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Coating of Poly(ethylene glycol) for Prevention of Biofilm Formation on the Surface of Polyethylene Ventilation Devices

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To improve the biocompatibility of the polyethylene (PE) ventilation tubes for its applications in otolaryngology, poly(ethylene glycol)-acrylate (PEG-acrylate) was coated for prevention of biofilm formation via proteins adsorption on the PE surface. The PE film was surface-modified in advance with plasma treatment with oxygen and argon gases on the PE surfaces in film and tube types. PEG-acrylates was radical polymerized on the plasma-treated PE samples. The surface-modified samples were analyzed chemically with ATR-FTIR and XPS, and physically with SEM observation and contact angle measurement. While ATR-FTIR, XPS and SEM results showed new chemical peaks and smooth surface morphologies similar to those of the untreated controls, *in vitro* cultures of fibroblasts and bacteria showed less adhesion *in vitro* on the film samples than those of the unmodified control. The evaluations of the PE samples in tube type also showed reduction of adhesion of both cells and bacteria in rats on both the plasma-treated and PEG graft-polymerized surfaces compared to those of the untreated PE surfaces, indicating reduction of biofilms. Surface modification with oxygen plasma treatment and graft polymerization of PEG-acrylate on the PE tubes seemed to be an excellent technique for prevention of biofilm in ventilation tubes.

Key words: ventilation tubes, biofilm, polyethylene, surface modification, plasma treatment

Introduction

Since Armstrong's 1954 publication reporting his success in the application of ventilation tubes, the procedure has become the most popular surgical operation in otolaryngology clinics with an increasing number of applications in recent years.[1-3] For this reason, there has been a numerous number of research reports on ventilation tubes from either different materials or different surfaces and designs to reduce the occurrence of complications such as otorrhea, plugging complications, contamination and biofilm formation.[3] Biofilm is an adhesive aggregating covering on the biomaterial surfaces, consisting of hydrated bacterial exo-biopolymers, glycoproteins and many other macromolecules, and its formation depends on the areas of tube applications and the shapes and surface properties of the tube materials.[4-6]

Plasma treatment among the surface modification techniques could be utilized to change surface properties of ventilation tubes, targeting to reduction of adverse cells and bacteria adhesion on and improvement of biocompatibility of biomaterials.[7-11] It could control an interaction of the plasma-generated excited chemical species with a solid interface leading to a physicochemical modification of the first few molecular layers of the ventilation tube surface, while keeping the bulk properties intact.[12-14] Furthermore it could induce the polymer surfaces more hydrophilic and resistant to physicochemical attacks of protein adsorption.[15,16] It does not also require the use of complicated wet chemicals for modifications, being considered as an sterile technology, convenient characteristics especially of ventilation tubes.[17]

Diverse polymeric biomaterials have been surface-modified by plasma treatment by employing various plasma gases and treatment conditions depending on their applications. Among the applications of plasma treatment on polymer surfaces, poly (dimethylsiloxane) surface was modified by with argon gas and then with acrylic acid for DNA hybridization by grafting oligo-

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nucleotides, showing decrease in contact angle values, changes in chemical structures and stability of its surface properties.[18] Poly(ethylene terephthalate) has been surface-modified with nitrogen gas to cross-link its surface and to control its surface.[19] Biodegradable poly(L-lactide) has been surface-modified with diverse gases including NH_3 by controlling atmosphere, electric power, pressure and treatment time.[20] Repellence of cell adhesion on the plasma-treated poly(L-lactide) surface was observed by controlling shear stress field on the parallel plate flow chamber. Biodegradable macroporous poly(L-lactide) and poly(lactide-co-glycolide) scaffolds have been surface-modified for its tissue engineering applications to induce increase in cell seeding density, delivery of nutrient and oxygen and increase in cell affinity.[21] Polyethylene (PE) has also been surface-modified by plasma treatment and characterized by measuring surface free energy and observation of oxygen-containing species.[22] Despite these excellent characteristics, PE is often unsuitable for use because of its low surface free energy, thus leading to formation of biofilms through microorganisms and cellular aggregations in ventilation tubes.[7,23-25] It also causes irreversible contamination of ventilation tubes and an increased resistance to antibiotic penetration in patients.[26-28]

To prevent biofilm formation and other complications in ventilation tubes, we here worked on surface modification of PE films and tubes, and then graft polymerization of polyethylene glycol (PEG). PEG has been known to have biocompatible properties to resist adsorption of proteins and bacterias.[29,30] Surface-modified and graft-polymerized surfaces of PE samples were evaluated by physicochemical analyses and *in vitro/in vivo* assays for their possible applications in ventilation tubes.

Materials and Methods

Materials

While polyethylene (PE) film was purchased from Namil Enpla Co., Ltd. (Korea), PE tube (1.1 mm I.D.) was obtained from Tecfen Corporation (St. Paul, MN 55121, USA). Benzene, acrylic acid (Ac), dicyclohexylcarbodiimide (DCC), dimethylsulfoxide (DMSO), poly(ethylene glycol) diacrylate (PEG-AC) and 2,2-dimethoxy-2-phenyl acetonphenone (DMPA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Argon and oxygen gas (99.9%, Daeheung Gas, Seoul, Korea) was purchased as plasma gases.

Surface treatment of polyethylene tube with oxygen plasma

After loading the PE tubes (n = 10) and films (n = 15) in a plasma reactor, low pressure plasma reactor (PTS-003IDT, I.D.T. ENG., Inc., Korea), the gas chamber was purged with argon gas at a flow rate of 100 sccm for 5 min. Gas concentration, pressure, treatment time, power were controlled for modifications of the PE samples.

Surface modification of PE samples with argon and oxygen plasma

After loading the PE samples in the low pressure plasma reactor at room temperature, and then purging the chamber with argon gas with 45 sccm flow rate for 5 min, surface modification of PE samples was processed by generating radio frequency glow discharge by supplying powder to the electrodes located outside the chamber, i.e. either oxygen or argon by generating either 100 W for 6 min with supplying 100 sccm or 200 W for 5 min with supplying 45 sccm, respectively. Gas concentration, pressure, treatment time, and power were controlled outside for their modifications.

Grafting of poly(ethylene glycol) on plasma-treated PE samples

PEG-diacrylate on the oxygen plasma-treated PE tube was graft-polymerized by irradiating ultraviolet light (λ_{max} = 352 nm, 40 W, F40T10BLB, Sankyo Denki, Japan) on the PEG-diacrylate solution for 5 min on the plasma-treated PE tube as below. PEG-diacrylate solution was in advance obtained by adding 6% PEG-diacrylate and 0.3% DMPA initiators in benzene. PEG-graft-polymerized PE samples were dried in vacuum oven overnight, and then the PEG-grafted PE samples were washed with 30 mL benzene for 1 hr by shaking with 70 rpm (Rotamax 120, Heidolph, Germany).

Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR)

To observe surface modification of PE samples, ATR-FTIR spectra of the PE filmes without treatment and with plasma treated and PEG-AC graft-polymerized were recorded at the wavelength of 650 to 4000 cm^{-1} with a spectrometer (Travel IR; Smiths, USA). While a diamond crystal refractive index was measured as 2.4 at 45° incidence angle, the ATR depth of penetration was controlled as about 2 μm.

Contact angle measurement

0.025 mL distilled water drops were placed by using 200 μl pipet (Eppendorf, Hamburg, Germany) on each PE film (n = 2) without any treatment, and with plasma treated and PEG-AC graft-polymerized surfaces (n = 2), thus measuring 4 locations on each conditions. Static contact angles were measured by using a contact angle meter (G-1, Erma Inc., Japan).

Scanning electron microscopy (SEM)

Dry PE tubes were gold sputter-coated in plasma for 60 sec, thus forming approximately 200°A thickness. The PE tubes fixed on the aluminum stage were inserted into a vacuum chamber of the SEM (JSM-6400; JEOL Ltd, Japan). Morphological images of both the outer and inner surfaces of the PE tubes were obtained by magnifying 10-500 times to observe their morphologies under vacuum.

X-ray photoelectron spectroscopy (XPS)

XPS was performed with an M-Probe Surface Spectrometer (K-α, Thermo Scientific, UK). A monochromatic Al K-α X-ray source was employed, and measurements were taken with a resolution 4 at a 55° take-off angle. A flood gun was employed for charge neutralization on the sample surfaces, using the minimum energy feed possible. Survey scans and high resolutions of the samples were observed and the 285.0 eV on a C1s peak was generally fixed as a reference for the other peaks.

In vitro cell culture

Fibroblasts were cultured in FGM-2 media (Lonza, Switzerland) containing 10% fetal bovine serum, 1% penicillin/streptomycin in the *in vitro* incubator with 5% CO_2 at 37°C. The film samples of untreated PE and PEG-AC graft-polymerized (r = 0.5) were in advance sterilized by ethylene oxide gas sterilizer (DWM-EOG 630L/310S, Daewoon Medical,Korea). *In vitro* cell culture was performed by seeding fibroblast on the film surface of untreated PE, PEG-AC graft-polymerized PE (oxygen plasma-treated), and PEG-AC graft-polymerized PE (argon plasma-treated) at a density of either 1×10^6 cells/well, respectively, for 7 days.

Bacterial strain and growth conditions

S. pneumoniae R6 strain (ATCC BAA-255) used in this study was obtained from the American Type Culture Collection (Manassas, VA, USA). Bacteria were grown on a blood agar plate with 5% sheep's blood. A fresh colony was transferred in trypticase soy broth (TSB) and grown at 37°C for 12 h in 5% CO_2.

In vitro identification of reduction activity of biofilm formation (Microplate assay)

In vitro test was employed using 96 well microplate (BD Falcon, Sparks, MD, USA). Streptococcus pneumonia was obtained at 1×10^8 CFU/ml (OD 600 = 0.4) in mid-logarithmic phase and diluted with 1:100. The 200 µl of that was inoculated in 96 well microplate, containing TSB culture medium. After inserting PE ventilation tube sample in the medium plate, *in vitro* culture was performed in the incubator with 5% CO_2 at 37°C for 18 hours. After removing the ventilation tube samples, the 96 well microplate was washed and dried by air. Using 0.2% crystal violet of 100 µl, ventilation tubes were dyed for 15 minutes and then washed by distilled water. Crystal violet dyed on ventilation tube was dissolved by 95% ethanol of 200 µl and the absorbance is measured at the wavelength of about 570 nm. (SpectraMax plus 384 microplate reader, Molecular Devices, Sunnyvale, CA, USA).

In vivo observation of inhibition activity of biofilm formation in animal model

In vivo test of the PE ventilation tubes was employed using SD rat (Orient bio, Kyeonggi, Korea) that was 150 to 200 g. The results showed no middle ear pathology. After anesthesia was employed by tiletamine/zolazepam (Zoletil, Virbac Lab, France) (0.02 ml/100 mg), culture medium of 50 µl containing Streptococcus pneumonia of 3×107 was injected into the middle ear cavity with implementing otoscopy by tuberculin syringe and 27-gauge needle. Using an operating microscope and pick, ventilation tube was inserted into tympanic membrane. After 2 weeks, ventilation tubes were removed and the 96 well microplate was washed and then dried by air. Using 0.2% crystal violet of 100 µl, ventilation tube samples were dyed for 15 minutes and then washed by distilled water. Crystal violet dyed on ventilation tube samples were dissolved by 95% ethanol of 200 µl and the absorbance was measured at the wavelength of about 570 nm (SpectraMax plus 384 microplate reader, Molecular Devices, Sunnyvale, CA, USA).

Results and Discussion

Chemical Analyses of PE samples

Surface modification of the PE samples such as unmodified control, oxygen plasma-treated and PEG-AC graft-polymerized PE samples was chemically analyzed with ATR-FTIR and XPS. ATR-FTIR spectra demonstrated its surface modification as shown by the new peaks on the oxygen plasma-treated PE samples (Figure 1B) over the unmodified control (Figure 1A). While the unmodified sample demonstrated its characteristic hydrocarbon peaks at 3000~2850 cm^{-1}, new weak peaks on the plasma-treated samples were observed at the wavelengths of 1680~1600 cm^{-1}, which may indicate C=C bonds. This weak peak may be from the effects of air exposure. Next, the

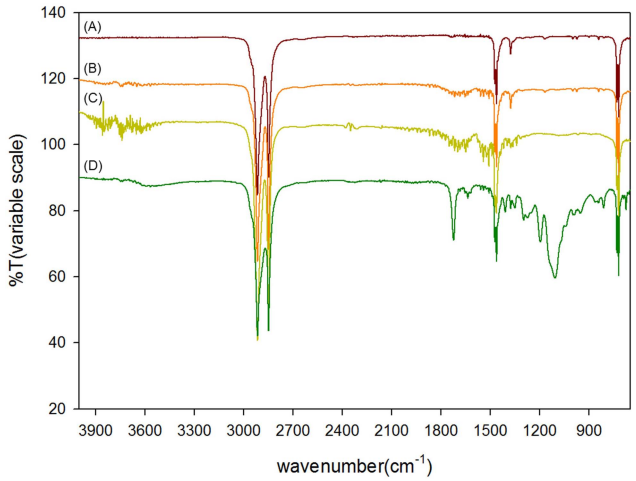

Figure 1. ATR-FTIR spectra of the PE films; untreated (A), oxygen plasma-treated (B), argon plasma-treated (C) and PEG-AC graft-polymerized on the sample B (D). The plasma was treated by generating either 100 W for 6 min with supplying 100 sccm of oxygen gas or 200 W for 5 min with supplying 45 sccm of argon gas, respectively.

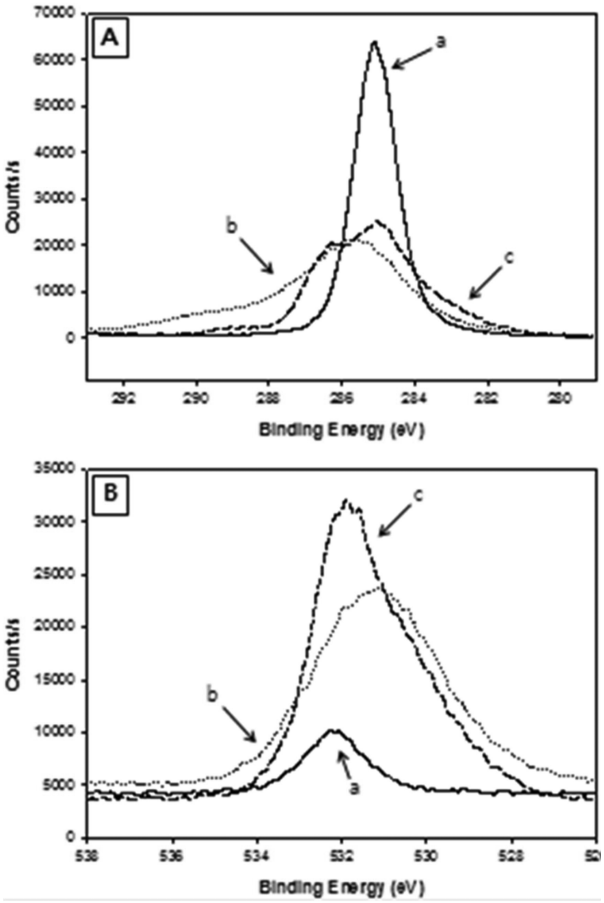

Figure 2. XPS spectra of C1s (A) and O1s (B) of the PE films: the untreated film (a), the PE films treated with oxygen plasma (b), and graft-polymerized with PEG-AC on sample B (c).

PEG-AC graft-polymerized PE samples demonstrated distinct amount of new oxygen peaks at the positions of 1726 and 2866 cm^{-1}, which were the peaks of ethylene oxide chemical structures of PEO-AC. Similar changes in the chemical structures of PE samples were observed by the analyses of XPS. While the unmodified PE sample showed its characteristic hydrocarbon peak at 285 eV (Figure 2A-a), the oxygen plasma-treated PE samples did new very broad C1s peak (Figure 2A-b), indicating incorporation of diverse oxygen species such as carboxylic acid, carbonyl groups, hydroxyl groups and etc. Graft polymerization of PEG-AC on the plasma-treated PE samples showed shift of the C1s peaks to characteristic poly (ethylene glycol) peaks at around 286.5 eV (Figure 2A-c). O1s peaks of the unmodified, plasma-treated and PEG-AC graft-polymerized PE samples indicated very small amount of oxygen peak, broad oxygen peak and narrow ether peak, corresponding to those of C1s spectra (Figure 2B-a, b, c). Small amount of oxygen species on the control PE samples may be from contaminants during analysis or/and in the possibly synthesis-related PE tubes.[31,32)]

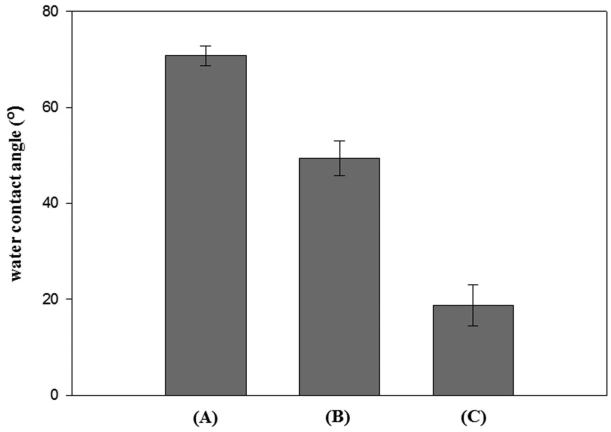

Figure 3. Water contact angles of the untreated PE film (A), the PE films treated with oxygen plasma (B), and graft-polymerized with PEG-AC on sample B (C).

Surface wettability of PE samples

Surface tensions of the PE samples were analyzed by measuring water contact angles. Plasma treatment turned the sample surface from hydrophobic to hydrophilic, i.e. while the control PE samples had 70.75° contact angle, the plasma-treated ones had 49.38°. The PEG-AC graft-polymerized samples demonstrated complete water spreading on its surface and its contact angle was measured as 18.75°. Lower contact angle values indicated higher surface interaction between water and sample surface, leading to hydrophylic (Figure 3).

Morphological analysis by scanning electron microscopy

Surface morphologies of PE tubes were investigated with SEM by comparing that of control with the plasma-treated and PEG-AC graft-polymerized ones. The unmodified control showed smooth surface (Figure 4A). While the surface of the oxygen-plasma-treated PE samples was changed to rough one (Figure 4B), the surface of the argon plasma-treated one was split (Figure 4C). It has been speculated that energy was strong enough to make the surfaces cracked when either plasma treatment was employed or the beam was irradiated on the sample surface during SEM observation. After graft polymerization with PEG-AC, the surface was observed to be smooth and on the surface PEG residue was also observed as PEG concentration as high to be grafted on the plasma-treated ones (Figure 4D).

Observation of *in vitro* biofilm formation

A quantitative analysis of *in vitro* biofilm formation by *S. pneumoniae* on ventilation tubes was performed. The mean OD values of the uncoated ventilation tube samples were measured as 0.34 ± 0.09. And while that from PEG-AC graft-polymerized one (oxygen plasma-treated) was measured as 0.22 ± 0.06, that from PEG-AC graft-polymerized one (argon plasma-treated) was as 0.23 ± 0.06. Their results between the

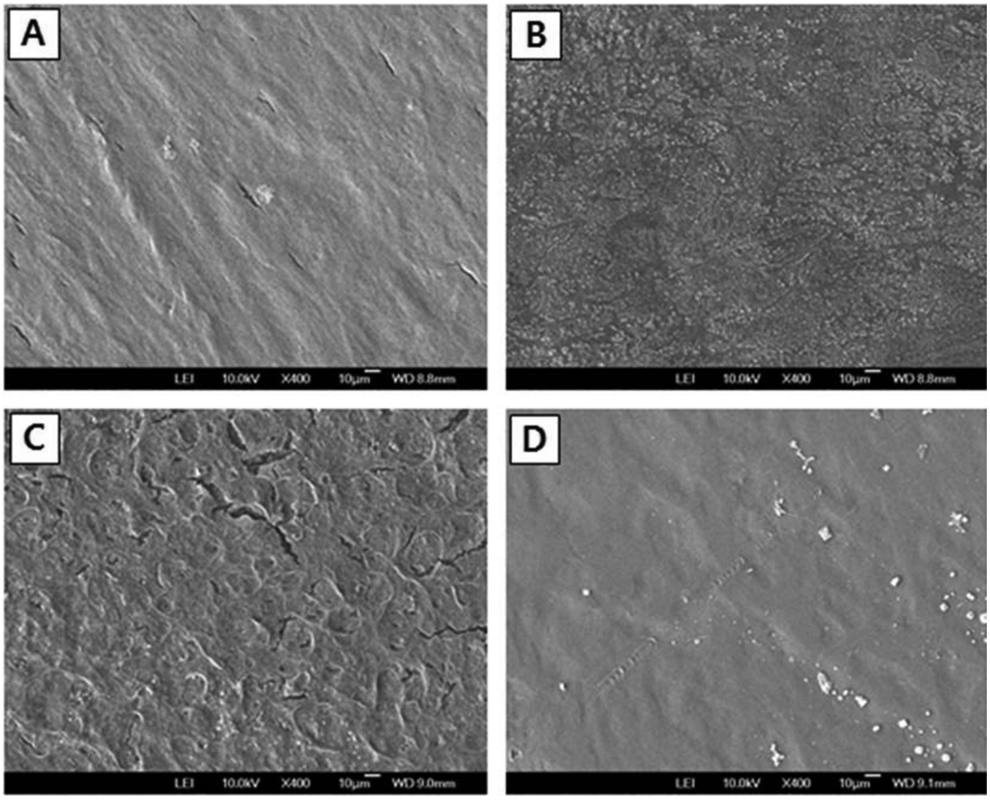

Figure 4. SEM Morphologies of the PE films: untreated films (A), the PE films plasma-treated with either oxygen (B) or argon (C) gas and graft-polymerized with PEG-AC on the sample B (D).

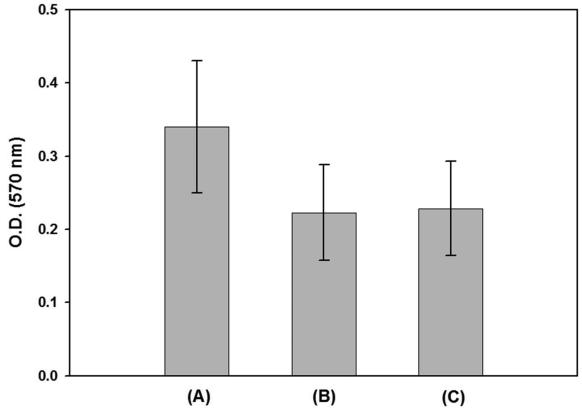

Figure 5. Cell proliferation of the PE films after *in vitro* culture of fibroblasts for 7 days: the untreated film (A), the PE films (oxygen plasma-treated) (B), and PEO-AC graft-polymerized on the oxygen plasma-treated sample (C) measured by CCK-8.

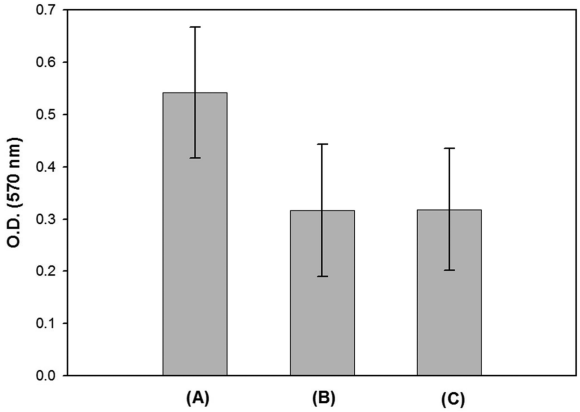

Figure 6. OD values of the PE tubes after implant in rats; untreated (A), PE films (oxygen plasma-treated) (B) and PEO-AC graft-polymerized on the oxygen plasma-treated sample (C) measured by CCK-8.

control and PEG-AC graft polymerized ones were significantly different in statistics ($p < 0.05$) (Figure 5), but those between plasma treated with oxygen and argon gases were not.

Observation of *in vivo* biofilm formation

A quantitative analysis of *in vivo* biofilm formation by *S. pneumoniae* on ventilation tube samples was performed. The mean OD values of the uncoated ventilation tubes were measured as 0.54 ± 0.12. And while the OD value of the PEG-AC graft-polymerized one (oxygen plasma-treated) was measured as 0.32 ± 0.13, that of the PEG-AC graft-polymerized one (argon plasma-treated) was measured as 0.32 ± 0.12. Their results between the control and PEG-AC graft polymerized ones were significantly different in statistics ($p < 0.05$), but those between plasma treated with oxygen and argon gases were not (Figure 6).

Conclusions

Surface modifications of PE films and tubes with both plasma treatment and PEG-AC graft polymerization induced changes in both chemical structures and physical properties such and surface tension and morphologies. While the oxygen plasma treatment induced chemical modifications of polyethylene with generation of oxygen-containing groups, further PEG-AC graft polymerization of the plasma-treated surfaces showed coating of PEG-AC on the PE tube/film surface. Furthermore, oxygen plasma treatment induced substantial reduction of water contact angles on their surfaces, and PEG graft polymerization did further reduction of static contact angles, leading to complete water spreading. The morphologies on their surface showed that each process of surface modifications of plasma treatment and PEG-AC graft polymerization induced significant changes in chemical structures and morphologies.

And in biological tests, we evaluated prevention of biofilm by *in vitro* seeding fibroblasts on the PE films and implanting the PE tubes in rats. It was confirmed that the results showed decrease in both adsorption of *in vitro* fibroblasts culture and *in vivo* biofilm formation by *S. pneumoniae* depending on the surface modification methods. The PEG-AC graft-polymerized samples showed the maximal prevention of cell adhesion and biofilm formation. These results indicated that the sequential surface modification of plasma treatment and graft polymerization of PEG-AC with radical polymerization may be a useful method for prevention of *in vivo* biofilm formation by *S. pneumoniae* on ventilation tube.

Acknowledgements

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