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The effect of morphologies of carbon nanotube-based membranes and their leachates on antibacterial property

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ABSTRACT

Carbon nanotube (CNT)-based membranes have been reported to have outstanding potential properties such as extraordinary water permeability, microbial toxicity, and chlorine resistance in water and wastewater treatment. However, aspects of antibacterial property for various morphologies of CNT-based membranes and for the membrane leachate are still unclear. In this study, CNT-based membranes having different morphologies were fabricated and were directly exposed to bacterial cultures to test for bacterial viability. In addition, the antibacterial effect of membrane leachates was evaluated by comparing the viability of bacteria in leached and unleached waters. Neither the CNT-based membranes nor any leaching media inhibited *Pseudomonas aeruginosa* PA14 (PA14) growth. PA14 grown on any of the CNT-based membranes or in their leaching medium were not inactivated, and only PA14 that directly contacted to the surface of vertically aligned (VA) CNT membranes were inactivated. VA-CNT solid membranes which have the highest packing density of CNTs showed higher antibacterial property. They showed 23 and 6.4 times higher antibacterial property than deposited CNT and even VA-CNT forest membranes, respectively. Acidified membrane surfaces showed two times higher antibacterial property than non-acidified membranes. Especially when VA-CNT solid membranes were acidified, their antibacterial property increased 23-fold. After leaching tests, all VA-CNT membranes showed a 10% increase in antibacterial property. This study provides the first comprehensive comparison of the antibacterial property of diverse CNT-based membranes morphologies and further details the effect of acidification and leaching of CNT membranes on their antibacterial properties.

Keywords: Antibacterial; Carbon nanotube; Leachate; Membrane; Vertically aligned

1. Introduction

Carbon nanotube (CNT) is a promising nanomaterial due to its outstanding properties such as extremely higher mechanical strength, electric and thermal conductivity. Environmental researchers have focused on other properties that make them suitable as membranes for water and wastewater treatment. Vertically aligned (VA) CNT membranes have shown extraordinary water permeability [1] through the CNT pore, and CNTs in

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dispersion state have shown microbial toxicity which allows possible anti-biofouling membrane properties.

On the other hand, their microbial toxicity has been a source of concern since it might not only have effect on fouling bacteria but also on the total microbial system and on the ecosystem. Even though it was simulated, that CNTs might not confer aquatic ecotoxicity [2], like other nanomaterials, the toxicity of CNT is still an issue of concern and studied in many areas. Human blood serum [3], fibroblast cells [4], epithelial [5], protozoa [6], algae [7,8], and bacteria [9,10] were studied to probe the degree of toxicity to the environment. Through such recent studies, the interaction between CNTs and bacteria which are ubiquitous in nature can serve as a more accurate predictor of ecotoxicity in the environment [2].

Antibacterial properties of CNTs are closely related to various membrane properties and morphologies. Of them, the diameter or wall number of the CNTs is the most critical. Kang et al. [11] reported for the first time the antibacterial activity of CNTs. They showed that single walled CNTs (SWCNTs) had stronger antibacterial activity than multi walled CNTs (MWCNTs). Moreover, they demonstrated that uncapped, debundled, short, and dispersed MWCNTs had antibacterial activity as well. The degree of dispersion of CNTs is another important factor. Individually dispersed CNTs were more toxic to bacteria than CNT aggregates [12]. The functionalization of the surface of CNTs can also affect microbial toxicity. Wang et al. [13] reported that SWCNTs which were functionalized by carboxylation were significantly more toxic than standard. In the case CNT suspension, surfactants themselves can affect the antibacterial property. Bai et al. [14] tested several kinds of surfactants with equivalent concentrations of MWCNTs and showed differences among them. That is, the surfactants-modified MWCNTs were not only capable of capturing bacteria and forming cell aggregates, but also capable of killing them. Dong et al. [15] used SWCNTs to compare the antibacterial properties of three surfactants. Finally, Yang et al. [16] studied the effect of length on the antimicrobial activity of SWSNTs. They revealed that longer SWCNTs exhibited stronger antimicrobial property at same weight concentration.

Previously, lots of parameters have been suggested as influential factors on the antimicrobial effect of CNTs. It is certain that dispersed CNTs constantly attack bacteria, degrade bacterial cell integrity, and eventually cause cell death [17]. However, the mechanisms and pathways followed by direct contact of CNTs to microbial cell walls are still unclear. Possibilities have included physical damage such as piercing, oxidative stress by higher electric conductivity of CNTs and chemical toxicity by impurities such as residual catalysts. The most convincing reason was physical puncture by CNT "Nano Darts" [17].

Until now, most of the studies on antibacterial properties of CNTs have been limited to dispersed CNTs; thus, there has been little understanding on the antibacterial property of VA-CNT membranes [1]. Even though the two morphologies are based on the same CNTs, the interaction of VA-CNT membranes and bacteria is different from that of dispersed CNTs and bacteria. Only the surface of VA-CNT membrane can directly contact with bacteria, so the bulk of planktonic bacteria that do not contact the CNTs should not show any antibacterial effects.

For CNTs to show antimicrobial property, there needs to be some range of bundling of CNTs, which may damage bacterial cell walls. The immobilized arrangement of VA-CNTs may show different antimicrobial activity than the freely moving CNT aggregates—the former showing less chance of the effective contact than the latter. Furthermore, VA-CNT solid configuration have higher CNT density than VA-CNT forest membranes [18], and they also may show different antimicrobial property.

Being membrane candidates for water treatment, CNT membranes in their various morphologies should be confirmed to possess no secondary effect of contamination by leached toxic components from the material during operation. However, there have been no studies done on the antibacterial property of leachates of CNT membranes.

In this study, we compared antibacterial property of the most possible morphologies of both pristine and leached CNT membranes and their leaching water using *Pseudomonas aeruginosa* as a model bacterium. The differences of bacterial growth when exposed to CNT membranes and their leaching water were monitored, and the ratio of dead and live cells on the surfaces of CNT membranes after the 16 h of growth test were characterized by confocal laser scanning microscope (CLSM).

To our knowledge this is the first comprehensive study on antibacterial property of all morphologies of CNTs and their leachates using water. It will be very helpful to understand the microbial toxicity of CNT membranes and their leachates as a predictor of biofouling properties of CNT membranes that would be used in water and wastewater treatment processes.

2. Materials and methods

2.1. Synthesis of VA-CNTs and CNT inks

Four-inch silicon wafers were used as substrates. They were P-type; boron was used as dopant. Their thickness was 0.525 mm. The polished side was used as the substrate face. The substrate face of the silicon wafer was coated by aluminum (Sigma Aldrich, USA) and iron (Sigma Aldrich, USA), in order, as catalyst layers. The thickness of the aluminum layer was 15 nm, and that of the iron layer was 1.5 nm. Catalyst coating was done by e-beam evaporation system (Dae Ki Hi-tech Co. Inc., Seoul, Korea). After coating they were chopped into rectangular chips which have the dimension of 9 mm width, and 9 mm length; then the chips were inserted into the cylindrical reactor of chemical vapor deposition (CVD) machine.

Fig. 1 shows (a) VA-CNT CVD machine and (b) its schematic diagram. Carbon source was ethylene gas and its flow rate was 100 sccm (standard cubic centimeters per minute indicating cc/min at a standard temperature and pressure). Carrier gas was argon and its flow rate was 300 sccm. 200 sccm of hydrogen and 50 sccm of water vapor from a bubbler by argon purging flowed. Purged water was deionized (DI) water (humanpower I+, Human Corporation, Seoul, Korea). Until the reactor temperature was increased up to 750˚C all gases except for ethylene flew through the reactor and after 40 min of heating, the carbon source began to flow for CNT growth. Reaction time was 30 min and after the reaction reactor was cooled in the air.

Fig. 2 shows the procedure of VA-CNT synthesis and preparation of CNT inks and CNT membranes. The densification procedure of VA-CNT forest (c in Fig. 2) by capillary forces reported by Futaba et al. [19] was used to make VA-CNT solid (d2 in Fig. 2). They used several volatile solvents for the densification of CNTs and ethanol (Sigma Aldrich, USA) was used in this study. CNT ink was originated from the same VA-CNT forest. The CNT ink preparation procedure from VA-CNTs was based on that of Yang et al. [20]. One part of VA-CNT forest which had 0.9 mm width and 0.9 mm length on substrate was added into 45 mL volume of conical tube and 0.020 mg of sodium dodecyl sulfate (SDS, Sigma Aldrich, USA) was added following to make in total 40 mL aqueous dispersion by DI water (d3 in Fig. 2). After the dissolution of SDS the CNT dispersion was sonicated for 60 min at 50˚C with 60% power to fully separate individual CNTs. After 6 h of sedimentation of sonicated dispersion, supernatant was collected and was used as CNT ink sample (e3 in Fig. 2).

2.2. Membrane Preparation

Membrane samples for comparing antibacterial property were fabricated using VA-CNTs and CNT inks which had the same morphology as VA-CNTs (c in Fig. 2). Deposited CNT membrane (f3 in Fig. 2) was fabricated by filtering CNT inks (e3 in Fig. 2) equivalent to one VA-CNT part by polycarbonate (PC) microfiltration (MF) membranes (Nuclepore Track-Etch membrane by Whatman®) which had 0.1 μm of pore size and was flat-sheet type of 47 mm diameter. After the deposition of CNT inks they were rinsed by DI water two times in order to remove residual surfactants (SDS) and dried in the air. The VA-CNT membrane was fabricated as forest state (f1 in Fig. 2) and solid state (f2 in Fig. 2). The interstitial space between CNTs was filled with polyurethane (UC-40 A, B,

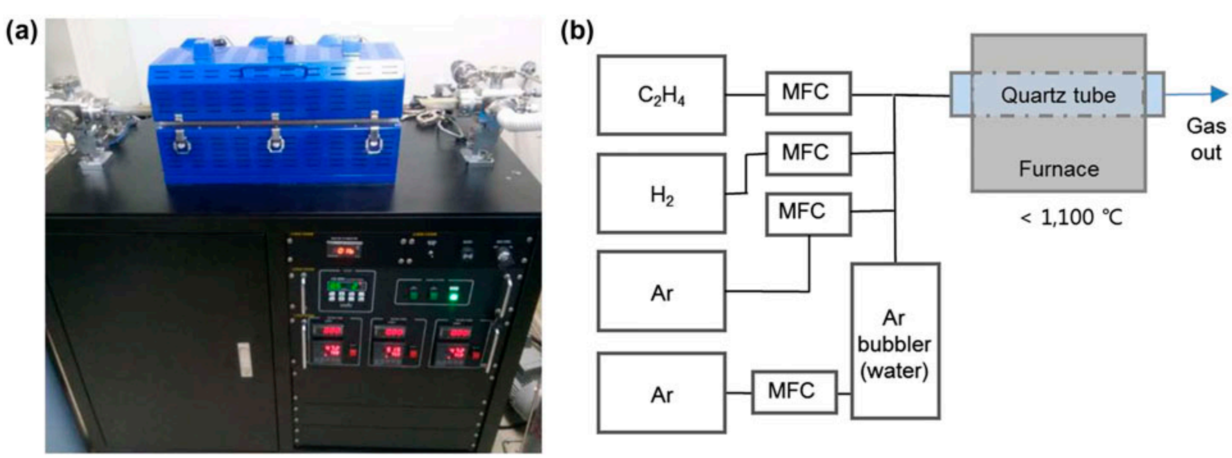

Fig. 1. (a) VA-CNT chemical vapor deposition (CVD) machine and (b) its schematic diagram.

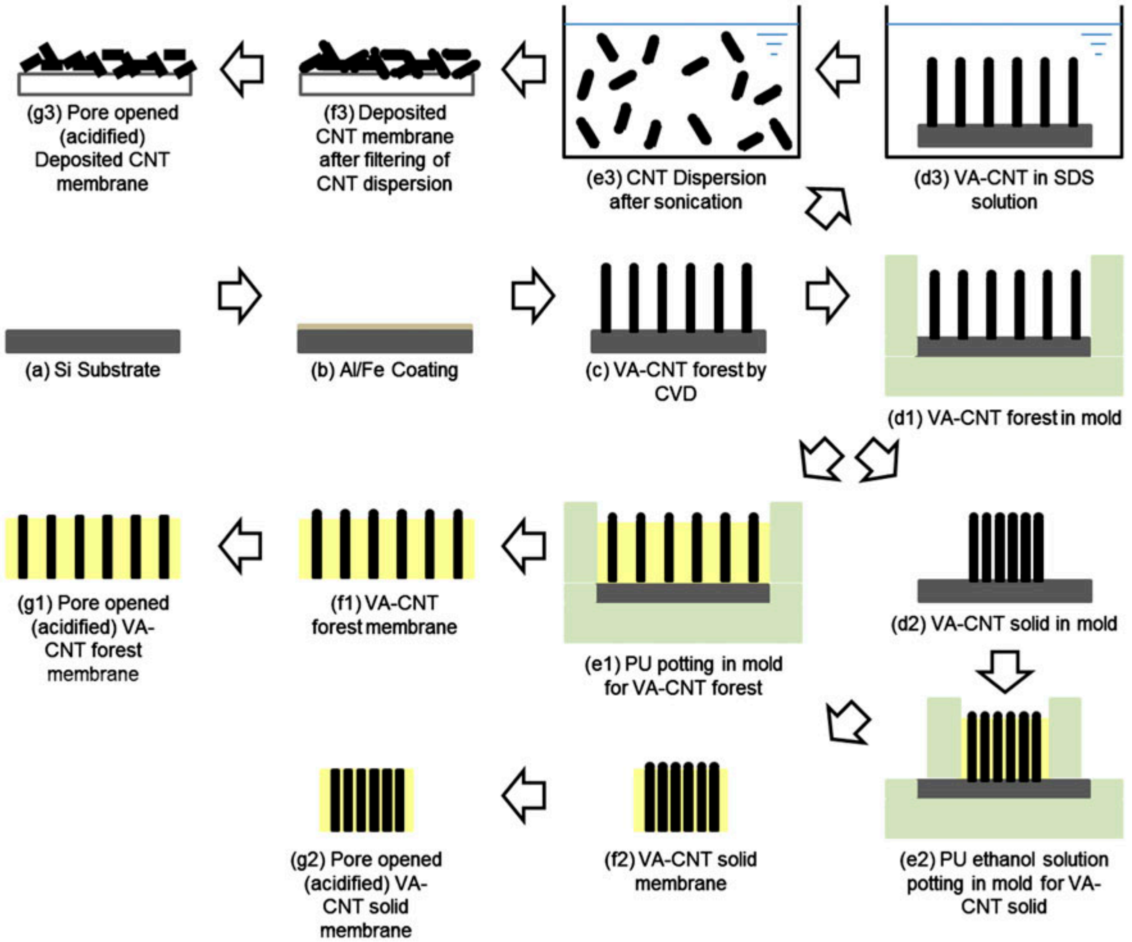

Fig. 2. CNT membrane fabrication procedure; Al and Fe (b) were deposited on Si substrate (a). CNTs were vertically aligned on the deposited substrate (c). VA-CNTs were dispersed in SDS solution (d3) after sonication (e3). CNT dispersion was filtered by MF membrane to be deposited CNT membrane (f3). After the deposited CNT membrane was in 5 M HNO_3 for 12 h most of caps were opened to be deposited CNT membrane (g3). After VA-CNT (c) was molded by pouring PU (e1) VA-CNT membrane was put out from the mold (f1). After the molded VA-CNT forest was in 5 M HNO_3 for 12 h most of pores were opened to be VA-CNT forest membrane (g1). When VA-CNT (c) was molded by pouring PU and ethanol (1:1, v/v, d) densified VA-CNT membrane (e2) was acquired. After put out from the mold (f2) the molded VA-CNT solid was in 5 M HNO_3 for 12 h most of pores were opened to be VA-CNT solid membrane (g2).

Cytec, Korea) in both states. In the case of VA-CNT solid membranes fabrication, densification (d2 in Fig. 2) process was done before polyurethane potting.

Three morphologies of membranes were acidified with 5 M of HNO_3 for 24 h in order to open gate. In total, six morphologies of CNT membranes were deposited; CNT membranes (f3, g3) and VA-CNT membranes had forest (f1, g1) and solid (f2, g2) state. g1, g2, and g3 had pores by removing caps from f1, f2, and f3 samples. Curing time of polyurethane was at least 12 h. PC MF membrane and polyurethane which were base material in fabrication of VA CNT membranes were tested together as references.

Table 1 listed all of membrane samples with abbreviations. All membrane samples were tested four times in order to provide averaged evaluation data.

2.3. Leaching

Leaching was performed using Luria-Bertani (LB) broth medium (10 g/L tryptone, 5 g/L yeast extract, 10 g/L NaCl) in DI water. LB broth is the most widely used medium for the growth of bacteria. The reason why pure DI water was not used as leaching medium was that nutrients in leaching medium were required when model bacterium was incubated.

Table 1
Membrane sample list and their abbreviations

Sample	Abbreviation		
	Membrane	Leaching water	Leached membrane
VA CNT forest	VF1	VFL	VF2
VA CNT forest acidified	VFA1	VFAL	VFA2
VA CNT solid	VS1	VSL	VS2
VA CNT solid acidified	VSA1	VSAL	VSA2
Deposited CNT	D1	DL	D2
Deposited CNT acidified	DA1	DAL	DA2
PC membrane	PC		
PC membrane acidified	PCA		
Urethane	PU		
Urethane acidified	PUA		
Control	C		

Note: “V” meant vertical, “F” forest, “S” solid, “A” acidified, “D” deposited, “1” before leaching, “2” after leaching, “L” leached water, “PC” polycarbonate, “PU” polyurethane, and “C” control bacteria.

Membrane samples and 10 mL of LB medium were added into 45 mL of conical tubes. Samples in tubes were sonicated for 1 h at 40˚C with 60% power. After the sonication membranes and leaching water were isolated and conducted to bacterial growth test individually. The same condition was repeated four times and average value of the results was acquired. Water temperature in general water and wastewater treatment plant is less than 40˚C, thus this leaching condition closely simulated conditions of real membrane operation.

2.4. Antibacterial effect of CNT-based membrane surfaces on growth of PA14

PA14 was used as a model bacterium in this experiment. After overnight culture of PA14 on the LB agar plate, a single colony was inoculated in 10 mL of LB medium. The bacterial strain was cultured in the incubator at 250 rpm of shaking rate at 37˚C for 12 h. Cultured bacterial solutions were diluted with 20 mL of fresh LB medium (1:99 (v/v)) to have 0.010 optical density values at 595 nm (OD_{595}). OD_{595} values were read by UV–VIS spectrophotometer (DR 5000, Hach, USA).

Bacterial growth and bacterial viability test procedure are shown in Fig. 3. Each membrane was added to bacterial solution (Fig. 3(b)) and the solution was cultured in the incubator at a 250 rpm of shaking rate at 37˚C for 16 h. During the incubation CNT membranes directly contacted a part of the bacteria in solution. The effect of partial contact on the growth of bulk bacteria was monitored by OD_{595} in the solution at every 2 h. At the end of exponential growth phase, monitoring interval was reduced to 1 h. PA14 culture was added in leaching media from each CNT membrane (Fig. 3(a)) and their growth was also measured.

In order to decide volume of bacterial solution per unit CNT-based membrane, the exposure intensity was considered as the effective membrane surface area per total volume of bacterial solution; the exposure intensity was determined 3 by the ratio of the effective membrane surface area of commercial submerged membrane per volume of membrane cassette. CNT-based membrane area was 0.2–1.0 cm^2 and bacterial LB solution volume was 20 mL. Accordingly, the membrane area per exposure volume was calculated at 100–500 m^2/m^3. Meanwhile the ratio of the effective membrane area to the cassette volumes—however for not membrane tanks whose volume was larger than that of the cassette—of ZeeWeed 500d (GE, Canada) series membranes, a highly popular commercial membrane, was 158 and 210 m^2/m^3.

In this study, the exposure intensity of bacteria to CNT membranes was similar to or higher than that of commercial membrane used in actual operation. Also, the concentration of microbial cell solution of this study was not less than that of a membrane bioreactor (MBR) plant. So bacteria in this study actually had higher chances to contact with membrane surface than they would in real membrane operation.

2.5. Antibacterial effect of CNT-based membrane surfaces on loss of viability of PA14

L7012 LIVE/DEAD® *Bac*Light™ Bacterial Viability Kits (Invitrogen, US) was used for comparison of influence of CNT membranes and their leaching

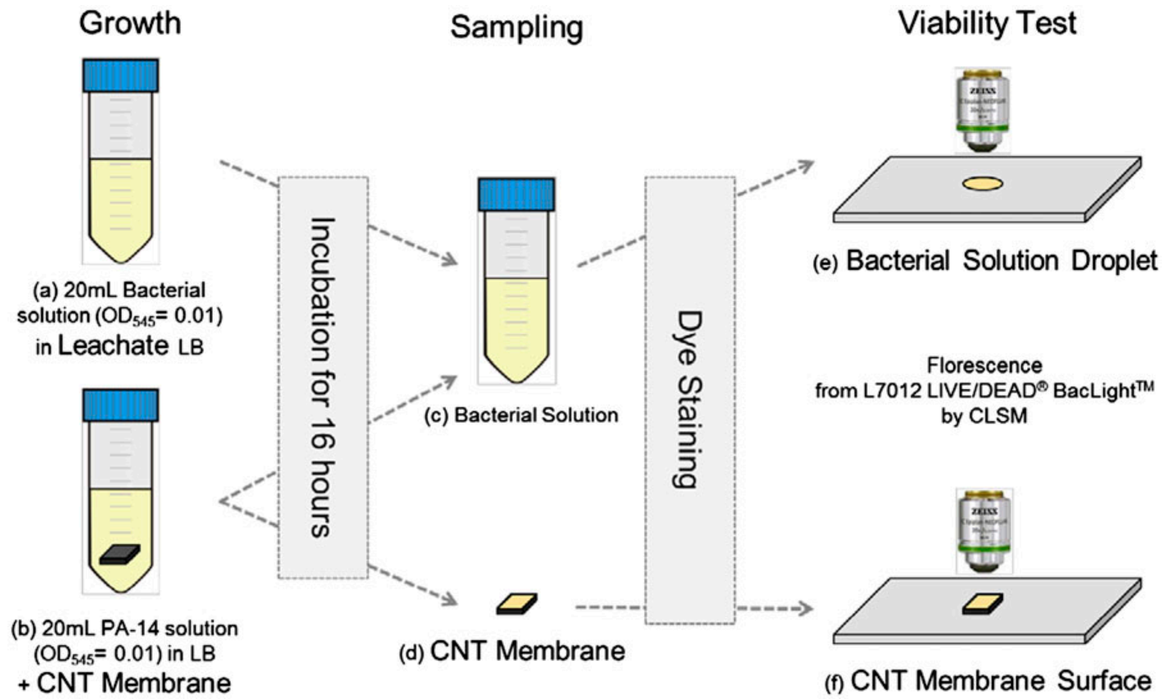

Fig. 3. Bacterial growth and bacterial viability test procedure: (a) PA14 growth test in leaching media from each CNT membrane, (b) PA14 growth test with diverse CNT membranes, (c) bacterial solution after 16 h of incubation, (d) CNT membranes after 16 h of incubation, (e) CLSM measurement of bacterial solution droplet on slide glass after staining, and (f) CLSM measurement of surface of CNT membrane on slide glass after staining.

solutions on the loss of viability on membranes surface and bacterial bulk solutions. It includes two dyes: SYTO® 9 which stains nucleic acid of all bacteria and shows green fluorescence; and propidium iodide (PI) which stains nucleic acid of damaged bacteria and shows red fluorescence. When both dyes are present, PI fluorescence is diminished, thus live cells show only green fluorescence and dead cells only red.

After the growth test, membranes (Fig. 3(d)) were taken out from the conical tubes and the surface of membranes were dyed in order and analyzed by CLSM (Fig. 3(f)). 3 μL of SYTO® 9 and PI were treated to stain nucleic acid of bacteria for 30 min in the dark at room temperature. CLSM images of the stained membranes were generated. A Zeiss CLSM model LSM 700 with lens of W N-Achroplan 20X/0.5 W(DIC) M27 was used for the analysis. Total magnification was 200×. All images had the same threshold value. Isolated bacterial solutions (Fig. 3(c)) after growth test were dropped on slide glasses (Fig. 3(e)), stained, and monitored by CLSM.

Leaching medium and control samples were analyzed after they were dropped on slide glass, stained according to the same method used for the membrane, and covered by cover glass. The intensities of green and red color of all images were evaluated in order for quantitative comparison of viability of PA14 by pixel counting of each hue using ImageJ program (National Institutes of Health, USA).

3. Results and discussions

3.1. CNTs and their membranes

Fig. 4(b) shows an image of VA-CNT solid (left) and forest samples (right). The dimension of the VA-CNT forest was 9 mm width, 9 mm length, and 1 mm thickness; while that of VA-CNT solid was reduced to 2 mm width, 2 mm length, and 1 mm thickness. Their dimensions were not changed in membranes and resulted in the reduction of total membrane surface area of VA-CNT forest membrane from 81 to 4 mm^2; likewise for the VA-CNT solid membrane. It means the pore-density of VA-CNT solid membrane was increased 20-fold compared with the forest membrane. The result of N_2 adsorption/desorption isotherm (ASAP 2010 BET Surface Area Analyzer, USA) data showed the average inner diameter of VA-CNTs was 4.1 nm. This results showed the VA-CNTs had a diameter 1 nm larger than that of

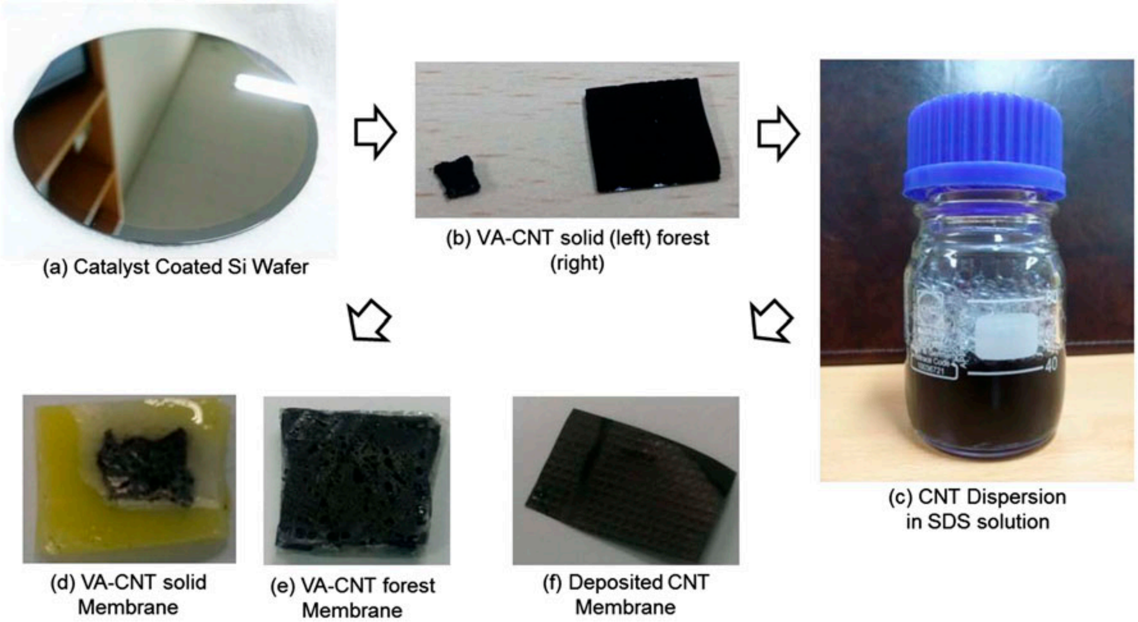

Fig. 4. CNT-based membranes: (a) catalyst coated SI wafer, (b) VA-CNT solid (left) and forest (right), (c) CNT dispersion of one piece of CNT forest sample in SDS solution after sonication, (d) VA-CNT solid membrane, (e) VA-CNT forest membrane, and (f) deposited CNT membrane on PC MF membrane.

Yu et al. [18]. Fig. 4(c) shows the CNT ink sample originated from the same VA-CNT forest. Fig. 4(e–f) show VA-CNT membranes which were fabricated with VA-CNT solid (d), VA-CNT forest (e), and CNT ink (f). Acidified and non-acidified CNT membranes were tested; leached and non-leached CNT membranes were tested as well.

3.2. Antibacterial effect on bacterial growth

Fig. 5 shows microbial growth curve of PA14 cultures exposed to diverse morphologies of CNT membranes, their leaching media, and their base materials. The violet dotted line represents the growth curve of the control—a pure culture of PA14 in LB

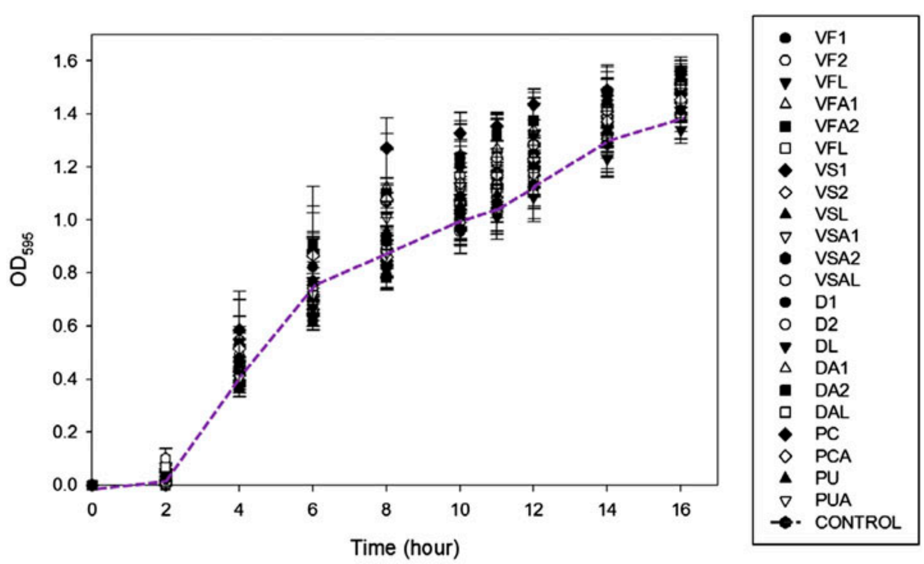

Fig. 5. Microbial growth of all planktonic PA14 exposed to each CNT membrane; All PA14 cultures except for that on acidified deposited CNT membrane showed similar or better growth pattern compared with control PA14 culture.

medium. Only the DL sample showed slower growth rate (<5%) than the control after 11 h while the rest showed equivalent or faster growth rate compared with the control; even retardation of DL was not significant. This showed that the growth of most PA14 cultures was neither inhibited by any CNT-based membranes nor by their leaching media including base materials such as polyurethane and PC membrane. It also signifies that whether CNT-based membranes were acidified or not, the membranes and their leaching media did not negatively influence the growth of PA14. The result of bacterial growth is closely indicated; suspended PA14 culture have a much lower chance to contact the CNT membrane than if they were grown on the surface. The viability of PA14 on the CNT membrane surface was compared by CLSM images after staining.

3.3. Antibacterial effect of CNT membranes and their leaching media on planktonic PA14 culture

Fig. 6(a)–(c) and (e)–(g) show the CLSM images that represent viability of PA14 cultures after growth reached at the end of exponential phase in leaching media of six types of CNT membranes. Viability could be shown as difference in color: green (viable) and red (non-viable). All six images show green color rather than red. Fig. 8 shows the ratio of green to red in each image, and the ratios of these six leaching media were nearly zero.

The pictures and color ratios show that regardless of morphologies of base CNTs, leaching media of all membranes did not show any antibacterial property to planktonic PA14 cultures, even though the exposure intensity of bacteria to CNT membranes was higher than that in commercial membrane operation.

All planktonic bacteria which might contact with CNT membranes during incubation remained viable; Fig. 6(h) is one of their CLSM images. The ratio of green to red is nearly zero in Fig. 8. Also planktonic control PA14 showed the same results.

Regardless of which CNT-based membrane contacted the planktonic PA14 during incubation, they did not inhibit bacterial growth and did not show any antibacterial properties; this result, which matches that of Liu et al. [17], showed that more chance of direct contact of CNTs with bacteria was needed for antibacterial property.

3.4. Antibacterial effect of CNT-based membrane surfaces on PA14

Fig. 7 shows PA14 stained by L7012 LIVE/DEAD® *Bac*Light™ Bacterial Viability Kits on various

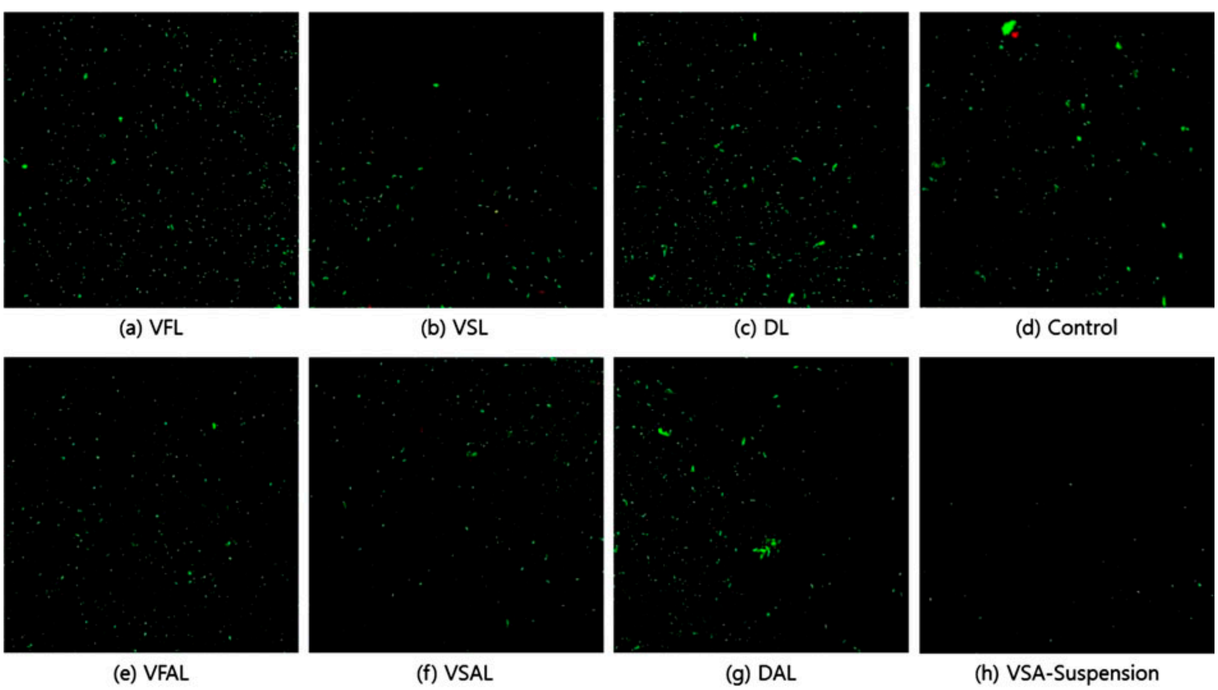

Fig. 6. CLSM images of a droplet of bacterial solution grown in leaching media, bacterial bulk solution directly contacted with CNT membrane, and control bacterial solution: All bacterial solutions showed little antibacterial properties like control solution.

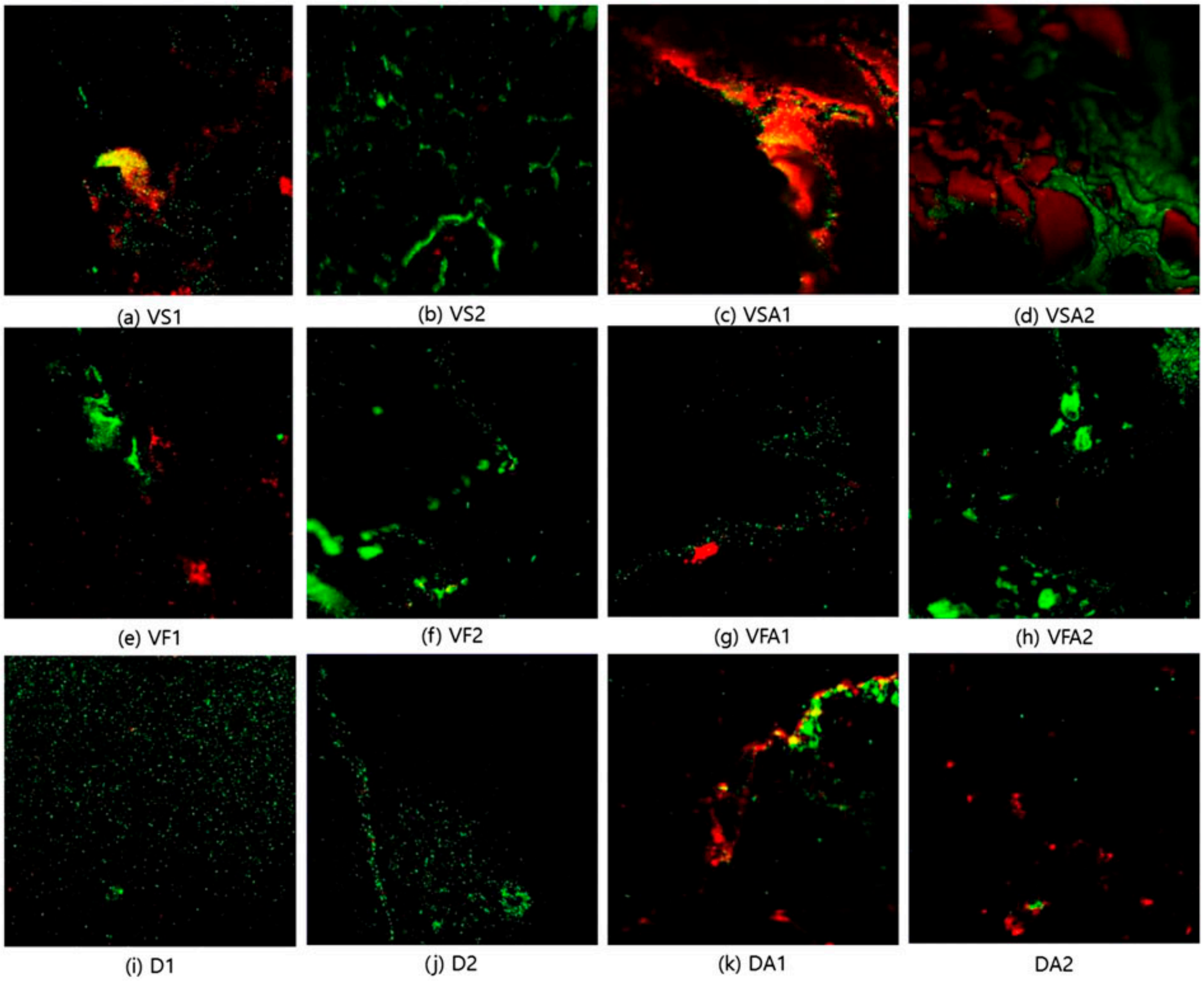

Fig. 7. CLSM images of CNT-based membranes surfaces stained: VA-CNT membranes showed higher antibacterial property than deposited CNT membranes and VA-CNT solid membranes showed the highest antibacterial property. The antibacterial property of VA-CNT solid membranes was enhanced after acidification.

CNT-based membrane surfaces after 16 h of growth. Compared to PA14 grown in bulk solution, PA14 on CNT membrane surfaces showed an increase in the area of red color. Also, it showed more non-viable PA14 were found on CNT-based membrane surfaces than in the bulk solution. The comparison of the results shows that the range of antibacterial effect of CNT-based membranes might be limited to the membrane surfaces.

In Fig. 7, there were different red to green color ratio for each of the membrane surfaces. The ratios were acquired by ImageJ program, shown in Fig. 8. The red to green color ratio can represent the number of dead to live cells and directly relates to antibacterial property. In Fig. 8, three main morphologies of CNT membranes showed different antibacterial properties. Acidification and leaching effects on antibacterial properties of CNT membrane were studied quantitatively as shown in Fig. 8. Exact average ratios and their standard deviations of red to green color pixels of all samples are listed in Table 2.

In comparison of antibacterial property of VA and Deposited morphologies of pristine CNT membranes the ratio of red to green pixel number of VF1, VS1, and D1 are 0.191 ± 0.007, 0.689 ± 0.012, and 0.030 ± 0.005. VA-CNT membrane surfaces showed higher antibacterial property, VA-CNT forest and solid membranes showed 6.4, 23 times higher antibacterial property than deposited CNT membrane. This tendency was similar whether the membranes were acidified or leached or not.

DA1 and DA2 which are acidified deposited CNT membrane and its leached membrane showed opposite results even though their pristine membrane did

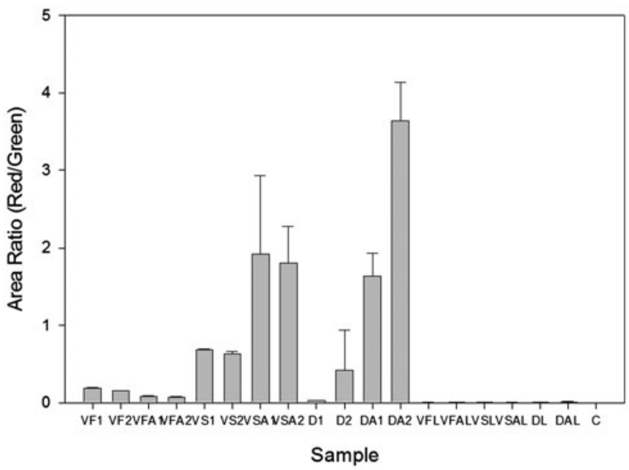

Fig. 8. Ratio of red (non-viable) to green (viable) of each sample: the results of Fig. 7 can be quantified.

Table 2
Ratio of red (dead) to green (live) of each sample

Sample	Average (Red/Green)	Standard deviation
VF1	0.191	0.007
VF2	0.155	0.006
VFA1	0.087	0.006
VFA2	0.076	0.006
VS1	0.689	0.012
VS2	0.637	0.025
VSA1	1.923	1.010
VSA2	1.810	0.463
D1	0.030	0.005
D2	0.079	0.028
DA1	1.640	0.294
DA2	3.635	0.497
VFL	0.007	0.001
VFAL	0.010	0.001
VSL	0.010	0.004
VSAL	0.007	0.001
DL	0.009	0.003
DAL	0.014	0.003
C	0.001	0.000

not show any antibacterial property. This might have resulted from "chopped" CNTs during the acidification and leaching process. Liu et al. [17] reported that shorter CNTs had higher antibacterial property because their tips would have higher chance to meet and pierce bacteria. Increasing chopping number of CNTs would also give rise to direct contacting chance. Thus, in order for the deposited CNT membrane to be used for water treatment, CNTs should be fixed onto the substrate surface.

In Fig. 8 and Table 2 VS1, VS2, VSA1, and VSA2 showed 3.6, 4.1, 22.1, 23.8 times higher antibacterial property than VF1, VF2, VFA1, and VFA2 each. Densified VA-CNT membranes showed higher antibacterial property than their pristine counterparts. Arias and Yang [12] reported that when CNT bundles in suspension met bacteria, highly aggregated CNT bundles showed higher antibacterial property. This showed that CNT density is closely related to antibacterial property and that VA CNTs showed a tendency similar to that of CNT suspension. This tendency of pristine membranes did not change whether they were acidified or leached or not.

In comparison of acidification effect of VA-CNT membranes, there were opposite results between VA-CNT forest and solid membranes. VA-CNT forest membranes before acidification, such as VF1 and VF2, showed 2.2 and 2.0 times higher antibacterial property than acidified VA-CNT membranes such as VFA1 and VFA2; also, acidified VA-CNT solid membranes such as VSA1 and VSA2 showed 2.8 times higher antibacterial property. Wang et al. [13] compared the influence of carboxyl, amino, and sulfonyl CNT surface moieties on antibacterial property and showed that the carboxyl group showed highest antibacterial property among the functional groups tested. Acidification introduced carboxyl groups to the membrane surfaces and accordingly, higher antibacterial effects resulted. Their results matched only VA-CNT solid membranes that had enough CNT packing density. When deposited CNT membranes were acidified, their morphologies were changed and yielded complex results. In this figure, when CNTs were acidified they were reduced into smaller fragments and had carboxyl functional groups added to their surfaces. These parts increased the ends and provided more opportunity for bacteria to contact; while the carboxyl groups conferred increased cytotoxicity.

In Fig. 8 and Table 2 VS1, VSA1, VF1, and VFA1 showed 1.2, 1.1, 1.1, and 1.1 times higher antibacterial property than VS2, VSA2, VF2, and VFA2 each. Leached VA-CNT membranes show 10% lower antibacterial property than pristine VA-CNT membranes. There has been no known study on the leaching effect of VA-CNT-based membranes on antibacterial property, thus these results could be the first of such study. Leaching media and leached VA-CNT membranes did not show additional antibacterial properties compared to their pristine membranes and thus demonstrated the safety of VA-CNT-based membranes in operation after potting by fixation.

4. Conclusions

CNT-based membranes have been actively studied because of their promising properties such as outstanding water permeability and anti-biofouling property. But the cytotoxicity of CNT membranes and their leachates are still a point of concern. In this study, antibacterial property of all morphologies of CNT-based membranes and their leaching media were compared after exposure to planktonic PA14 cultures grown for 16 h. Also, acidification and leaching effects on antibacterial property of CNT membranes were studied.

All morphologies of CNT-based membranes were prepared with the same VA-CNTs; morphologies included deposited CNTs, VA-CNT forests, and VA-CNT solids. Acidified and leached CNT membranes were also prepared for comparison with pristine CNT membranes.

Neither the CNT-based membranes nor any of their leaching media inhibited PA14 growth in bulk.

When PA14 grew in the presence of any CNT-based membrane or their leaching medium, they were not inactivated—only PA14 which directly contacted the surface of VA-CNT membranes were inactivated. On the other hand, the surface of deposited CNT membranes did not show any antibacterial affect to bacteria.

VA-CNT solid membranes which have the highest packing density of CNTs showed highest antibacterial property. They showed 23 and 6.4 times higher antibacterial property than deposited CNT and even VA-CNT forest membranes. The results of a growth and viability test of PA14 grown on each CNT membranes showed that VA-CNT solid membranes have the highest potential of anti-biofouling property in membrane operation without bacterial toxicity in source water.

Acidified membrane surfaces showed twofold higher antibacterial property than non-acidified membranes. Most notably, VA-CNT solid membranes when acidified showed a 23-fold increase in their antibacterial property. This result may be explained by a synergistic effect of densification and carboxylation, which enhances piercing power as fixed “Nano Darts”.

After leaching tests were conducted, all VA-CNT membranes showed a 10% increase of antibacterial property. There has been no known study on the leaching effect of CNT-based membranes, thus these results can be the first step of the consideration regarding leaching effects of CNT-based membranes.

This study provides the possibility that when CNT-based membranes are operated in water and wastewater treatment systems, disturbance or disruption of microbial system in source water by membrane exposure and leached CNT fragments may not be as great a concern as commonly believed. Also from this study, highly densified VA-CNT solid membranes were shown to possess the highest anti-biofouling property among the various morphologies tested as well as high water permeability [18].

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