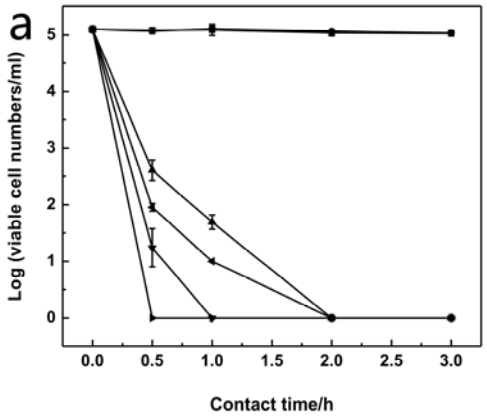

Figure 1: Viable cell numbers of (a) *S. aureus* in contact with (■) blank control, (●) PAN, (▲) PAN-COO^--(PHGH/HP)5, (▼) PAN-COO^--(PHGH/HP)5.5, (◄) PAN-COO^--(PHGH/HP)10 and (►) PAN-COO^--(PHGH/HP)10.5 nanofibrous membranes.

Conclusions: Layer-by-layer alternative deposition of PHGH/HP is an effective approach to construct antibacterial and anti-fouling multilayer films based on PAN nanofibrous membranes. The novel modified nanofibrous membranes not only killed bacteria effectively but also released the dead cells through antifouling process.

References:

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