

## REFERENCES

- [1] Decher, G. "Fuzzy nanoassemblies: toward layered polymeric multicomposites" *Science* **1997**, *277*, 1232-1237.
- [2] Shahidi, F.; Kamil, J.; Arachchi, V.; Jeon, Y.J. "Food applications of chitin and chitosans" *Trends Food Sci. Technol.* **1999**, *10*, 37-51.
- [3] Sashiwa, H.; Saimoto, H.; Shigemasa, Y.; Ogawa, R.; Tokura, S. "Lysozyme susceptibility of partially deacetylated chitin" *Int. J. Biol. Macromol.* **1990**, *12*, 295-296.
- [4] Shigemasa, Y.; Saito, K.; Sashiwa, H.; Saimoto, H. "Enzymatic degradation of chitins and partially deacetylated chitins" *Int. J. Biol. Macromol.* **1994**, *16*, 43-49.
- [5] Iwasaki, N.; Yamane, S. T.; Majima, T.; Kasahara, Y.; Minami, A.; Harada, K. "Feasibility of polysaccharide hybrid materials for scaffolds in cartilage tissue engineering: evaluation of chondrocyte adhesion to polyion complex fibers prepared from alginate and chitosan" *Biomacromolecules* **2004**, *5*, 828-833.
- [6] Rabea, E.I.; Badawy, M.E.T.; Stevens, C.V.; Smagghe, G.; Steurbaut, W. "Chitosan as antimicrobial agent: applications and mode of action" *Biomacromolecules* **2003**, *4*, 1457-1465.
- [7] Seong, H. S.; Whang, H. S.; Ko, S. W. "Synthesis of a quaternary ammonium derivative of chito-oligosaccharide as antimicrobial agent for cellulosic fibers" *J. Appl. Polym. Sci.* **2000**, *76*, 2009-2015.
- [8] Lim, S. H.; Hudson, S. M. "Synthesis and antimicrobial activity of a water-soluble chitosan derivative with a fiber-reactive group" *Carbohydr. Res.* **2004**, *339*, 313-319.
- [9] Kato, Y.; Onishi, H.; Machida, Y. "N-succinyl-chitosan as a drug carrier: water-insoluble and water-soluble conjugates" *Biomaterials* **2004**, *25*, 907-915.

- [10] Amiji, M. M. "Platelet adhesion and activation on an amphoteric chitosan derivative bearing sulfonate groups" *Colloids Surf., B* **1998**, *10*, 263-271.
- [11] Vongchan, P.; Sajomsang, W.; Subyen, D.; Kongtawelert, P. "Anticoagulant activity of a sulfated chitosan" *Carbohydr. Res.* **2002**, *337*, 1239-1242.
- [12] Channasanon, S.; Graisuwan, W.; Kiatkamjornwong, S.; Hoven, V. P. "Alternating bioactivity of multilayer thin films assembled from charged derivatives of chitosan" *J. Colloid Interface Sci.* **2007**, *316*, 331-343.
- [13] Brugnerotto, J.; Lizardi, J.; Goycoolea, F. M.; Argüelles-Monal, W.; Desbrières, J.; Rinaudo, M. "An infrared investigation in relation with chitin and chitosan characterization" *Polymer* **2001**, *42*, 3569-3580.
- [14] Lavertu, M.; Xia, Z.; Serreqi, A.N.; Berrada, M.; Rodrigues, A.; Wang, D.; Buschmann, M.D.; Gupta, A. "A validated  $^1\text{H}$  NMR method for the determination of the degree of deacetylation of chitosan" *J. Pharm. Biomed. Anal.* **2003**, *32*, 1149-1158.
- [15] Jiang, X.; Chen, L.; Zhong, W. "A new linear potentiometric titration method for the determination of deacetylation degree of chitosan" *Carbohydr. Polym.* **2003**, *54*, 457-463.
- [16] Rinaudo, M.; Pavlov, G.; Desbrieres, J. "Influence of acetic acid concentration on the solubilization of chitosan" *Polymer* **1999**, *40*, 7029-7032.
- [17] Sorlier, P.; Denuziere, A.; Viton, C.; Domard, A. "Relation between the degree of acetylation and the electrostatic properties of chitin and chitosan" *Biomacromolecules* **2001**, *2*, 765-772.
- [18] Strand, S. P.; Tommeraas, K.; Varum, K. M.; Ostgaard, K. "Electrophoretic light scattering studies of chitosans with different degrees of N-acetylation" *Biomacromolecules* **2001**, *2*, 1310-1314.

- [19] Anthonsen, M. W.; Smidsrod, O. "Hydrogen-ion titration of chitosans with varying degrees of *N*-acetylation by monitoring induced  $^1\text{H-NMR}$  chemical-shifts" *Carbohydr. Polym.* **1995**, *26*, 303-305.
- [20] Varum, K. M.; Ottoy, M. H.; Smidsrod, O. "Water-solubility of partially *N*-acetylated chitosans as a function of pH: Effect of chemical composition and depolymerization" *Carbohydr. Polym.* **1994**, *25*, 65-70.
- [21] Kim, J. Y.; Lee, J. K.; Lee, T. S.; Park, W. H. "Synthesis of chitooligosaccharide derivative with quaternary ammonium group and its antimicrobial activity against *Streptococcus mutans*" *Int. J. Biol. Macromol* **2003**, *32*, 23-27.
- [22] Sashiwa, H.; Shigemasa, Y. "Chemical modification of chitin and chitosan 2: preparation and water soluble property of *N*-acylated or *N*-alkylated partially deacetylated chitins" *Carbohydr. Polym.* **1999**, *39*, 127-138.
- [23] Aoki, N.; Nishikawa, M.; Hattori, K. "Synthesis of chitosan derivatives bearing cyclodextrin and adsorption of *p*-nonylphenol and bisphenol A" *Carbohydr. Polym.* **2003**, *52*, 219-223.
- [24] Aiping, Z.; Tian, C.; Lanhua, Y.; Hao, W.; Ping, L. "Synthesis and characterization of *N*-succinyl-chitosan and its self-assembly of nanospheres" *Carbohydr. Polym.* **2006**, *66*, 274-279.
- [25] Aiping, Z.; Lanhua, Y.; Tian, C.; Hao, W.; Feng, Z. "Interactions between *N*-succinyl-chitosan and bovine serum albumin" *Carbohydr. Polym.* **2007**, *69* 363-370.
- [26] Zhang, C.; Ping, Q.; Zhang, H.; Shen, J. "Synthesis and characterization of water-soluble *O*-succinyl-chitosan" *Eur. Polym. J.* **2003**, *39*, 1629-1634.
- [27] Muzzarelli, R. A. A. "Modified chitosans carrying sulfonic acid groups" *Carbohydr. Polym.* **1992**, *19*, 231-236.
- [28] Gregorio, C.; Giangiacomo, T.; Marco, G.; Michel, M.; Marek, W.; Bernard, M. "NMR characterization of *N*-benzyl sulfonated derivatives of chitosan" *Carbohy. Polym.* **1997**, *33*, 145-151.

- [29] Jayakumar, R.; Nwe, N.; Tokura, S.; Tamura, H. "Sulfated chitin and chitosan as novel biomaterials" *Int. J. Biol. Macromol.* **2007**, *40*, 175-181.
- [30] Fleer, J.G.; Cohen, S.A.M.; Scheutjens, M.H.M.J.; Cosgrove, T.; Vincent, B. "Polymer at interfaces" London: Chapman and Hall **1993**.
- [31] Caruso, F.; Furlong, D.N.; Ariga, K.; Ichinose, I.; Kunitake, T. "Characterization of polyelectrolyte-protein multilayer films by atomic force microscopy, scanning electron microscopy, and Fourier transform infrared reflection-absorption spectroscopy" *Langmuir* **1998**, *14*, 4559-4565.
- [32] Krasemann, L.; Tieke, B. "Composite membranes with ultrathin separation layer prepared by self-assembly of polyelectrolytes" *Mater. Sci. Eng., C* **1999**, *8-9*, 513-518.
- [33] Wu, A.; Yoo, D.; Lee, J.K.; Rubner, M.F. "Solid-state light-emitting devices based on the tris-chelated ruthenium (II) complex: 3. high efficiency devices via a layer-by-layer molecular-level blending approach" *J. Am. Chem. Soc.* **1999**, *121*, 4883-4891.
- [34] Mao, J. S.; Cui, Y. L.; Wang, X. H.; Sun, Y.; Yin, Y. Ji.; Zhao, H. M.; Yao, K. D. "A preliminary study on chitosan and gelatin polyelectrolyte complex cytocompatibility by cell cycle and apoptosis analysis" *Biomaterials* **2004**, *25*, 3973-3981.
- [35] Serizawa, T.; Yamaguchi, M.; Matsuyama T.; Akashi, M. "Alternating bioactivity of polymeric layer-by-layer assemblies: anti-vs procoagulation of human blood on chitosan and dextran sulfate layers" *Biomacromolecules* **2000**, *1*, 306-309.
- [36] Serizawa, T.; Yamaguchi, M.; Akashi, M. "Alternating bioactivity of polymeric layer-by-layer assemblies: anti-vs procoagulation of human blood on chitosan and dextran sulfate layers" *Biomacromolecules* **2002**, *3*, 724-731.

- [37] Elbert, D. L.; Herbert, C. B.; Hubbell, J. A. "Thin polymer layers formed by polyelectrolyte multilayer techniques on biological surfaces" *Langmuir* **1999**, *15*, 5355-5363.
- [38] Fischer, P.; Laschewsky, A. "Layer-by-layer adsorption of identically charged polyelectrolytes" *Macromolecules* **2000**, *33*, 1100-1102.
- [39] Schoeler, B.; Kumaraswamy, G.; Caruso, F. "Investigation of the influence of polyelectrolyte charge density on the growth of multilayer thin films prepared by the layer-by-layer technique" *Macromolecules* **2002**, *35*, 889-897.
- [40] Dubas, S.T.; Schlenoff, J. "Factors controlling the growth of polyelectrolyte multilayers" *Macromolecules* **1999**, *32*, 8153-8160.
- [41] Chen, W.; McCarthy, T. J. "Layer-by-layer deposition: a tool for polymer surface modification" *Macromolecules* **1997**, *30*, 78-86.
- [42] Du, W.W. "Electrostatic self-assembly of biocompatible thin films" *Thesis; M.Sc. Mat. Sci. Eng. Virginia Polytechnic Institute and State University*, **2000**, 22.
- [43] Cai, K.; Rechtenbach, A.; Hao, J.; Bossert, J.; Jandt, K. D. "Polysaccharide-protein surface modification of titanium via a layer-by-layer technique: Characterization and cell behaviour aspects" *Biomaterials* **2005**, *26*, 5960-5971.
- [44] Elbert, D.L.; Hubbell, J.A. "Surface treatments of polymers for biocompatibility" *Annu. Rev. Mater. Sci.* **1996**, *26*, 365-394.
- [45] Drotleff, S.; Lungwitz, U.; Breunig, M.; Dennis, A.; Blunk, T.; Tessmar, J.; Göpferich, A. "Biomimetic polymers in pharmaceutical and biomedical sciences" *Eur. J. Pharm. Biopharm.* **2004**, *58*, 385-407.
- [46] Ma, Z.; Mao, Z.; Gao, C. "Surface modification and property analysis of biomedical polymers used for tissue engineering" *Colloids Surf., B* **2007**, *60*, 137-157.

- [47] LeBaron, R.G.; Athanasiou, K.A. "Extracellular matrix cell adhesion peptides: functional applications in orthopedic materials" *Tissue Eng.* **2000**, *6*, 85-103.
- [48] Massia, S.P.; Hubbell, J.A. "An RGD spacing of 440 nm is sufficient for integrin alpha V beta 3-mediated fibroblast spreading and 140 nm for focal contact and stress fiber formation" *J. Cell Biol.* **1991**, *114*, 1089-1100.
- [49] Boudreau, N.J.; Jones, P.L. "Extracellular matrix and integrin signaling: the shape of things to come" *Biochem. J.* **1999**, *339*, 481-488.
- [50] Guy, L.; Csilla, G.; Bernard, S.; Gero, D.; Jean-Claude, V.; Pierre, S.; Frederic, J. G. C. "Protein interactions with polyelectrolytes multilayers: interactions between human serum albumin and polystyrene sulfonate/polyallylamine multilayers" *Biomacromolecules* **2000**, *4*, 674-687.
- [51] Boura, C.; Menu, P.; Payan, E.; Picart, C.; Voegel, J.C.; Muller, S.; Stoltz, J.F. "Endothelial cells grown on thin polyelectrolyte multilayered films: an evaluation of a new versatile surface modification" *Biomaterials* **2003**, *24*, 3521-3530.
- [52] Boura, C.; Muller, S.; Vautier, D.; Dumas, D.; Schaaf, P.; Voegel, J. C.; Stoltz, J. F.; Menu, P. "Endothelial cell-interactions with polyelectrolyte multilayer films" *Biomaterials* **2005**, *26*, 4568-4575.
- [53] Zhu, H.; Ji, J.; Shen, J. "Construction of multilayer coating onto poly-(DL-lactide) to promote cytocompatibility" *Biomaterials* **2004**, *25*, 109-117.
- [54] Köstler, S.; Delgado, A.V.; Ribitsch, V. "Surface thermodynamic properties of polyelectrolyte multilayers" *J. Colloid Interface Sci.* **2005**, *286*, 339-348.
- [55] Kolasińska, M.; Warszyński, P. "The effect of support material and conditioning on wettability of PAH/PSS multilayer films" *Bioelectrochemistry* **2005**, *66*, 65-70.

- [56] Lin, Y.; Wang, L.; Zhang, P.; Wang, X.; Chen, X.; Jing, X.; Su, Z.; "Surface modification of poly(L-lactic acid) to improve its cytocompatibility via assembly of polyelectrolytes and gelatin" *Acta Biomater.* **2006**, *2*, 155-164.
- [57] Serizawa, T.; Yamaguchi, M.; Kishida, A.; Akashi, M. "Alternating gene expression in fibroblasts adhering to multilayers of chitosan and dextran sulfate" *J. Biomed. Mater. Res.* **2003**, *67A*, 1060-1063.
- [58] Zhu, Y.; Gao, C.; He, T.; Liu, X.; Shen, J. "Layer-by-layer assembly to modify poly(L-lactic acid) surface toward improving its cytocompatibility to human endothelial cells" *Biomacromolecules* **2003**, *4*, 446-452.
- [59] Feng, Q.; Zeng, G.; Yang, P.; Wang, C.; Cai, J. "Self-assembly and characterization of polyelectrolyte complex films of hyaluronic acid/chitosan" *Colloids Surf., A* **2005**, *257-258*, 85-88.
- [60] Fu, J.; Ji, J.; Yuan, W.; Shen, J. "Construction of anti-adhesive and antibacterial multilayer films via layer-by-layer assembly of heparin and chitosan" *Biomaterials* **2005**, *26*, 6684-6692.
- [61] Liu, Y.; He, T.; Gao, C. "Surface modification of poly(ethylene terephthalate) via hydrolysis and layer-by-layer assembly of chitosan and chondroitin sulfate to construct cytocompatible layer for human endothelial cells" *Colloids Surf., B* **2005**, *46*, 117-126.
- [62] Baba, A.; Kaneko, F.; Advincula, R.C. "Polyelectrolyte adsorption processes characterized in situ using the quartz crystal microbalance technique: alternate adsorption properties in ultrathin polymer films" *Colloids Surf., A* **2000**, *173*, 39-49.
- [63] Marx, K.A. "Quartz crystal microbalance: a useful tool for studying thin polymer films and complex biomolecular systems at the solution-surface interface" *Biomacromolecules* **2003**, *4*, 1099-1120.

- [64] Sauerbrey, G. "Use of quartz crystal vibrator for weighting thin films on a microbalance" *Z. Phys.* **1959**, *155*, 206-222.
- [65] QSENSE-Application[Online].  
Available from: <http://www.q-sense.com/applications--2.asp> [2008, February 19].
- [66] Xiao, S.J. "Tailored organic thin films on gold and titanium: peptide-grafting, protein resistance and physical characterization" *Thesis; M. Sc. Chemistry, Fudan University*, **1999**.7.
- [67] FT-IR spectroscopy: Attenuated total reflectance (ATR) technical note[Online]. Available from: <http://www.perkinelmer.com> [2008, April 8].
- [68] J.W. Cross. Review of SPM techniques: Scanning probe microscopy[Online]. Available from: <http://www.mobot.org/jwcross/spm/> [2008, April 8].
- [69] Elliott, W.C.; Miceli, P.F.; Tse, T.; Stephens, P.W. "Temperature and orientation dependence of kinetic roughening during homoepitaxy: a quantitative X-ray scattering study of Ag" *Phys. Rev. B* **1996**, *54*, 17938-17942.
- [70] Mosmann, T. "Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays" *J. Immunol. Methods.* **1983**, *16*, 55-63.
- [71] Lvov, Y.; Ariga, K.; Onda, M.; Ichinose, I.; Kunitake, T.; "A careful examination of the adsorption step in the alternate layer-by-layer assembly of linear polyanion and polycation" *Colloids Surf., A*, **1999**, *146*, 337-346.
- [72] Lojou, E.; Bianco, P. "Buildup of polyelectrolyte-protein multilayer assemblies on gold electrodes. Role of the hydrophobic effect" *Langmuir* **2004**, *20*, 748-755.
- [73] Notley, S.M.; Eriksson, M.; Wågberg, L. "Visco-elastic and adhesive properties of adsorbed polyelectrolyte multilayers determined in situ



- with QCM-D and AFM measurements” *J. Colloid Interface Sci.* **2005**, *292*, 29-37.
- [74] Lowman, G. M.; Buratto, S. K. “Nanoscale morphology of polyelectrolyte self-assembled films probed by scanning force and near-field scanning optical microscopy” *Thin Solid Films* **2002**, *405*, 135-140.
- [75] Mattanavee, W. “Immobilization of biomolecules on surface of polycaprolactone for artificial skin application” *Thesis; M. Sc. Petrochemistry and Polymer Science, Chulalongkorn University*, **2005**, 76-77.

## **APPENDICES**

## APPENDIX A

**Table A-1** Frequency shift,  $\Delta F$  (Hz) of QCM of three pairs of multilayer film in the presence of 0.5M NaCl.

Number of Layers	Frequency Shift, $\Delta F$ (Hz)		
	(HTACC-PAA)	(PAH-SCC)	(PAH-SFC)
1	20	29	12
2	28	68	46
3	61	93	67
4	74	153	140
5	128	181	161
6	143	277	250
7	241	317	281
8	270	417	413
9	478	472	465
10	498	630	616

**Table A-2** Water contact angle of treated PET-(HTACC-PAA)<sub>n</sub> assemblies, 0.5 M NaCl was added to both polyelectrolyte solutions.

Top layer	Number of layers (n)	Water contact angle (°)
Treated PET	0	63.6 ± 1.4
HTACC	9	73.4 ± 6.8
PAA	10	58.7 ± 4.6
HTACC	13	73.6 ± 6.8
PAA	14	56.8 ± 2.6
HTACC	15	72.7 ± 2.9
PAA	16	56.6 ± 3.0

**Table A-3** Water contact angle of treated PET-(PAH-SCC)<sub>n</sub> assemblies, 0.5 M NaCl was added to both polyelectrolyte solutions.

Top layer	Number of layer (n)	Water contact angle (°)
Treated PET	0	63.6 ± 1.4
PAH	9	107.1 ± 4.2
SCC	10	97.2 ± 4.3
PAH	13	111.0 ± 6.1
SCC	14	96.4 ± 6.0
PAH	15	110.5 ± 2.2
SCC	16	95.0 ± 3.2

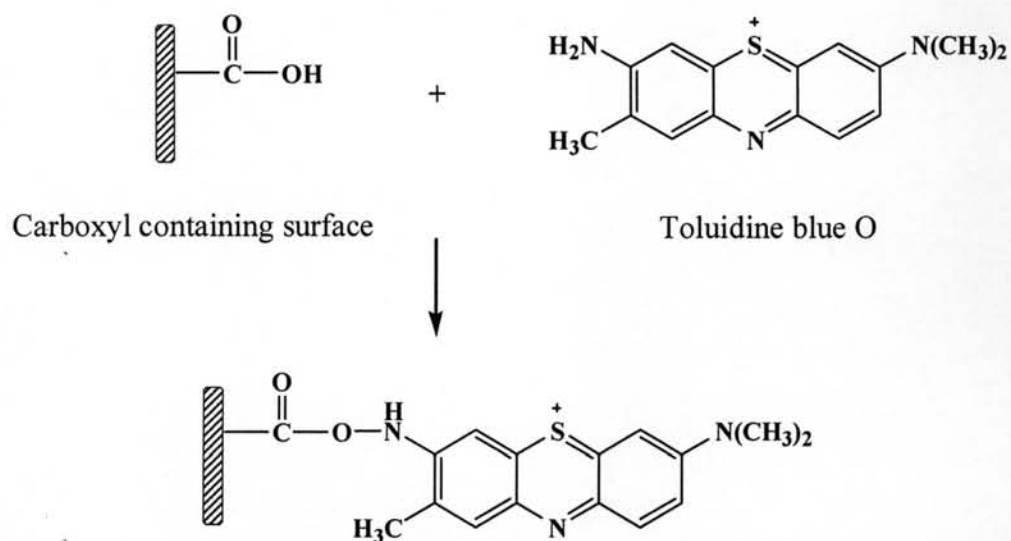
**Table A-4** Water contact angle of treated PET-(PAH-SFC)<sub>n</sub> assemblies, 0.5 M NaCl was added to both polyelectrolyte solutions.

<b>Top layer</b>	<b>Number of layer (n)</b>	<b>Water contact angle (°)</b>
Treated PET	0	63.6 ± 1.4
PAH	9	101.4 ± 7.6
SFC	10	85.4 ± 8.4
PAH	13	106.6 ± 3.3
SFC	14	87.1 ± 8.0
PAH	15	108.1 ± 4.0
SFC	16	89.6 ± 4.2

## APPENDIX B

### Toluidine blue O assay

Toluidine blue O assay is a method used for determination of the amount of carboxyl groups. The complex formed by the carboxyl group and toluidine blue O has the  $\lambda_{\text{max}}$  at 633 nm.



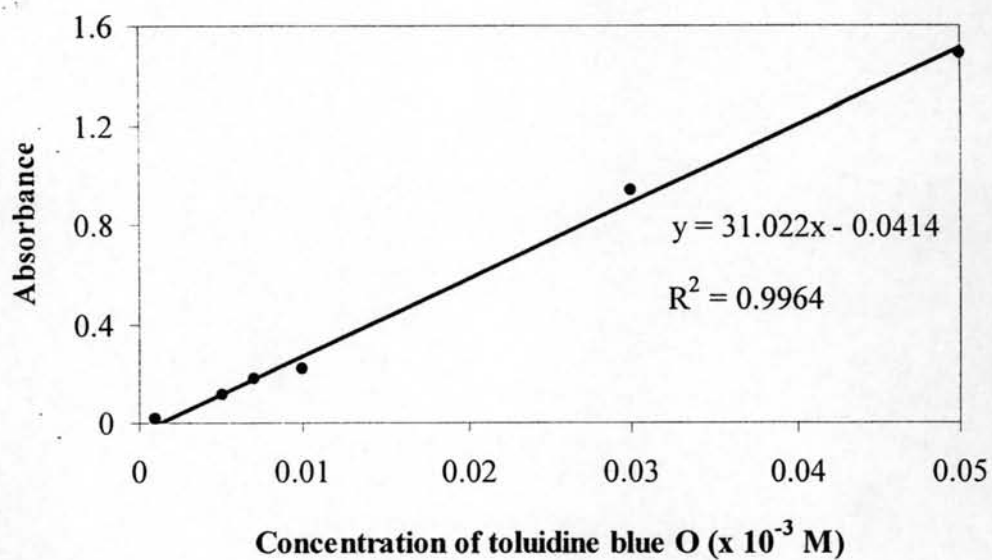
**Scheme B-1** Formation of the complex between toluidine blue O and carboxyl group

### Determination of Carboxyl Groups on Plasma-treated PET

A number of carboxyl (COOH) groups on the plasma-treated PET substrate after the pretreatment was determined by a reaction with toluidine blue O, generating the absorbance at 633 nm. The COOH group was obtained from a calibration plot of the optical density versus dye concentration assuming a 1:1 stoichiometric ratio (Figure B-1) between the dye and the COOH group.

**Table B-1** UV absorbance at  $\lambda = 633$  nm as a function of toluidine blue O concentration [77].

Toluidine blue O concentration ( $\times 10^{-3}$ M)	Absorbance
0.001	0.013
0.005	0.112
0.007	0.177
0.010	0.216
0.030	0.939
0.050	1.490



**Figure B-1** Calibration curve of UV absorbance as a function of toluidine blue O concentration [77].

## APPENDIX C

**Table C-1** Fibroblast (L929) cell adhesion and proliferation on treated PET substrates and treated PET substrates with multilayer films.

Materials	12-h Adhesion		2-day Proliferation		4-day Proliferation	
	OD	% relative to TCPS	OD	% relative to TCPS	OD	% relative to TCPS
TCPS	0.240	100.0	0.287	100.0	0.699	100.0
Treated PET	0.207	86.3	0.279	97.2	0.586	83.8
HTACC-PAA~9	0.208	86.7	0.292	101.7	0.554	79.3
HTACC-PAA~10	0.254	105.8	0.257	89.5	0.636	91.0
HTACC-PAA~15	0.219	91.3	0.232	80.8	0.482	69.0
HTACC-PAA~16	0.244	101.7	0.290	101.0	0.759	108.6
PAH-SCC~9	0.213	88.8	0.341	118.8	0.626	89.6
PAH-SCC~10	0.174	72.5	0.270	94.1	0.565	80.8
PAH-SCC~15	0.216	90.0	0.376	131.0	0.723	103.4
PAH-SCC~16	0.173	72.1	0.254	88.5	0.557	79.7
PAH-SFC~9	0.295	122.9	0.364	126.8	0.719	102.9
PAH-SFC~10	0.239	99.6	0.279	97.2	0.581	83.1
PAH-SFC~15	0.248	103.3	0.243	84.7	0.642	91.8
PAH-SFC~16	0.191	79.6	0.211	73.5	0.610	87.3

For HTACC-PAA~n, PAH-SCC~n, and PAH-SFC~n, n is the number of layers.



## VITAE

Miss Wilaiporn Graisuwan was born in Nakhon Sri Thammarat, Thailand, on April 12<sup>th</sup>, 1982. She received Bachelor Degree of Science Industrial Chemistry from the Faculty of Science and Technology, Prince of Songkla University, Pattani in 2005. In the same year, she started as a Master Degree student with a major in Program of Petrochemistry and Polymer Science, Faculty of Science, Chulalongkorn University and completed the program in 2008.