Biofunctionalized nanofibrous membranes mimicking carnivorous plants

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A novel biofunctionalized nanofibrous membrane is developed through immobilization of protein ligands on the surface of nanofibers. The biofunctionalization not only enhances the membrane's structural properties including mechanical and thermal ones but also makes the membrane capable to separate nanoparticles and biomolecules much smaller than the pore size from water efficiently. Upon contact with water, the conformational change of the protein immobilized leads to its swelling, thereby an enlarged functional surface area and a higher steric hindrance capturing the filtrates. In case of filtration of a plasmonic nanoparticle containing suspension, decoration of the membrane with the plasmonic nanoparticles forms a smart bionanocomposite biosensor for detection of protein denaturation.

1. Introduction

Membrane technology for water treatment is steadily gaining very high importance worldwide. This is primarily due to water pollution and dwindling fresh water supplies leading to water scarcity. Water quality has to be controlled to ensure a safer environment by implementing efficient technologies such as advanced membranes offering more output with less input, that is, efficient energy saving membranes. Electrospun nanofibrous membranes (ENMs) that have the potential to be used as advanced membrane systems will be able to remove pollutants from the environment at lower energy and hence cost.¹ Energy saving by ENMs derives from their high interconnected porosity leading to a very high permeability.² Despite an extraordinary permeability, ENMs suffer from low size selectivity. Microfiltration (MF) range pore size of ENMs makes them efficient in removing relatively coarse particles and suspended solids but not tiny substances smaller

than the pore size.^{3–7} Considering the importance of separation of nanoparticles also organics that can be detrimental to the quality of water systems, optimizing the selectivity of such membranes could be crucial. Accordingly, not only the high permeability of the membrane is preserved but also on the basis of selectivity, the application domain would be extended from MF to ultrafiltration (UF) and even nanofiltration. Long-term functionality, that is, longevity of ENMs with regard to their extraordinary surface area thereby a higher exposed surface to the water streams is dependent on their mechanical stability. Hence, to maximize the efficiency of an ENM, besides the optimization of the selectivity, its mechanical stabilization should be also stressed.

Here, we show that through one-single approach, that is, protein functionalization, an ENM can acquire mechanical and thermal stability while showing more optimum selectivity. Inspired by the

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nature, similar to a carnivorous plant, which spreads the leaves equipped with some sticky mucilage to attract and capture insects, here, we show that through protein functionalization, that is, immobilization, an ENM can acquire more optimum selectivity in an analogous way. Thanks to water–protein interaction, subsequent conformational change and protein–metal and protein–protein interactions, the protein immobilized onto the nanofibers is assumed to capture not only very small nanoparticles⁸ but also tiny biomolecules normally passing through the pores leading to a higher selectivity. This behavior brings about a breakthrough in applicability of the ENMs and extends the application area beyond MF to UF. This approach through cross-linking of the nanofibers could be positively influential on mechanical stability of the membrane as well. As another promising outcome, decoration of ENMs with metal nanoparticles filtered out from the respective aqueous suspension would give rise to a nanohybrid with synergetic optical, electrical, catalytic and biological properties.⁹ Thus, for the first time, we also report a filtration-based approach for fabrication of a nanohybrid with well-dispersed nanoparticles applicable for a naked eye plasmonic detection of protein denaturation/renaturation.

2. Experimental

2.1 Materials

Poly(acrylonitrile-co-glycidyl methacrylate) (PANGMA) as the polymer to be electrospun and to be formed as nanofibers was synthesized at Helmholtz-Zentrum Geesthacht (Geesthacht, Germany; previously GKSS Forschungszentrum GmbH)¹⁰ with a molecular weight (M_n) of ca. 100,000 g/mol and glycidyl methacrylate (GMA) content of 13 mol%. Bovine serum albumin (BSA; dried powder) and phosphate-buffered saline (PBS) were purchased from Sigma-Aldrich Co., (USA). N,N-dimethylformamide (DMF) was obtained from Merck (Germany). As the model for the nanoparticles to be filtered out, the Au nanoparticles of 40 nm were made by sodium citrate reduction method.¹¹

2.2 Electrospinning and biofunctionalization of PANGMA nanofibers

DMF was used to dissolve PANGMA (20 wt.%). The solution was fed with a constant rate of 1.1 ml/h into a needle by using a syringe pump (Harvard Apparatus, USA). The electrospinning was performed using aluminum (Al) foil located 25 cm above the needle tip for 4 h by applying a voltage of 15–20 kV (Heininger Electronic GmbH, Germany). After all, the nanofibrous mat was peeled off from Al foil and dried under vacuum at 30°C for 24 h to remove the residual solvent.

In the next step, the PANGMA nanofibrous mats were immersed into the BSA/PBS buffer (5 mg/ml; pH 6.8) while gently shaken at 55°C for 24 h. Subsequently, to remove all the unbound BSA, the membranes were taken out and washed several times by PBS buffer (pH 6.8) and then deionized water and dried under vacuum at 30°C for 24 h.

2.3 Characterization of structural properties

The morphology of the PANGMA ENMs as neat and biofunctionalized was observed with a scanning electron microscope (SEM; LEO Gemini 1550 VP, Zeiss) at 10 kV accelerating voltage after gold coating. The diameter of the electrospun nanofibers was determined from the SEM images using the Adobe Acrobat v.07 software.

To investigate the eventual influence of the biofunctionalization on thermal and mechanical properties of the membranes, differential scanning calorimetry (DSC), thermal gravimetric analysis (TGA) and tensile tests were performed. DSC was performed by a Netzsch DSC 204 Phoenix[®] using indium standards. The glass-transition temperature (T_g) was determined by means of a dynamic scan at 10°C/min from 20 to 300°C. The thermal stability of the neat and biofunctionalized ENMs was evaluated by TGA. TGA of the ENMs was carried out with a thermogravimetric analyzer of Netzsch 209 TG. TGA analysis was performed at 20–700°C with a heating rate of 10°C/min under argon. The decomposition temperature (T_d) was defined as the temperature at 5% weight loss.

The ENMs' mechanical properties were also characterized by a tensile machine (Zwick/Roell Z020-20KN, Germany) equipped with a 20 N load cell at ambient temperature. The cross-head speed was 2 mm/min and the gauge length was 20 mm. The reported tensile moduli and tensile strengths represent average results of ten tests.

2.4 Characterization of filtration properties

2.4.1 Filtration of protein

The ability of the biofunctionalized ENMs in removing proteins from water was investigated via a filtration experiment of BSA.

To run the experiment, 20 ml protein aqueous solution (2 mg/l) was passed through the membranes with an active filtration area of 2 cm². The driving force for the filtration was a minor hydrostatic pressure of 20 mPa supplied by an installed column on the module. The experiment was performed in a cyclic style, that is, every permeate was considered as the new feed for the new filtration cycle until completion of five successive cycles.

Already constructed the standard protein calibration curve, the concentration of the proteins in the original feed and permeates was specified by a UV-Vis spectroscopy. The filtration efficiency (FE) was determined according to the Equation 1:

$$1. \quad FE = \left(1 - \frac{C_p}{C_f}\right) \times 100\%$$

where C_p and C_f are the concentrations of protein in the permeates and feeds, respectively.

2.4.2 Filtration of nanoparticles

The retention capability of the neat and biofunctionalized PANGMA ENMs was determined using aqueous suspensions (20 ml) containing gold nanoparticles of 40 nm. The filtration test was performed in a same manner as the protein filtration experiment. This process was performed in three successive cycles. Noteworthy, the filtration was performed under a negligible hydrostatical pressure, that is, no considerable feed pressure. The retention ability of the ENM for the gold nanoparticles was characterized by UV-Vis spectroscopy.

3. Results and discussion

As a new polymeric material to be used for nanofiber production, PANGMA is a copolymer of acrylonitrile (AN) and glycidyl methacrylate GMA.⁶ The main advantages of this polymer include the chemical stability from the sturdy backbone of polyacrylonitrile and the optimum functionality, that is, further reacting ability owing to presence of the free and active epoxy group on GMA. The latter possibility offers the opportunity in a variety of activation/coupling chemistries for the covalent binding of capturers. PANGMA nanofibers were functionalized through surface immobilization with BSA. BSA acting as the capturer component is a cheap serum albumin protein, widely used in numerous biochemical applications.

As shown in Figure 1, the biofunctionalized ENM possesses beadless nanofibers with smooth surface. Moreover, as seen in this figure, the biofunctionalization does not sacrifice the porosity of the mat, that is, clogging the pores. On the basis of the measurement of nanofibers' diameter (Figure 2), the fibers after the biofunctionalization are slightly enlarged in diameter. This growth of nanofibers certainly does not alter the porous structure, that is, pore size of the membranes.

The functionalization of the nanofibers is according to the extremely simple and extensively used epoxy amino reaction between the amine groups on BSA and epoxy groups on PANGMA.^{6,12} Figure 3 shows a schematic sketch of the functionalization process illustrating how BSA ligands can concurrently bond to two adjacent nanofibers, thereby form a chemical cross-linking, which is expected to improve the mechanical and thermal stabilities of the membrane.

Accordingly as seen in Figure 4, the biofunctionalized membranes possess a higher level of thermal resistance. The main reason could be cross-linking of the PANGMA chains stimulated by the functionalization. The cross-linking restricts local chain mobility thereby increases T_g .¹³ Moreover, a higher thermal stability is induced by the cross-linking,¹⁴ implying the feasibility of application of the biofunctionalized membrane at higher temperatures than ambient temperature but certainly lower than denaturation temperature of the BSA.

Figure 1. Surface morphology of the PANGMA nanofibers as BSA biofunctionalized. BSA, bovine serum albumin; PANGMA, poly(acrylonitrile-co-glycidyl methacrylate).

Figure 2. Average diameter of the PANGMA nanofibers as neat and BSA biofunctionalized. BSA, bovine serum albumin; PANGMA, poly(acrylonitrile-co-glycidyl methacrylate).

As a prerequisite for pressure-driven liquid filtration applications,¹⁵ as seen in Figure 5, the biofunctionalization of the PANGMA ENMs could lead to a more optimum mechanical performance in terms of stiffness (elastic modulus) also strength. As mentioned earlier, BSA ligands act as a cross-linker forming a network structure that restricts movement of the nanofibers tending to pass by each other thereby optimize the mechanical performance of the biofunctionalized membrane.

Applicability of ENMs is revolutionized when they are able to filter out not only coarse suspended solids but also nanosolids and organics much smaller than the pore size. Here, we aim to show that the biofunctionalization approach brings about a high selectivity to nanoparticles also biomolecules. As the model for the filtrates, Au nanoparticles (40 nm) and BSA were selected and their respective

Figure 3. Schematic diagram of the immobilization of BSA onto PANGMA nanofibers. BSA, bovine serum albumin; PANGMA, poly(acrylonitrile-co-glycidyl methacrylate).

Figure 4. Variation of thermal properties of the PANGMA ENMs before and after the biofunctionalization. ENM, electrospun nanofibrous membranes; PANGMA, poly(acrylonitrile-co-glycidyl methacrylate).

suspension and solution, respectively, were passed through the neat and biofunctionalized ENMs.

As shown in Figure 6, based on the UV-Vis graph, in contrary to the neat PANGMA ENM that is able to reject a negligible part of the nanoparticles, the biofunctionalized ENM holds them significantly. Such an efficient removal as adsorption of the nanoparticles to the surface of nanofibers is obviously observed in Figure 7.

The SEM image shown in Figure 7 implies that the metal nanoparticles are arranged uniformly on the biofunctionalized nanofibers. It is assumed that the immobilized protein plays an important role in the uniform dispersion of the gold nanoparticles.

The reason for such an optimum FE by the BSA/PANGMA ENMs is assumed to be pH- dependent conformational change of

Figure 5. Tensile properties of the PANGMA ENMs before and after the biofunctionalization. ENM, electrospun nanofibrous membranes; PANGMA, poly(acrylonitrile-co-glycidyl methacrylate).

BSA. As soon as wetting, pH increases to above isoelectric point of BSA, that is, 4-7, therefore the folded state, that is, “ α -helix,” is unfolded as β -turn, and tryptophan and cysteine residue groups also amine-based groups which due to strong hydrogen bondings between the peptide groups were less available emerge. This alteration is traceable through attenuated total reflectance-fourier transform infrared measurements (Figure shown at Ref. 8) as replacement of the bands assigned to α -helix secondary structure of BSA ($1650\text{--}1658\text{ cm}^{-1}$) predominated in dry state with those of turns ($1666\text{--}1673\text{ cm}^{-1}$)^{16,17} in wet state. When previously buried peptide bonds and amino acid side chains are exposed and become available for interaction with the aqueous environment, the higher functionality leads to binding considerably more water¹⁸ thereby a swollen structure. The swollen functional protein on the surface of the nanofibers makes a smaller pore size and more steric hindrance by growing nanofibers. On the other hand, through emergence of the new functional groups upon conformational change, a higher chance of interaction between the

Figure 6. Separation of nanoparticles (40 nm) from water by the biofunctionalized PANGMA nanofibrous membranes (the left and right graphs are about the filtration performance of the neat and biofunctionalized ENMs, respectively). ENM, electrospun nanofibrous membranes; PANGMA, poly(acrylonitrile-co-glycidyl methacrylate).

Figure 7. Adsorption of nanoparticles (Au) to the surface of the biofunctionalized PANGMA nanofibers. PANGMA, poly(acrylonitrile-co-glycidyl methacrylate).

protein immobilized and the filtrates comes to existence. Strong protein–metal nanoparticle interactions, such as ionic interaction, hydrogen binding, Van der Waals and hydrophilic interactions,^{19–22} make Au nanoparticles stick to the biofunctionalized nanofiber. Combination of these effects reminds us the hunting behavior of a carnivorous plant and results in a significantly high retention efficiency for the membrane being studied.

Depending on the pH of the aqueous medium, different behaviors could be observed. When pH is lowered down to 4, the conformational change does not occur thereby capturing of the nanoparticles will be suppressed.

Figure 8. The plasmonic red color of the bionanohybrid at the naturated (pH 7 – left) and denaturated (pH 3 – right) states.

It is well known that under highly acidic conditions, that is, a pH below 4, the acidic moiety of the protein component is highly protonated, thereby the protein is denaturated. The secondary and tertiary structures of the protein are disrupted leading to an unfolding or straightening effect. These transitions bring about an increased local separation of the domains as the conversion of the compact heart shape of the protein to a linear bead form. Such an expansion could alter the plasmonic state of the nanoparticles stuck to the protein, thereby acting as signal transduction. In the ideal case, this transition should be reversible upon increase of pH, that is, a switchability in plasmonic behavior of the metal nanoparticles. To investigate this switchable plasmonic effect representative of denaturation/renaturation of the protein, the nanohybrid was immersed in an acidic aqueous solution (pH 3) and subsequently in a neuter aqueous solution (pH 7). As shown in Figure 8, while the plasmonic red color of the bionanohybrid after exposure to the acidic medium is drastically weakened, it is recovered as soon as the pH rises to 7 in a neuter aqueous medium.

The weak and intensive plasmonic red colors observed at different pH values represent denaturation and renaturation of the protein,

respectively. This color change occurs owing to coupling/decoupling between the nanoparticles induced by the interparticle distances. Figure 9 schematically illustrates this effect. While in the naturated state, a strong near field coupling between closely spaced nanoparticles occurs, in the denaturated state as the distance between the nanoparticles changes, the effect is diminished. This hypothesis is proved through UV-Vis measurements. Figure 10 shows the reflection spectra of the bionanocomposite in both the states (for a better clarity, only a portion of the full reflectance curves are shown). While the denaturated state is recognized by a single plasmonic dip located at 530 nm, it is obvious that, the intense red color of the naturated membrane shows a wide resonance with a plasmonic shoulder at 572 and a central dip at 523 nm, which is shifted to a lower wavelength compared with the denaturated one. This observations are in good agreement with the expectation from the plasmon hybridization of the individual nanoparticle plasmons.^{23,24} Five successive cycles of exposure of

Figure 9. The sketch illustrates plasmonic coupling between nanoparticles in the naturated state (pH 7) left and plasmonic decoupling for the denaturated one (pH 3), right.

Figure 10. The reflection spectra of the bionanocomposite in the naturated and denaturated states.

the bionanohybrid to the aqueous media with different pH values, verified optimum switchability of the plasmonic color and the spectral shift of the samples.

The promising results about the nanofluid filtration of nanoparticles as small as 40 nm encouraged us to evaluate the filtration potential of the biofunctionalized ENM for other kind of water pollutants. Therefore, filtration of a protein model such as BSA using the biofunctionalized ENM was performed. Owing to a macroporosity, the nanofibrous membranes can hardly retain soluble substances; however, this study showed that the biofunctionalized ENM is also able to separate a biomolecule such as BSA from water efficiently. As seen in Figure 11, the soluble protein of BSA is optimally separated from water. Surprisingly, after five cycles of continuous filtration, the protein rejection efficiency of the membrane reaches to almost 90%. This value is in contrast to that obtained by the nonfunctionalized ENM. The same principle as that in filtration of metal nanoparticles, that is, the “carnivorous plant” concept is applicable here as well.

The amazing filtration and smart plasmonics properties obtained through the adopted biofunctionalization approach made us optimistic about the high potentials of the carnivorous concept. This is why our research on the concept of biofunctionalized ENMs for filtration of main water pollutants and bridging the filtration area to other advanced technological domains such as plasmonics is going on.

4. Conclusion

Biofunctionalization of nanofibers was adopted as an efficient approach for not only optimization of structural properties including thermal and mechanical ones but also water filtration selectivity. The protein immobilization did not change the size of the nanofibers considerably and the porosity remained unchanged.

Figure 11. Protein filtration efficiency of the biofunctionalized ENM. ENM, electrospun nanofibrous membranes.

An operative filtration and selectivity to nanosized and soluble substances while possessing an extraordinary interconnected porosity for the nanofibrous membranes could be amazing potentials for the development of new generation of energy saving but efficient membranes. Moreover, decoration of the nanofibers by the functional filtrates such as metal nanoparticles could bring about a novel approach for the fabrication of smart bionanocomposite sensors, for example, for detection of protein denaturation.

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