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A two-step bioluminescence assay for optimizing antibacterial coating of hollow-fiber membranes with polydopamine in an integrative approach

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ABSTRACT

Pure-water filtration membranes are often fouled by bacterial biofilms. Antibacterial coatings for preventing biofilm formation on such membranes should not rely on leaching of inhibiting compounds but should only be effective on surface contact. Certified assays for antibacterial coatings do not sufficiently exclude leaching effects and involve nutrient-rich cultivation media that do not correspond to conditions in pure-water systems. In this study, a two-step bioluminescence assay was developed for optimizing an antibacterial coating of PES/PVP ultrafiltration hollow-fiber membranes with a polydopamine as a sustainable, bio-inspired material for preventing bacterial biofilm formation. In the first step, leaching of the antimicrobial coating was analyzed by a bioluminescence assay with supernatants generated by washing coated membranes. In the second step, bioluminescence of bacterial biofilms on coated and uncoated membranes was measured using a nutrient-poor medium resembling site-specific conditions. Based on this bioluminescence assay, an optimized protocol for the coating process could be established by acidic polymerization of dopamine using 2 g/L sodium periodate and 4 g/L dopamine at 40 °C for 20 min reaction time. With coatings produced in this way, bioluminescence was reduced on coated membranes only while the corresponding supernatants exhibited no inhibitory effects.

1. Introduction

Membranes in technical pure- and potable water settings are regularly subject to bacterial biofilm formation (Vrouwenvelder et al., 1998; Xiong and Liu, 2010). The colonization of filtration membranes used for water purification or retention of microorganisms can lead to plugging of the membranes and subsequent loss of permeability; biofilms on membranes in medical applications can cause severe hygienical problems such as nosocomial infections. The use of antimicrobial agents or coatings for such membranes can therefore be a reasonable strategy to prolong the lifetime of such membrane filtration systems and to prevent biofilm-related outbreaks of pathogenic bacteria. As in many antimicrobial coatings the inhibitory effect is based on the release of antibacterial substances (Adlhart et al., 2018), which causes environmental pollution, there is a clear need for more sustainable solutions (Batista-Andrade et al., 2016). Especially in filtration systems for drinking water and beverages, in which the products are aimed for consumption by humans, intrinsically antimicrobial surfaces without the release of compounds are clearly required. Apart from copper-based materials (Grass et al., 2011), other coatings aim to avoid bacterial adhesion by physicochemical properties, like superhydrophobic surfaces (Cheng et al., 2019) or the use of polyethylene glycol coating which provides a steric barrier (Swartjes et al., 2015).

Polydopamine (PDA) is a bio-inspired synthetic polymer that shows antibacterial activity (Kasemset et al., 2013). PDA is prepared by the pH-induced oxidative polymerization of dopamine (DOPA, 3,4-dihydroxy-*L*-phenylalanine) under alkaline conditions (Lee et al., 2007). It was reported that a simple dip-coating of an object in an aqueous solution of DOPA led to the formation of a thin and surface-adherent PDA film on a large variety of inorganic and organic surfaces. The polymerization of DOPA often is called 'mussel-inspired', since DOPA is found to be responsible for the strong underwater adhesion of mussels to wood and

Abbreviations: DOPA, Dopamine; PES, polyether sulfone; PVP, polyvinyl pyrrolidone; FESEM, Field Emission Scanning Electron Microscopy; PDA, Polydopamine.

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stones, even under high water flow shear stresses (Ding et al., 2016). In recent years, PDA has been used for membrane modification, either to increase the hydrophilicity of membranes for water- and wastewater treatment, as well as to increase their antifouling and antibiofouling properties (Yan et al., 2020). PDA modifications are reported for all types of pressure-driven membranes used in water and wastewater treatment: microfiltration, ultrafiltration, nanofiltration, reverse osmosis and forward osmosis. Parameters that can be used to fine-tune and determine the properties of the PDA coatings include the DOPA concentration, pH, coating time, the applied oxidant, temperature and the substrate it is applied on (Yang et al., 2018). To meet all demands for the desired coating properties, many studies report the application of PDA just as a linker for attachment of a second component onto the membrane surface, referred to as co-deposition (Yang et al., 2018).

Previous research has not unambiguously shown why PDA coatings are antibacterial. The hydrophilicity of PDA coatings is thought to play a role, as well as the surface roughness and the surface charge. However, Karkhanechi et al. found no significant increase in hydrophilicity and negative charge after a PDA layer was applied on a reverse osmosis membrane and concluded that the presence of protonated PDA amine groups on the surface caused the antibacterial effect (Karkhanechi et al., 2014). For the evaluation of antimicrobial efficiencies of newly developed surfaces, standard tests like the "*Japanese* industrial standard" JIS Z 2801 exist (Japanese Standards Association, 2010). However, whereas the standardization of tests can help providing methods easy to exert and replicate, it also leads to an oversimplification and potential misinterpretation of the real setting in which a surface will be finally implemented. Regarding tap water filtration, the use of a complex medium like in the JIS Z 2801 tests is very different from the nutritional conditions at the respective technical settings. In addition, the counting of viable cells as colony forming units (CFUs) after a certain exposure time, like it is also done in the JIS Z 2801 tests (Japanese Standards Association, 2010), does not only target bacterial cells within the biofilm but also in the suspended cells from the supernatant. To this end, an inhibitory effect revealed by this certified assay might strongly rely on the release of antimicrobial compounds from the coating rather than on the intended inhibition upon surface contact. The ASTM E2149 assay also uses the counting of CFUs from a liquid medium surrounding putative antimicrobial material (ASTM International, 2020). Both Assays, the JIS Z 2801 and the ASTM E2149, but also several other standard assays as well are known not to discriminate between leaching and contact killing and always need additional assays (van de Lagemaat et al., 2017). Apparently, for avoiding potential misinterpretations there is a need for alternative assays for identifying antimicrobial coatings that act only upon surface contact and do not cause environmental pollution by leaching.

A suitable method for evaluating negative effects of putative antimicrobials on bacteria is measuring bacterial bioluminescence. As biologic light production is dependent on cellular energy levels, inhibitory effects, such as a disruption of the cellular integrity, result in a loss of bioluminescence (Girotti et al., 2008). This phenomenon is the functional principle of the ISO 11348 test, in which water samples are added to cultures of the marine bioluminescent bacterium *Aliivibrio fischeri* to test for the presence of inhibitory compounds (International Organization for Standardization, 2007). This assay serves for detecting contaminations harboring a risk for eukaryotic organisms, but as the monitored effect is based on a negative effect on the bacterium, this strategy is also suited for tests of antimicrobial effects. However, the use of the *A. fischeri* excludes its use for settings in which marine growth conditions cannot be applied. Recombinant bacteria expressing heterologous luminescence genes constitutively can help to overcome this limitation and are known to be suited for finding inhibitory effects, too (Kurvet et al., 2011), (Jagmann et al., 2015), (Motealleh et al., 2020). The recombinant bioluminescent bacteria in combination with a nutrient-poor incubation medium for testing of newly-developed coatings for pure-water environments could therefore provide a more realistic assay, in which the metabolic state of the bacteria on the antibacterial surface can be directly visualized. Such an ISO 11348-derived assay would also allow to detect the leaching of inhibitory compounds from the coating into the surrounding medium.

2. Materials and methods

2.1. Chemicals

Dopamine.HCl was purchased from Sigma Aldrich and used as received. 15 mM Tris buffer at pH 8.8 was prepared by dissolving Trizma® (Sigma Aldrich) in water and adapting the pH to 8.8 using diluted NaOH. All other chemicals were of analytical grade and purchased from Carl Roth (Carl Roth GmbH+Co Kg., Karlsruhe, Germany).

2.2. Coating of the membranes

PDA coating experiments were carried out on hollow-fiber microfiltration membranes, kindly provided by Pentair X-Flow (Enschede, the Netherlands). The PES/PVP based hollow fiber membranes had an inner diameter of 0.7 mm and an outer diameter of 0.9 mm. According to field emission scanning electron microscopic (FESEM) analysis, the diameter of the pores at the outside (shell) was app. 1.5–3.5 μm while the inside surface had pores of app. 0.2 μm diameter. For membrane modification purposes, the fibers were cut into 4–12 cm length parts prior to coating. After washing with ethanol and drying the fibers were closed at both ends by hot melting.

Both auto-oxidation (with dissolved oxygen) as well as chemical oxidation (using e.g. sodium periodate or potassium permanganate as oxidant) have been investigated extensively for the application of PDA coatings, with different thicknesses, homogeneities and reaction times as result (Ryu et al., 2018). Thick and homogeneous PDA layers were formed at much shorter reaction times under acidic conditions than under alkaline conditions (Ponzio et al., 2016). Roughened PDA coatings could be applied to different substrates when shaking the solution during the application (Su et al., 2016). For coating of the hollow fiber membranes, an adaptation of the previously reported methods was used. An acidic method using an oxidant was applied, which was carried out either static or under mechanical shaking ('shaking method'). The shaking method was carried out in a mechanical shaker. For this procedure, fibers of 4–12 cm length were closed at both ends (using hot melt polyethylene glycol (PE)), placed in a beaker glass with the dopamine/buffer/$NaIO_4$ solution at pH 5.0 (2 g/L dopamine, 2 g/L or 4 g/L $NaIO_4$), and shaken (250 shakes/min) for 2.5–60 min. Reaction temperature initially was 21 °C, to speed up the process later on reaction temperatures of 40 °C and 60 °C were tested. In the results section, the exact PDA coating conditions always are given.

2.3. Characterization of the membranes

The first check for a successful PDA coating process was a visual inspection of the fiber. The more PDA was coated on the fiber surface, the darker brown the fiber became. Then, Field Emission Scanning Electron Microscopy (FESEM) was used to examine the structure of the deposited PDA on the membrane (pore) surface. Hollow fiber samples were mounted onto regular FESEM holders, coated with 5 nm of chromium, and examined using a Jeol JSM-7610F FESEM at 1 kV accelerating voltage. Water fluxes of the (un)modified fibers were determined to elucidate the effect of the PDA coating on the water flux. Fibers with a length of approximately 10 cm were tested in outside-in mode using demineralized water as feed at a transmembrane pressure of 0.1 bar.

2.4. Preparation of the membrane samples for bioluminescence testing

Three replicates of membrane pieces of a length of 4 cm were fixed to the bottom of a petri dish by melting the hot-melt PE ends with the

polystyrene surface. The samples fixed in the petri dishes were sterilized by irradiation with a Philips TUV 30 W/G30T8 UV lamp for 10 min from each side at a distance of about 50 cm.

2.5. Bacterial strains and media

Assays were performed with *Escherichia coli* MG1655 pBBR1MCS-2::*luxCDABE* (Motealleh et al., 2020). A modified M9 medium (Klebensberger et al., 2006) was used for precultures. The incubation medium for leaching test and biofilm formation on membranes was composed of ultrapure water (Puranity PU 15, VWR International, Leuven, Belgium) supplemented with 1% M9 medium, 2.5 mM sodium bicarbonate, 0.025 g/L yeast extract and 0.05 g/L tryptone.

2.6. Leaching assay

For obtaining samples with potentially leached compounds from PDA-coatings, 30 mL incubation medium was added to a petri dish with a coated membrane-piece mounted and incubated for 24 h with shaking at 100 rpm and 30 °C in the Minitro incubator (Infors AG, Bottmingen, Switzerland). Three times 1 mL of supernatants from these leaching treatments was transferred to wells of a standard polystyrene TC-24 well microtiter plate (Sarstedt AG and Co. KG, Nümbrecht, Germany). *E. coli* MG1655 [pBBRMCS-4::*luxCDABE*] was grown as overnight culture in M9 minimal medium with 100 µg/mL ampicillin and 20 mM glucose. Growth was determined by measuring optical density at 600 nm (OD_{600}) with the UV-mini 1240 photometer (Shimadzu, Kyōto, Japan), and the bacterial culture according to OD_{600} = 0.1 was added to the 1 mL samples from the leaching treatment. As control, the bacterial culture was added to 1 mL fresh incubation medium that has not had contact to membrane samples. If bioluminescence was altered significantly after 1 h of incubation, the leaching treatment was repeated with fresh medium, until no more reduction of bioluminescence was detected.

2.7. Biofilm assay

E. coli MG1655 [pBBRMCS-4::*luxCDABE*] was grown as described above. Cell material according to OD_{600} = 0.01 was washed once by centrifuging at 10,000 *x g* for 2 min, resuspending in fresh medium and centrifuging again. After resuspension in fresh medium the cell material was added to the petri dishes containing the membrane samples in 30 mL incubation medium. The samples were incubated with shaking at 100 rpm and 30 °C for 24 h in the Minitro incubator (Infors AG, Bottmingen, Switzerland) and washed twice with 30 mL sterile incubation medium before quantifying the bioluminescence on the surface by the Bio-Rad ChemiDoc imaging system using the Bio-Rad ImageLab software (Bio-Rad Laboratories, Hercules, California, USA; Fig. 1A and D). For ensuring that bioluminescence of the whole membrane area is used for quantification the membranes first were visualized with white light pictures (Fig. 1B) and marked (Fig. 1C). These marks were overlaid with the bioluminescence picture and used to record the bioluminescence within the covered area (Fig. 1D) Bioluminescence was calculated as signal/mm^2 *exposure time [s].

3. Results

3.1. Optimization of the different PDA coating procedures

A combination of an acidic coating using an oxidant under ultrasonic agitation or mechanical shaking conditions at room temperature was applied. Four oxidants were tested for coating the hollow fibers: $NaIO_4$, H_2O_2, $Na_2S_2O_8$ and $2Na_2CO_3.3H_2O_2$., all at 20 mM concentration and 2

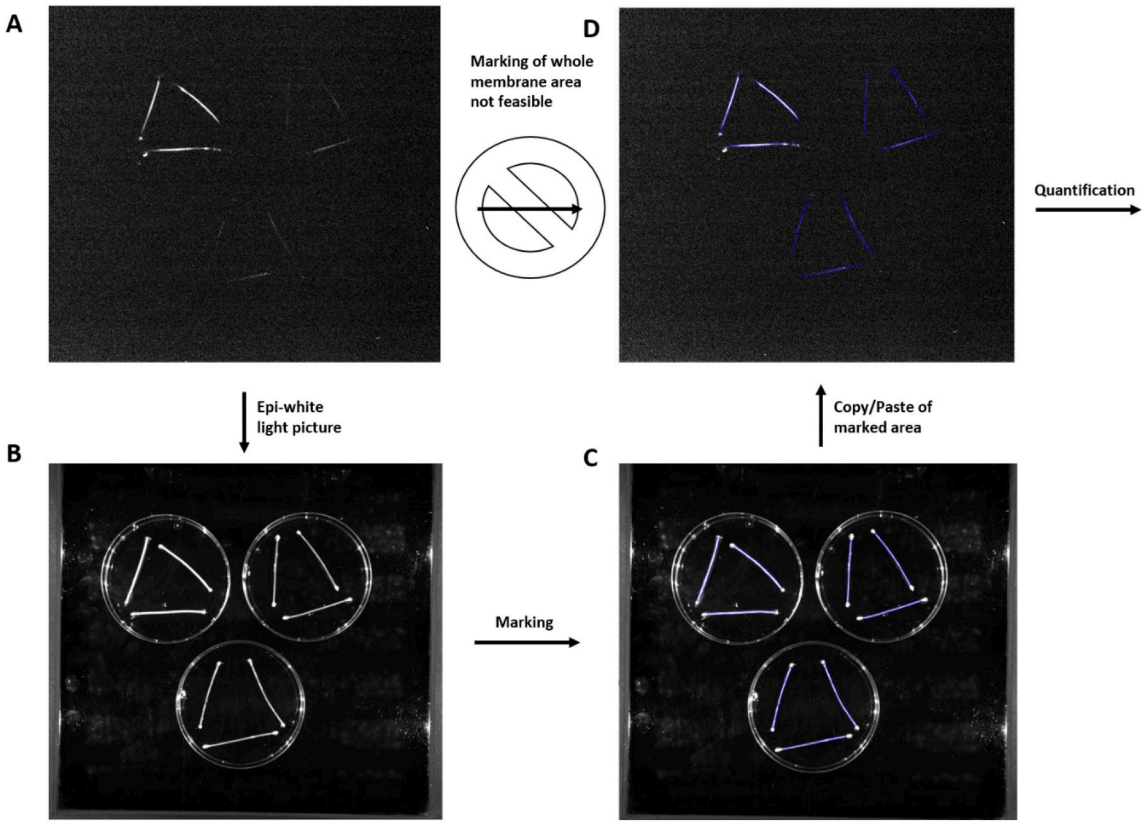

Fig. 1. Illustration of the procedure for membrane marking. Since the whole membrane area is not visible at low bioluminescence levels, epi-white light pictures were taken in the same position as the bioluminescence pictures. The membranes were marked, and these marks were overlaid with the bioluminescence pictures for quantifying the signal intensity within this area.

g/L (10.5 mM) PDA. Out of these 4, a preliminary set of experiments at fixed reaction times showed that $NaIO_4$ appeared to be the best oxidant: it resulted in the thickest PDA layer on the fiber when the brownish colors were compared. This is in agreement with the findings of Ponzio et al. (2016). For this reason, further work on the PDA coating was only carried out using $NaIO_4$ as oxidant. Subsequently, the influence of the reaction time on the coating for the acetate/$NaIO_4$ system using mechanical shaking was investigated. A visual inspection of the fibers (Fig. 2) showed a color change from white (starting fiber) via light brown (30 min) to dark brown (60 min) for the conditions 2 g/L DOPA/50 mM acetate buffer (pH 5.0)/4 g/L $NaIO_4$, clearly showing the reaction time influences the amount of PDA on the fiber.

FESEM examination (Fig. 3) clearly indicated a microstructured deposition of PDA on the membrane and a porous surface (compare Fig. 3A with 3B and 3C). Unlike static deposition, which resulted in a smooth layer of PDA on the surface, application of mechanical shaking generated a much rougher surface. It is reported in literature that a rough surface can be beneficial for the antimicrobial activity of PDA since surface roughing increases the contact between bacteria and PDA, which might enhance its killing efficiency (Su et al., 2016).

Another coating parameter is the reaction temperature. Tests were carried out using the 'mechanical shaking' acidic $NaIO_4$ system, but now temperature was increased from room temperature (21 °C) to 40 °C and 60 °C. At the same time, also a range of reaction times at these temperatures was investigated. Visual analysis of the brownish color of the fibers after coating revealed that an increase in temperature accelerated the reaction. At 40 °C, a reaction time of 10–20 min was sufficient to obtain a maximum in brown intensity, while for 60 °C this time was even shorter (below 10 min). However, FESEM analysis revealed that at 60 °C the layer became smoother again (not the microstructured morphology typical for the room temperature shaken coating) which might result in loss of antimicrobial activity.

An important parameter for membranes used in water- and wastewater treatment is their water flux. If the PDA layers significantly lower the water flux, more membrane area needs to be installed to obtain the same treatment capacity. For that reason, water fluxes were measured for the microfiltration hollow fibers before and after coating with PDA via the acidic $NaIO_4$ route. The PDA coating did not affect the water flux: both the bare fiber as well as the PDA fiber showed pure water permeabilities of around 12,000–14,000 L/m^2 h bar.

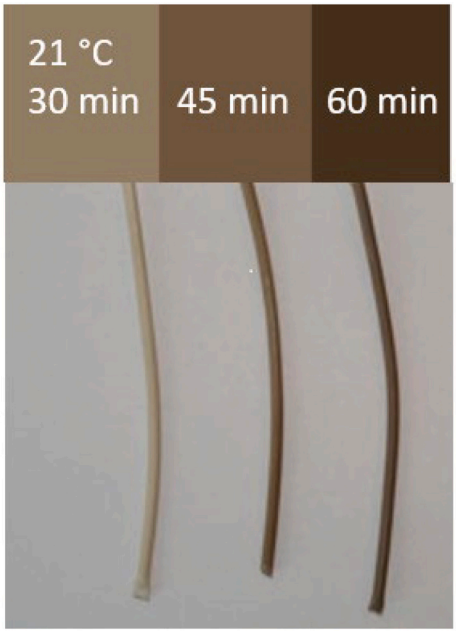

Fig. 2. Visualization of PDA reaction carried out on the hollow fiber membranes. System: 2 g/L DOPA/50 mM acetate buffer (pH 5.0)/4 g/L $NaIO_4$, reaction time 30, 45 and 60 min. The higher the brown intensity, the more PDA was deposited on the fiber. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.2. Establishment of a bioluminescence assay

To provide a test system resembling a nutrient-limited environment and yet supply the used test strain of *E. coli* MG1655 [pBBRMCS-4:: *luxCDABE* with sufficient nutrients for biofilm formation and sustained bioluminescence production, different aqueous media with and without a mixture of low amounts of tryptone/yeast extract were tested as a preliminary assay (data not shown). Ultrapure water with 0.025 g/L yeast extract and 0.05 g/L was found most appropriate for the further development of these assays. Additionally, 2.5 mM sodium bicarbonate was added to simulate moderate water hardness, as well as 1% of fully complemented M9-Medium to provide minimal amounts of inorganic nutrients and trace elements. Compared to maximal bioluminescence values of suspended cells of the same strain in undiluted M9 medium with 20 mM glucose (Motealleh et al., 2020), the bioluminescence values with this medium were about 50% and 90% lower for suspended cells and for biofilms formed on the uncoated fibers, respectively.

3.3. Evaluation of the coating efficiency with bioluminescence assays in ana integrated approach

For optimizing the PDA-coating for antibacterial activity the coating procedure were evaluated in a two-step assay applying bacterial luminescence. In the first step, it was analyzed whether the coatings released any constituents that inhibit bacterial bioluminescence (leaching assay; Figs. 4A, 5A and 6A). In the second step, it was analyzed whether bioluminescence within biofilms on the membranes was inhibited (biofilm assay; Figs. 4B, 5B and 6B).

Comparing static and shaking incubation differences in bioluminescence levels in the leaching assay were not observed (Fig. 4A). Compared to the reference samples, static and shaking polymerization procedures both caused a reduction of the bioluminescence in the formed biofilm after 24 h for both, in which the reduction in samples obtained from the shaking polymerization was more pronounced (Fig. 4B). This tendency was also observed in a long-term assay in which the medium was above the fibers was exchanged and re-inoculated daily over 7 days (Fig. S1). While the bioluminescence of the biofilms on the uncoated reference fluctuated strongly, the values for the biofilms on the coated membranes was clearly lower.

Furthermore, the effect of the amounts of oxidant sodium periodate used and the reaction time in the acetate polymerization solution was tested for the shaking-polymerization method. Both parameters are known to influence the PDA coating and the layer properties. The leaching assay showed some fluctuations of bioluminescence, but no significant changes in medium samples incubated in the presence of the coated fibers (Fig. 5A). Fibers coated in the presence of 2 g/L sodium periodate resulted in a significant reduction of the bioluminescence when coated for 45 min or more, but not for 30 min. Coating in the presence of 4 g/L sodium periodate on the other hand caused a significant reduction of the bioluminescence in the formed biofilm at all tested polymerization times (Fig. 5B). More oxidant, thus, resulted in an increase in antimicrobial activity at shorter reaction times.

FESEM examination of the fibers showed that all membranes were coated with a roughened layer of PDA (data not shown). A longer polymerization time at identical sodium periodate concentrations appeared to result in a rougher surface (more small PDA particles on the surface). Increasing the sodium periodate concentration at equal polymerization times showed a rougher surface by FESEM for the higher $NaIO_4$ amounts. The sample produced with 2 g/L sodium periodate and 30 min incubation clearly was smoother than the other samples, which might explain the smaller reduction in the bioluminescence compared to

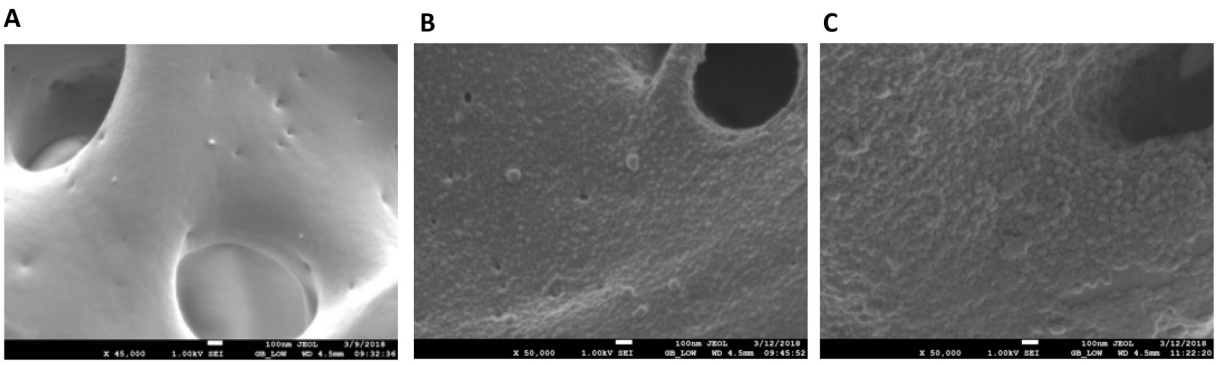

Fig. 3. FESEM of uncoated (A) 30 min coated (B) and 60 min coated (C) fibers. Coating was performed with 2 g/L DOPA/50 mM acetate buffer (pH 5.0)/4 g/L $NaIO_4$, reaction time 60 min. The scale bar is 100 nm.

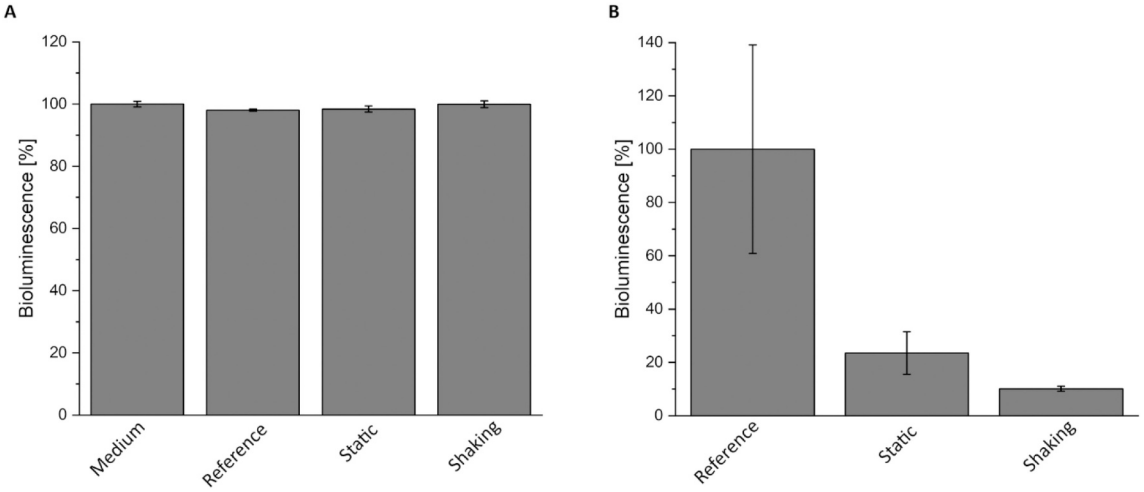

Fig. 4. Bioluminescence assays for inhibitory effects of hollow-fiber membranes coated with polydopamine produced by polymerization under static or shaking conditions using the bioluminescent *E. coli* MG1655 [pBBRMCS-4::*luxCDABE*]. (A) Leaching assays: bioluminescence after 1 h in medium that was incubated for 24 h together with membrane samples as well as medium without sample contact (B) Biofilm assays: bioluminescence at the surface of the hollow fiber membranes after 24 h of biofilm formation. Error bars indicate the standard deviation ($n = 3$).

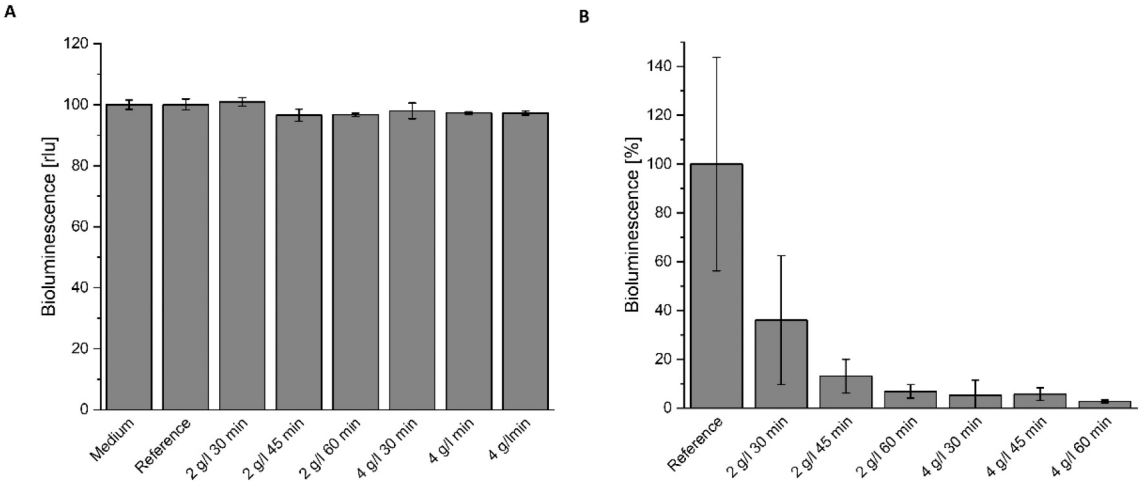

Fig. 5. Bioluminescence assays for inhibitory effects of hollow-fiber membranes coated with polydopamine using either 2 g/L or 4 g/L sodium periodate with different reaction times for polymerization. (A) Leaching assay: bioluminescence after 1 h in medium that was incubated for 24 h together with membrane samples as well as medium without sample contact (B) Biofilm Assay: bioluminescence at the surface of the hollow fiber membranes after 24 h of biofilm formation. Error bars indicate the standard deviation (n = 3).

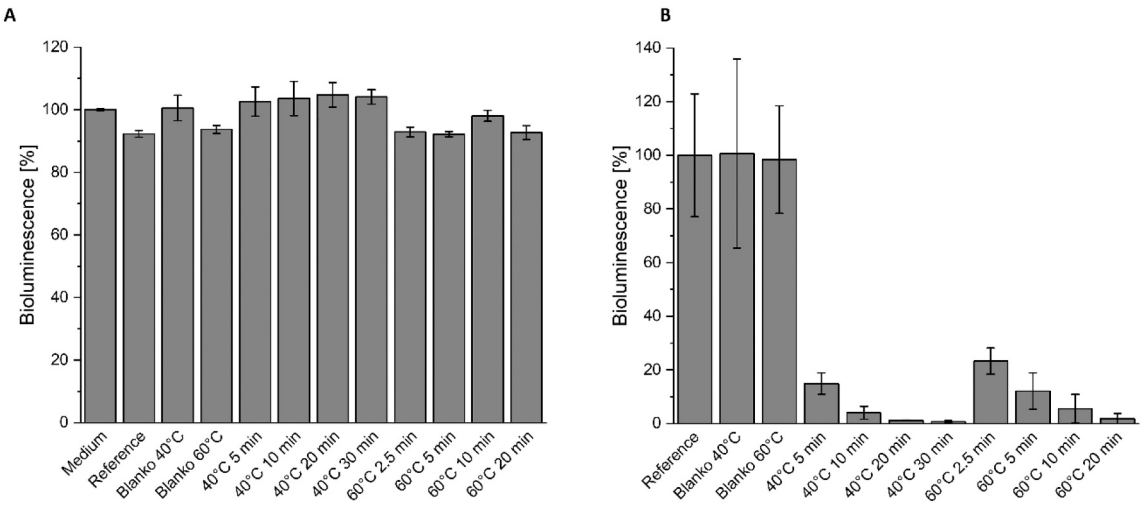

Fig. 6. Bioluminescence assays for inhibitory effects of hollow-fiber membranes coated with polydopamine using either 40 °C or 60 °C as reaction temperature with different reaction times for polymerization. Blanko samples represent Reference materials treated equivalently, but without DOPA. (A) Leaching Assay: bioluminescence after 1 h in medium that was incubated for 24 h together with membrane samples as well as medium without sample contact (B) Biofilm Assay: bioluminescence at the surface of the hollow fiber membranes after 24 h of biofilm formation. Error bars indicate the standard deviation ($n = 3$).

the blank fiber. Water flux measurements on all PDA coated fibers did not show a significant change in water flux compared to the blank (not shown).

Subsequently, temperature and coating time were varied for the 4 g/L sodium periodate shaking method in acetate buffer, and these fibers were assayed for their influence on bioluminescence. Coating with different temperatures and reaction times led to coated fibers causing again some fluctuations of bioluminescence in the leaching assay, but no clear reductions hinting to inhibitory effects aside surface contact (Fig. 6A). All coated fibers caused a clear reduction of the bioluminescence in the biofilm compared to the control samples. The effect increases with increasing reaction time with almost no detectable signal in the highest reaction times. Fibers coated for the same duration, but at different temperatures (40 °C/60 °C), caused comparable levels of bioluminescence (Fig. 6B). Increasing the temperature speeded up the coating process, but the final decrease in bioluminescence was identical (within standard deviation). Apparently, coating for 20 min at 40 °C was the ideal coating condition for antibacterial effects of this setting.

4. Discussion

The aim of this work was to develop intrinsic antibacterial surfaces with inhibitory properties upon surfaces contact without releasing biocidal substances to the environment. To achieve this, a PDA coating was applied to PES/PVP hollow-fiber membranes typically used for water- and wastewater filtration applications, and the antibacterial activity was analyzed with a bioluminescent strain of *E. coli* in two steps. In the first step, it was analyzed whether bioluminescence-inhibiting compounds were leached from the surfaces; in the second step, effects on bioluminescence of the firmly attached coating were measured. Even though in some cases a change in the bioluminescence levels was detected in leaching assays, a clear bioluminescence reduction on membrane surfaces was consistently observed after sufficiently often repeated leaching assays to wash off all residual influencing material. Therefore, we propose that reductions in bioluminescence levels on the membrane surface is not caused by a release of inhibitory material, but rather is exhibited upon direct contact with the surface.

This would be a clear advantage compared to the currently-used copper-based coatings. Although copper surfaces and copper alloys inactivate bacteria upon surface contact by e.g. generating reactive oxygen species, copper ions are also released from such coatings and have been shown to damage bacterial cells by their penetration (Grass et al., 2011). The release of copper ions is apparently enhanced by the presence of bacterial cells, suggesting the involvement of complexing compounds produced by the bacteria (Hahn et al., 2017).

The exact antibacterial mode of action of polydopamine coatings is not elucidated, but a reduction of the adhesion of albumin for at least short terms is known, indicating a low affinity of proteins to polydopamine (McCloskey et al., 2010). Other studies suggest the involvement of hydrogen peroxide release upon PDA oxidation (Tyo et al., 2019). Additionally, the surface structure could also play a role (Su et al., 2016).

Efficient PDA coating polysulfone ultrafiltration membranes is reported with polymerization times of up to 4 h (Kasemset et al., 2017), but this was considered to be too long for the foreseen upscaling. Shorter reaction times, however, resulted in lower PDA layer thicknesses on the fiber. For this reason, the procedure was adapted. A shaking-assisted acidic PDA application at slightly elevated temperatures resulted in considerably shorter reaction times, a roughened PDA layer, and enhanced antimicrobial activity. (Ponzio et al., 2016; Su et al., 2016). The permeability of these fibers did not change compared to the blank fiber (without PDA coating). A molar ratio 2: 1 of $NaIO_4$/DOPA in an acetate buffer at pH 5.0 turned out to give the roughest surface within relatively short reaction times (up to 1 h), while this time could even be shortened without loss in PDA coating roughness when the coating was carried out at 40 °C. Such conditions are promising and realistic for application in commercial membrane manufacturing.

Apparently, the coating method influenced the reduction of the bioluminescence on the surface. Using shaking-polymerization with different amounts of the oxidizing agent sodium periodate (2 g/L vs 4 g/L) led to a greater reduction of bioluminescence within the biofilm at 4 g/L sodium periodate, even when shorter reaction times were used. This indicates an acceleration of the polymerization speed. When using 4 g/L sodium periodate and varying the time and temperature for polymerization, the temperature seems to have lesser effects on the outcome of the inhibitory effects of the coating, leaving coating at 40 °C for 20 min the fastest and easiest polymerization method with maximal detectable effect. The stronger inhibiting effect might be explained by the fact that shaking during PDA coating leads to a rougher PDA morphology. This is in agreement with literature describing the stronger antibacterial effect of roughened PDA coatings compared to smooth surfaces (Su et al., 2016).

Apart from the fact that bacterial bioluminescence could be used to localize antibacterial effects in a non-invasive way, our two-step assay

displayed further advantages in comparison to the currently-used standard assays. As a correlation between bioluminescence loss and growth inhibition is known (Menz et al., 2013) a total loss of bioluminescence is likely to indicate that the bacteria are not able to grow on the antibacterial surface. A further advantage of our test system is that it was possible to operate it with a low-nutrient mineral medium that is more similar to conditions at the sites in which such membranes are being used. As the production of bioluminescence requires energy, supplying low amounts of yeast extract and tryptone could not be avoided. The use of authentic tap water or of synthetic tap water also led to dissatisfying results, leaving ultrapure water with the addition of bicarbonate and diluted M9 medium as an appropriate compromise between an oligotrophic medium like tap water and a medium enabling bioluminescence.

It has to be considered that PDA coatings are typically used as a basis for other antimicrobial agents (Fan et al., 2019) and the antifouling effects of pure polydopamine is rather limited to short term effects (Miller et al., 2012). Nevertheless, the contact-based inhibition of bacterial activity clearly showed that this bio-inspired material has the potential of being used as a sustainable and effective coating for membranes in pure- and potable-water settings. Additionally, a first long-term stability assay showed that the coating can repeatedly inhibit bioluminescence (Fig. S1) indicating that the antibacterial effect is not masked by an inactive layer of bacteria. Nevertheless, further research on PDA-coated membranes is necessary to elucidate its long-term stability in more detail and its resistance towards cleaning chemicals often applied in industry for removing membrane fouling.

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Author contributions

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Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mimet.2022.106452.

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