Fabrication and anti-biofouling properties of alumina and zeolite nanoparticle embedded ultrafiltration membranes

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HIGHLIGHTS
- Alumina and LTL zeolite nanoparticles suited the in situ embedment method.
- The nanoparticle embedded membranes had higher hydrophilicity and filterability.
- Embedded nanoparticles exhibited anti-adhesion ability but no bacteriocidal function.
- Anti-adhesion ability was responsible for the anti-biofouling performance.

GRAPHICAL ABSTRACT

ABSTRACT
Nanoparticle embedded polysulfone ultrafiltration (UF) membranes were prepared by using the in situ embedment method, and the anti-biofouling properties of the prepared membranes were evaluated by conducting bacteria adhesion test, bacterium inactivation test and biofilm formation test separately. Among the several aluminum and/or silicon oxide nanoparticles tested, alumina (Al₂O₃) and Linda type L (LTL) zeolite nanoparticles were successfully embedded which could be evenly dispersed on membrane surface with high coverage ratio (38% and 49%, respectively) and were resistant to hydraulic shear detachment. The water contact angles for the nanoparticle embedded membranes (UF-Al₂O₃ and UF-LTL) and the control membrane (UF-C) were 57°, 40° and 66°, respectively. Owing to the higher surface hydrophilicity, both UF-Al₂O₃ and UF-LTL demonstrated a higher filterability than UF-C. Biofouling was inhibited on both UF-Al₂O₃ and UF-LTL, indicated by the lower Pseudomonas aeruginosa biofilm formation rate. Further investigation showed that both UF-Al₂O₃ and UF-LTL exhibited a high anti-adhesion efficiency to both Escherichia coli and P. aeruginosa, but no bacteriocidal effect on E. coli. The anti-biofouling ability of UF-Al₂O₃ and UF-LTL mainly benefited from the anti-adhesion ability attributed to the embedded nanoparticles. The improved anti-adhesion ability could not be simply explained by the enhanced hydrophilicity.

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1. Introduction
Low-pressure membrane filtration has experienced accelerated growth over the past decades in applications for drinking water production and wastewater treatment. Membrane fouling, however, restricts
its wider application [1]. Membrane fouling can be roughly divided into organic fouling, inorganic fouling and biological fouling (i.e., biofilm formation). Previous studies showed that membrane fouling rate is closely related with the hydrophilicity of the membrane. Under otherwise identical conditions, a more hydrophilic membrane is usually less susceptible to fouling. As such, a lot of efforts from both the industrial and scientific societies have been devoted to increase the membrane hydrophilicity. It was estimated that over 50% of the commercial low-pressure membranes were surface-modified [2], mainly for increasing hydrophilicity.

In general, membrane modification approaches can be grouped into four categories, i.e., blending additives, physical coating, chemical coating, and heterogeneous reactions [2,3]. Minor amount of hydrophilic additives such as polyvinylpyrrolidone (PVP) and polyethylene glycol (PEG) blended into the membrane cast solution seems to be the simplest modification method. Physical coating refers to the coating process by which a coating layer is formed but no chemical bonds exist between the coating layer and the base membrane. Due to its high hydrophilicity and good film-forming properties, polyvinyl alcohol (PVA) is often used in physical coating to improve membrane hydrophilicity forming a thin layer on the original surface [4]. Chemical coating, in contrast to physical coating, is a process by which a polymer layer is coated on membrane surface where chemical bonds form through “grafting to” or “grafting from” approaches. For instance, low-fouling UF membranes could be achieved by simultaneous photo-graft (UV irradiation) copolymerization of PEG methacrylate (PEGMA) onto membrane surface [5]. Heterogeneous reactions, including sulfonation, carboxylation, hydroxylation and physical activation by high energy radiation, plasma and UV irradiation, take including sulfonation, carboxylation, hydroxylation and physical activation by high energy radiation, plasma and UV irradiation, take place on the membrane surface and alter membrane surface properties usually not generating a coating layer. A synergy of different modification strategies broadens the dimensions to improve the hydrophilicity of the modified membranes.

With the development of nanotechnology, nanomaterials emerge up as a novel “functional material” in membrane modification area. The nanomaterials are either blended in the membrane matrix or coated on the membrane surface usually with the aid of tethering chemicals. Previous studies showed that inorganic nanoparticles, including alumina (Al2O3) [6–10], zinc oxide (ZnO) [11–14], silicon dioxide (SiO2) [15–17], titanium dioxide (TiO2) [18–20] and zeolites [21,22], could endow membranes with more hydrophilic surface. In addition, nanomaterials might be able to impart membranes with excellent anti-biofouling ability [23]. Silver nanoparticles are one of the most extensively investigated nanoparticles that might show the bacteriocidal ability [24,25]. The bacteriocidal ability of silver nanoparticle-modified membranes was primarily attributed to the release of silver ions from the membranes. Copper, long known for its bactericidal activity and relatively low cost, was also used for membrane modification [26,27]. In contrast, the antibacterial potential of TiO2 hybrid membranes is manifested when exposed to UV illumination. Recently, carbon nanotubes [28] and graphene oxide nanosheets [29,30] are the hotspots in membrane modification fields. It is reported that both nanomaterials could show antimicrobial abilities.

Except for silver and copper nanoparticles, the anti-biofouling properties of surface modified membranes by using other nanoparticles have seldom been investigated. The less expensive and more chemically stable nanoparticles such as alumina and zeolite might also exhibit anti-biofouling capability owing to the high specific surface area and some other unique but unknown physico-chemical properties [31]. In addition, our previous study [6] showed that alumina nanoparticles could be easily embedded onto polyvinylidene fluoride (PVDF) membrane surface simultaneously when the membrane was formed, by using the distinct in situ embedment method. The method is easy to apply, and the embedded particles were all exposed on the membrane surface likely by physical anchoring (rather than buried in the membrane matrix). It was expected that alumina and other similar nanoparticles (e.g., zeolite) can also be embedded onto membrane surface of other materials such as polysulfone (PSf). Both PVDF and PSf are the common polymers used for low-pressure membrane fabrications. The anti-biofouling properties were disintegrated into the anti-adhesion and bacteriocidal properties and evaluated by a number of different techniques such as bacteria adhesion test, bacterium inactivation test and biofilm formation test. The primary purpose of this study is to further elucidate the functions of the embedded nanoparticles on the membrane surface.

2. Materials and methods

2.1. Membrane fabrication

Each nanoparticle embedded ultrafiltration membrane was fabricated via the in situ embedment method [6], which is a slight modification of the most popularly adopted non-solvent induced phase separation method. The cast solution was prepared by dissolving 15 g of polysulfone (PSf) (average molecular weight (MW) ~ 22 kDa, Aldrich, USA) and 8 g of polyvinylpyrrolidone (PVP) (average MW ~ 10 kDa, Sigma-Aldrich, USA) into 77 g of anhydrous 1-methyl-2-pyrrolidinone (NMP) (Sigma-Aldrich, USA) under vigorous stirring at room temperature for 24 h. The air bubbles in the cast solution, if any, could be dissipated after about 12 h at room temperature. A thin film (200 μm) of the obtained cast solution was casted onto a flat glass plate (DF-GP-2B, Paul N. Gardner, USA) by using a doctor blade (AP-G06/10, Paul N. Gardner, USA). The film was exposed in the air for about 10 s before it was immersed into the water bath in which nanoparticles were uniformly dispersed (after sonication for 2 h) at a weight concentration of 500 mg/L. In this way, the nanoparticles were expected to spontaneously embed onto the membrane top surface. The temperature of the water bath was controlled at 35 °C. The membrane was removed from the water bath, thoroughly rinsed with Milli-Q water and stored in regularly replaced Milli-Q water (4 °C) prior to future tests. Nanoparticles tested in this study included alumina (Al2O3, ~50 nm according to the supplier, Aldrich, USA), three types of zeolite which were Linde type A zeolite (LTA, 100 nm, NanoScape AG, Germany), Linde type L zeolite (LTL, 80 nm, NanoScape AG, Germany), and Faujasite type X zeolite (FTX, 150 nm, NanoScape AG, Germany), silicon dioxide (SiO2, <50 nm, Aldrich, USA), and single wall carbon nanotube (SWNT, <50 nm, Aldrich, USA). The ultrafiltration membranes fabricated with nanoparticles embedded on membrane surface were assigned as UF-X, where X stood for the name or acronym of the nanoparticles (Al2O3, LTA, LTL, FTX, SiO2 and SWNT). The possible agglomeration of the nanoparticles in the water bath was evaluated by comparing the mean particle size in Milli-Q water and that provided by the suppliers. The particle size distribution (PSD) of the nanoparticles in water was determined by using a PSD analyzer (Delsa Nano Analyzer, Beckman Coulter, USA).

Control ultrafiltration membrane (with no nanoparticle embedded) was fabricated according to the same procedures described above, except that the water bath contained only Milli-Q water but no nanoparticles. The membrane was denoted as UF-C.

2.2. Membrane characterization

Membrane resistance (or filtrability) was determined by conducting dead-end filtration of Milli-Q water using a stirred filtration cell (Amicon 8050, Millipore, USA) operated at 0.1 MPa of applied pressure. The membrane coupons were first pre-compacted at 0.15 MPa for 30 min. The filtration flux (Jw) was obtained from the recorded filtrate weight as a function of the filtration time. The membrane resistance (Rm) was calculated according to the Darcy equation,

$$R_m = \frac{\Delta P}{J_w}$$

where ΔP is the applied pressure and J is the water viscosity.
The membrane surface hydrophilicity was indicated by water contact angle, which was measured by using a video-supported measuring instrument (OCA20, Dataphysics, Germany) based on the sessile-drop method [12]. Membranes were dehydrated in a desiccator with silica gel used as the desiccant prior to the measurement. A volume of 1 μL Milli-Q water was dropped onto the membrane surface and the Ellipse Fitting method was employed to fit the shape profile of the water drop so as to calculate the value of contact angle. For each membrane, more than 10 measurements were carried out at different spots and the average of all contact angle values was reported in this paper.

Morphologies of the top and bottom surfaces as well as the cross section of each membrane were observed under a scanning electron microscope (SEM) (Quanta 200F, FEI, USA) in high vacuum mode after sputter-coating with ~20 Å of Au/Pd (Precision Etching and Coating System 682, Gatan, USA). The coverage ratio of nanoparticles embedded on membrane surface was determined by analyzing the SEM images using the ImageJ software.

Atomic force microscopy (AFM, Veeco NanoScope 5, USA) was used to measure the membrane surface roughness. Related parameters (including Rq and Rz) were determined through the AFM analysis software (NanoScope Software 7.20, USA). Rq is the arithmetic average of the absolute values of the surface height deviations (Z) measured from the mean plane, while Rz is the standard deviations of the Z values.

The stability of nanoparticle membranes on membrane surface was evaluated by comparing the nanoparticle coverages before and after strong hydraulic shear applied to the membrane surface. The membrane was fixed in a stirred cultivation cell (Amicon 8200, Millipore, USA) in which 150 mL Milli-Q water was vigorously stirred for 2 d. The membrane coupons were observed under SEM (Quanta 200F, FEI, USA).

2.3. Anti-biofouling property evaluation

2.3.1. Model bacteria suspension

Two model bacteria species were used for the evaluation of anti-biofouling property of the different membranes. Escherichia coli (E. coli, ATCC 25922) was selected in that E. coli is among the most commonly found bacteria in natural water and wastewater, and Pseudomonas aeruginosa (P. aeruginosa, ATCC 27853) was chosen because it has been often reported to promote or accelerate membrane biofouling via biofilm formation [32,33].

Luria–Bertani (LB) liquid medium was used for the cultivation of both bacteria species. E. coli was cultivated in fresh LB medium at 37 °C for 3–4 h to the mid-log-growth phase (with an optical density value at 600 nm (OD600) of 0.5–0.6). The cultivated E. coli were separated from the LB medium by 5 min centrifugation (5000 rpm, 4 °C). The pellet was then re-suspended in PBS (phosphate buffered saline) solution (KH2PO4 0.24 g/L, Na2HPO4 1.44 g/L, NaCl 8.0 g/L, KCl 0.2 g/L). This centrifugation and re-suspension procedure was repeated for three times to remove any residual LB medium. The obtained medium-free E. coli suspension, with concentration approximately at 0.5 × 10^7 CFU/mL, is hereafter denoted as E. coli suspension. P. aeruginosa was cultivated with shaking (120 rpm) in LB medium at 37 °C for 12 h to reach the mid-log-growth phase (with an OD600 value of 2.0–3.0). The cultivated bacteria were also harvested by the centrifugation and re-suspension procedure that was repeated for three times. The medium-free P. aeruginosa suspension, with concentration approximately at 1.5 × 10^7 CFU/mL, is hereafter denoted as P. aeruginosa suspension. Both bacteria suspensions were prepared no more than 1 h prior to use.

2.3.2. Bacteria adhesion test

The adhesion test of model bacteria E. coli and P. aeruginosa onto the membrane surface was conducted by immersing the membrane specimen into the respective bacteria suspension. A piece of each membrane (approximately 15 × 40 mm²), which had been sterilized by UV radiation (40 W, 253.7 nm) for 30 min, was placed into a test tube containing 10 mL bacteria suspension and incubated at 37 °C for 12 h. The membrane specimen was then step-dehydrated with 25%, 50%, 75% and 100% ethanol, respectively. It was ready for SEM observation after an overnight air-drying. Bacteria coverage on the membrane surface, expressed as cell density, was determined by counting cell numbers per unit area. The bacteria coverages on the control membrane and on the nanoparticle embedded membranes were compared to evaluate the anti-adhesion property of the nanoparticle embedded membranes [32].

2.3.3. Bacterium inactivation test

The bacterium inactivation test was conducted by using E. coli. The E. coli suspension was diluted with PBS to the concentration approximately at 50 CFU/mL. A volume of the diluted suspension (10 mL) was filtered onto the membrane surface, which was then transferred into a petri dish in which 15 mL chromogenic solid medium for E. coli (CHROMAgar™ ECC) had been added. After incubation at 37 °C for 24 h, the blue colonies grown on membrane surface (about 1 mm in diameter) were counted. The colony number on nanoparticle embedded membranes was compared with that on the control membrane to indicate the possible bacterioidal ability of the embedded nanoparticles. Four replications were performed.

2.3.4. Biofilm formation test

The rate of biofilm formation on membrane surface was evaluated using P. aeruginosa [34]. Each membrane specimen, approximately 10 × 10 mm² in size, was affixed to a glass slide with the top surface facing up, which was immersed into the P. aeruginosa suspension (approximately at 1.5 × 10^9 CFU/mL) that was then incubated at 37 °C for 24 h or 72 h. During the 72 h incubation, P. aeruginosa suspension was replaced every 24 h to maintain a high concentration of bacteria. Following the gentle rinse with PBS, the membrane sample was stained with SYTO9® green fluorescent nucleic acid dye (LIVE/DEAD® Biofilm Viability Kit, Invitrogen, Ltd., UK) for 15 min and then washed by PBS for three times to remove any remaining dye. The formed biofilm, if any, was observed under a confocal laser scanning microscope (CLSM, LSM 710, Zeiss, Germany). Images were taken from at least five randomly selected areas of each membrane. The average thickness of the P. aeruginosa biofilm on the nanoparticle embedded membrane was compared with that on the control membrane to indicate the anti-biofouling ability of the nanoparticle embedded membrane.

In addition to CLSM, the crystal violet staining method [35] could also be used for the quantification of the P. aeruginosa biofilm formation on the various membranes. According to the method, the formed biofilm was stained by crystal violet, which was then extracted by ethanol/acetone mixture followed by absorbance determination at 595 nm. The method was widely applied to the evaluation of the antimicrobial activity of many new (nano)materials [35]. In our case, however, the membrane material (primarily PSf) could also be strongly stained by crystal violet, which greatly impaired the quantification accuracy especially when the biofilm thickness was low. It was therefore not possible to supplement the CLSM method by using the crystal violet staining method.

3. Results and discussion

3.1. In situ embedment of nanoparticles

The in situ embedment approach was employed for fabrication of nanoparticle embedded ultrafiltration membranes. Among the several types of nanoparticles tested in this study, only Al2O3 and LTL zeolite...
suited the approach most. A first requisite when applying the approach is that the nanoparticles must be well dispersed in the water bath (i.e., coagulation bath for phase separation). Probably due to the high hydrophobicity, SWNT could not be dispersed well in water. Though SiO$_2$ nanoparticles would not settle from the water in a couple hours, particle size distribution (PSD) measurement result showed that they agglomerated to a high extent; the measured mean particle size was 1191 nm, much larger than that for the primary nanoparticles (<50 nm). The remaining four types of nanoparticles were very stably dispersed in the water (some of which would not settle in a few months). Nevertheless, PSD measurements indicated that these nanoparticles might slightly aggregate in the water. The measured mean particle sizes for Al$_2$O$_3$ and LTA, LTL and FTX zeolite were 302, 390, 211 and 340 nm, respectively, 2–6 times larger than that for the primary nanoparticles specified by the suppliers. It appears that the spontaneous aggregation of nanoparticles in water is fairly common, which might be driven by the long-range surface–surface interactions (e.g., van der Waals attraction, electrostatic interaction and acid–base interaction), hydrogen bonding and other forces. The rate of aggregation however differed substantially among the nanoparticles. In this study, sonication was applied to disperse the nanoparticles in the water bath just before cast film immersion.

The second requisite when applying the approach is that the nanoparticles in the water bath could transport to the cast solution film surface and be tightly attached when the polymer (PSf in this study) was solidified. SEM observation showed that all four types of nanoparticles which dispersed well in water could be embedded to the PSf membrane surface. However, the nanoparticle coverage ratios differed substantially among the different nanoparticles, although the nanoparticle concentrations in the water bath were all 500 mg/L. In comparison, Al$_2$O$_3$ (Fig. 1a) and LTL zeolite (Fig. 1c) nanoparticles showed excellent dispersion on membrane surface with fairly high coverage ratio at 38% and

Fig. 1. SEM images of ultrafiltration membranes in situ embedded with (a) Al$_2$O$_3$, (b) LTA, (c) LTL and (d) FTX nanoparticles, and (e) UF-Al$_2$O$_3$ and (f) UF-LTL membranes after being subjected to 2 d of hydraulic shear.
49%, respectively, while LTA zeolite (Fig. 1b) and FTX zeolite (Fig. 1d) nanoparticles poorly distributed and the coverage ratios were only 4.7% and 6.4%, respectively. In our previous study [6], γ-alumina was successfully embedded to PVDF membrane surface. It was argued that the free energy change for the nanoparticle attachment determined whether or not the nanoparticles could be embedded. A negative free energy change was favorable. However, the surface tension and its components could not be accurately determined for any type of the zeolite nanoparticles in this study. It was possibly due to the porous structure of zeolite. Therefore, the exact underlying reason for that LTL zeolite nanoparticles differed from the other two zeolite nanoparticles could not be proposed. Although theoretical analysis could not be made, this study experimentally showed that alumina and LTL zeolite nanoparticles were suitable for PSf membrane surface modification by using the in situ embedment approach.

The stability of the embedded nanoparticles on the membrane surface was assessed by exposing the membrane to hydraulic shear force generated by magnetic stirring of the above water in a filtration cell. Results (Fig. 1e and f) showed that no discernable nanoparticle detachment was observed after a duration of 2 d. It was argued that most of the nanoparticles were physically anchored into the membrane matrix and as such they were resistant to hydraulic shear force.

3.2. Membrane characterization

Ultrafiltration membranes were expected to form under the conditions adopted for membrane fabrication (cast solution recipe, film thickness, water bath temperature, etc.). Based on the SEM images, the membrane pore size and thickness for UF-C were determined to be 30 nm and 180 μm, respectively. The membrane resistance to filtration was determined to be 1.3 × 10¹² m⁻¹ (Fig. 2). The water droplet contact angle was measured to be 66°, indicating that the membrane was fairly hydrophilic. It was due to the blending of PVP as hydrophilic additive in the cast solution. Three-dimensional AFM images of the membrane top surface (Fig. 3) showed that the membrane had a fairly smooth surface, with Ra and Rq values being 15.4 ± 3.0 and 19.0 ± 3.6 nm, respectively.

Embedment of nanoparticles could substantially increase the hydrophilicity of the membranes. The water droplet contact angles for UF-Al₂O₃ and UF-LTL were measured to be 57° and 40°, respectively (Fig. 2). The higher hydrophilicity for UF-LTL was primarily due to the higher coverage ratio of LTL zeolite nanoparticles on the membrane surface (see above). Embedment of nanoparticles did not change the membrane thickness and morphology significantly (SEM images not shown). As SEM observation revealed, it might block the membrane pores to some extent. However, contrary to as expected, the membrane resistances for UF-Al₂O₃ and UF-LTL were determined to be 9.1 × 10¹¹ and 9.4 × 10¹¹ m⁻¹, respectively (Fig. 2), both of which were less than that for UF-C. It indicates that the embedded particles might not actually block the membrane pores for water passage. In addition, membrane resistance is determined not only by membrane pore size and membrane structure (i.e., microvoids and macrovoids) but also by membrane hydrophilicity [21]. The increased permeability of nanoparticle embedded membranes could be attributed to the enhanced membrane hydrophilicity.

In addition to increasing membrane permeability, hydrophilicity is one of the most important properties of membranes that determine the antifouling ability [16,36,37]. A number of previous studies have attempted to increase membrane hydrophilicity by doping nanoparticles mostly through the blending additive method. For example, Liao et al. [38] blended with NaY zeolite particles and Ag⁺ exchanged NaY zeolite particles at a concentration of 0.8 wt.%, which resulted in the contact angle value dropping from 91° to 82°. Leo et al. [21] illustrated that by increasing the concentration of SAPO-44 zeolites from 5 wt.% to 15 wt.%, the contact angle value decreased from 70.6° to 64.5°. It also enhanced the antifouling ability by reducing the adsorption of humic acid in membrane pores and thus alleviating fouling initiated by pore blocking. Compared to the blending method adopted in previous studies [9,39,40], the in situ embedment approach adopted in this study demonstrated more potential in the improvement of membrane hydrophilicity (especially when LTL zeolite nanoparticles are used).

The surface roughness of the nanoparticle embedded membranes was also measured from the AFM images (Fig. 3). The Ra and Rq values for UF-Al₂O₃ were 85.3 ± 11.7 and 111.4 ± 18.6 nm, respectively, while those for UF-LTL were 29.8 ± 6.6 and 37.4 ± 7.6 nm, respectively. It revealed that the embedment of nanoparticles would lead to a more rough membrane surface. It was as expected because the nanoparticles were embedded onto rather than buried into the membrane polymer matrix. Noted is that the particle size for the primary Al₂O₃ and LTL zeolite nanoparticles was <50 and 80 nm, respectively. The higher surface roughness of UF-Al₂O₃ was due to the agglomeration of the Al₂O₃ nanoparticles before they were embedded to the membrane surface (see the above measured particle size in water). It appears that agglomeration of the LTL zeolite particles was minimal. Noted is that in addition to surface roughness, surface rigidity and elasticity could be determined by using AFM as well [41]. However, only surface roughness was measured because it is more related to membrane fouling. Though the membrane surface rigidity and elasticity could be changed due to the embedment of nanoparticles, the effect was however not considered in this study.

3.3. Anti-adhesion property of the membranes

The anti-adhesion property was assessed by comparing the coverage ratios of model bacteria on the nanoparticle embedded membranes with that on the control membrane (UF-C). The attachment of model bacteria E. coli and P. aeruginosa on membrane surface were observed under SEM.

It was found that both E. coli and P. aeruginosa could easily adhere onto the control membrane (UF-C) (Fig. 4a and d). After a 12 h contact with each bacterium suspension, the membrane surface was almost fully covered by the bacteria cells (ca. 2.4 × 10¹¹ cells/m² of E. coli and 5.4 × 10¹¹ cells/m² of P. aeruginosa) (Fig. 5). Embedment of nanoparticles could greatly reduce the adhesion of both types of bacteria onto the membrane surface (Fig. 4b, c, e and f), demonstrated by the results that the coverage ratios of either E. coli or P. aeruginosa cells on UF-Al₂O₃ and UF-LTL were substantially less than that on UF-C. If we define an anti-adhesion efficiency as the reduced percentage of the bacteria coverage rate compared with the control membrane, the UF-Al₂O₃ membrane exhibited an anti-adhesion efficiency of 78% to E. coli and 94% to P. aeruginosa, while UF-LTL membrane manifested an anti-adhesion efficiency of 61% to E. coli and 97% to P. aeruginosa (Fig. 5). Though it may
not be appropriate to simply compare the anti-adhesion efficiencies, the above results showed that Al₂O₃ and LTL zeolite nanoparticles had similar anti-adhesion ability to both E. coli and P. aeruginosa. The improved anti-adhesion ability could be attributed to the increased membrane surface hydrophilicity due to nanoparticle embedment. A number of previous studies [33,42–44] have reported a clear correlation between the increase in hydrophilicity and corresponding decrease of bacteria attachment. Noted is that the control membrane was hydrophilic in nature. It was thus argued that the attachment/adhesion of bacteria on the membrane surface was a gradual process. The membrane surface property could be modified by the bacteria and their secreted matter. It could be expected that the nanoparticle embedded membranes would be fully covered by the bacteria if a longer contact time was allowed. It was demonstrated by using P. aeruginosa (see below). However, hydrophilicity only might not explain the enhanced anti-adhesion ability of the nanoparticle embedded membranes, considering that the anti-adhesion efficiencies were similar but the water droplet contact angles differed substantially between UF-Al₂O₃ and UF-LTL.

3.4. Bacteriocidal property of the membranes

It was proposed that nanoparticle may have bacteriocidal ability by physically disrupting the bacterial cell wall due to its extremely small particle size and high surface tension energy [45]. The bacteriocidal property of the embedded nanoparticles was evaluated by comparing the colony numbers formed on different membranes. It was hypothesized that if a bacterial cell was inactivated by its contacted nanoparticle(s), the bacterial cell could not develop into a colony in the subsequent cultivation step. E. coli was used for the assessment. Results (Figs. 6 and 7) showed that E. coli could grow on either UF-Al₂O₃ or UF-LTL as well as on the control membrane (UF-C). A total of four replicates were conducted, and the normalized survival rate was calculated by dividing the colony numbers on the nanoparticle embedded membrane by that on the control membrane which was set as a control for every filtration process. The normalized survival rates were both equal to 100%, indicating that the embedded nanoparticles had no bacteriocidal ability.

3.5. Anti-biofouling property of the membranes

Biofouling is initiated by the adhesion of bacteria on the membrane surface followed by the development into a biofilm. The anti-biofouling property of the membranes was assessed by comparing P. aeruginosa biofilm formation rates on the different membranes. The membrane specimens were immersed in P. aeruginosa suspension at 37 °C for up to 72 h to allow biofilm formation. The P. aeruginosa biofilm was stained and then observed under CLSM. As shown in Figs. 8 and 9, P. aeruginosa could easily develop into a biofilm on the control membrane; the biofilm thickness was measured to be 34.0 μm after incubation for 24 h. In contrast, the biofilm formation on either UF-Al₂O₃ or UF-LTL was substantially inhibited; the biofilm thicknesses were 3.7 and 8.5 μm, respectively. When the incubation was extended to 72 h (during which the P. aeruginosa suspension was replaced every 24 h), the biofilm thickness on the control membrane was further increased to 55.8 μm, while the biofilm thickness on UF-Al₂O₃ and UF-LTL was kept almost unchanged at 6.3 and 5.8 μm, respectively. In accordance with the fact that either...
Al₂O₃ or LTL zeolite nanoparticles had no bacteriocidal ability, the embedment of these nanoparticles could not prevent the formation of *P. aeruginosa* biofilm (i.e., biofouling). The inhibited biofilm formation on the nanoparticle embedded membranes was mostly due to the enhanced anti-adhesion ability caused by the embedment of nanoparticles. In actuality, improvement of anti-adhesion ability which could slow down the initial attachment of bacteria on membrane surface might be a more effective approach to reducing biofouling than using bacteriocidal agents which aim at killing/inactivating bacteria already attached on membrane surface. The dead or inactivated bacteria may serve as anchors for the next layer of bacteria, promoting further development of biofouling. Although the biofilm thickness on UF-LTL was slightly higher than that on UF-Al₂O₃, it may not be appropriate to conclude that Al₂O₃ nanoparticles had a better anti-biofouling performance than LTL zeolite nanoparticles. The results described earlier showed that the two types of nanoparticles had similar anti-adhesion ability.

### 4. Conclusions

The in situ embedment approach was employed for nanoparticle embedded ultrafiltration (UF) membrane fabrication, and the anti-biofouling ability of the fabricated membranes was then assessed. Results showed that Al₂O₃ and LTL zeolite nanoparticles could be successfully embedded with high surface coverage (38% and 49%, respectively), which were fairly resistant to hydraulic shear detachment. Water contact angles for UF-Al₂O₃ and UF-LTL were measured as 57° and 40°, respectively, significantly lower than that for the control membrane (UF-C) (66°). UF-Al₂O₃ and UF-LTL had lower intrinsic membrane resistance than UF-C, which was attributed to the higher membrane surface.
hydrophilicity. However, the membrane surface roughness was higher due to the embedded nanoparticles. UF-Al2O3 and UF-LTL exhibited similarly high anti-adhesion efficiency to either E. coli or P. aeruginosa. The adhesion of E. coli could be reduced by 78% and 61%, respectively, while the adhesion of P. aeruginosa could be reduced by 94% and 97%. In contrast, the embedded Al2O3 and LTL nanoparticles showed no bactericidal effect on E. coli. Nevertheless, embedment of nanoparticles could inhibit biofouling development, demonstrated by the results that the biofilm thickness of P. aeruginosa grown on UF-Al2O3 and UF-LTL membranes was much less than that on UF-C. It is concluded that the enhanced anti-biofouling ability of nanoparticle embedded membranes mainly benefited from the anti-adhesion ability attributed to the embedded nanoparticles. The improved ability might not be simply due to the enhanced hydrophilicity, given that the water contact angles for UF-Al2O3 and UF-LTL were far from identical.

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