REFERENCES

1. Bourke, S. L., Kohn, J. Polymers derived from the amino acid L-tyrosine:polycarbonates, polyarylates and copolymers with poly(ethylene glycol) Advanced Drug Delivery Reviews 55 (2003): 447-466.

2. Kohn, J., Langer, R. Polymerization reactions involving the side chains of α-L-amino acids J. Am. Chem. Soc. 109 (1987): 817-820.

3. Pulapura, S., Kohn, J. Tyrosine derived polycarbonates: backbone modified, 'pseudo'-poly(amino acids)designed for biomedical applications Biopolymers 32 (1992): 411-417.

4. Tangpasuthadol, V., Pendharkar, S. M., Peterson, R. C. Kohn, J. Hydrolytic degradation of tyrosine-derived polycarbonates, a class of new biomaterials. Part I: study of model compounds Biomaterials 21 (2000): 2371-2378.

5. Tangpasuthadol, V., Pendharkar, S. M., Peterson, R. C. Kohn, J. Hydrolytic degradation of tyrosine-derived polycarbonates, a class of new biomaterials. Part II: 3-yr study of polymeric devices Biomaterials 21 (2000): 2379-2387.

6. Yu, C.; Kohn, J. Tyrosine-PEG-derived poly(ether carbonate)s as new biomaterials Part I: synthesis and evaluation Biomaterials 20 (1999): 253-264.

7. Pierschbacher, M.D., Ruoslahti, E. Cell attachment activity of fibronectin can be duplicated by small synthetic fragments of the molecule Nature 309 (1984): 30-33.

8. Ruoslahti, E., Pierschbacher, M. D. New perspectives in cell adhesion: RGD and integrins Science 238 (1987): 491-497.

9. Xiao, S. J. Tailored organic thin films on gold and titanium: peptide-grafting, protein resistance and physical characterization (Doctoral dissertation, Department of Materials, Swiss Federal Institute of Science and Technology Zürich, 1999)

10. Craig, W. S., Cheng, S., Mullen, D. G., Blevitt, J., Pierschbacher, M. D. Concept and progress in the development of RGD-containing peptide pharmaceuticals Biopolymers 37 (1995): 157-175.

11. Hersel, U., Dahmen, C., Kessler, H. RGD modified polymers: biomaterials for stimulated cell adhesion and beyond Biomaterials 24 (2003): 4385-4415.

12. Quirk, R. A., Chan, W. C., Davies M. C., Tendler, S. J. B., Shakesheff, K. M. Poly(L-lysine)-GRGDS as a biomimetic surface modifier for poly(lactic acid) Biomaterials 22 (2001): 865-872.

13. Jo, S.; Engel, P. S.; Mikos, A. G. Synthesis of poly(ethylene glycol)-tethered poly(propylene fumarate) and its modification with GRGD peptide Polymer 41 (2000): 7595-7604.

14. Morpurgo, M., Bayer, E. A., Wilchek, M. N-hydroxysuccinimide carbonates and carbamates are useful reactive reagents for coupling ligands to lysines on proteins J. Biochem. Biophys. Methods. 38 (1999): 17-28.

15. Marchand-Brynaert, J., Detrait, E., Noiset, O.; Boxus, T., Schneider, Y. J., Remacle, C. Biological evaluation of RGD peptidomimetics, designed for the covalent derivatization of cell culture substrata, as potential promotors of cellular adhesion Biomaterials 20 (1999): 1773-1782.

16. Xiao, S. J., Textor, M., Spencer, N. D. Covalent attachment of cell-adhesive, (Arg-Gly-Asp)-containing peptides to titanium surfaces Langmuir 14 (1998): 5507-5516.

17. Davis, D. H., Giannoulis, C. S., Johnson, R. W., Desai, T. A. Immobilization of RGD to <111> silicon surfaces for enhanced cell adhesion and proliferation Biomaterials 23 (2002): 4019-4027.

18. Yoon, J. J., Songa, S. H., Leeb, D. S., Park, T. G. Immobilization of cell adhesive RGD peptide onto the surface of highly porous biodegradable polymer scaffolds fabricated by a gas foaming/salt leaching method Biomaterials 25 (2004): 5613-5620.

19. Neff, J. A., Tresco, P. A., Caldwell, K. D. Surface modification for controlled studies of cell–ligand interactions Biomaterials 20 (1999): 2377-2993.

20. Marchand-Brynaert, J., Detrait, E.; Noiset, O., Boxus, T., Schneider, Y. J., Remacle, C. Biological evaluation of RGD peptidomimetics, designed for the covalent derivatization of cell culture substrata, as potential promotors of cellular adhesion Biomaterials 20 (1999): 1773-1782.

21. Neff, J. A., Tresco, P. A., Caldwell, K. D. A novel method for surface modification to promote cell attachment to hydrophobic substrates J. Biomed. Mater. Res. 40 (1998): 511-519.

22. Banerjee, P., Irvine, D.J., Mayes, A. M., Griffith, L. G. Polymer latexes for cell-resistant and cell-interactive surface J. Biomed. Mater. Res. 50 (2000): 331-339.

23. Alberts, B., Bray, J. L., Raff, M.; Roberts, K., Watson, J. D. Molecular biology of the cell. 3rd ed. New York: Garland Publishing, 1994.

24. Karp, G. Cell and molecular biology: concepts and experiments. 3rd ed. New York: John Wiley & Sons, 2002.

25. Andrade, J. D. Surface and interfacial aspects of biomedical polymer: Volume 1 surface chemistry and physics. New York: Plenum Press: 1985.

26. Ho, M. H., Wang, D. M., Hsieh, H. J., Liu, H. C., Hsien, T. Y., Lai, J. Y., Hou, L. T. Preparation and characterization of RGD-immobilized chitosan scaffolds Biomaterials 26 (2005): 3197-3206.

27. Mccaldin, D. J. The chemistry of ninhydrin Chemical Reviews 60 (1960): 39-51.

28. Wang, N. S. Amino acid assay by ninhydrin colorimetric method [online]. Available from: http://www.glue.umd.edu/~nsw/ench485/lab3a.htm [2004, February 1]

APPENDICES

APPENDIX A

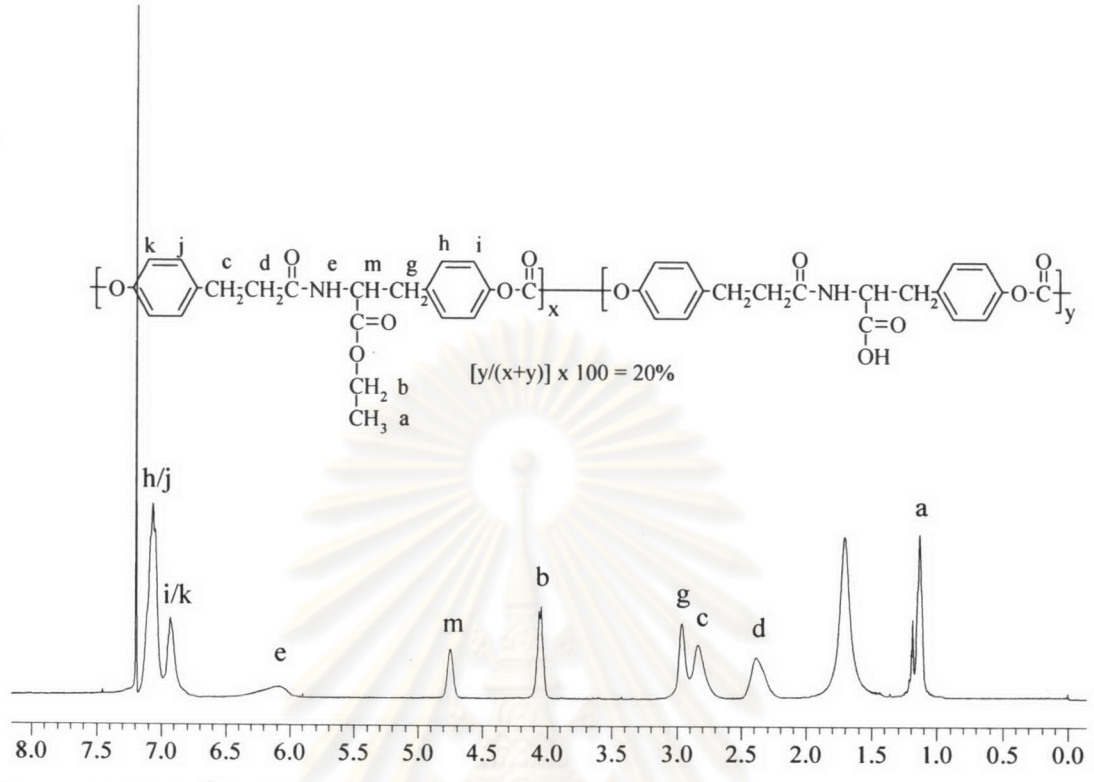

Figure A-1 The ^{1}H-NMR (400 MHz $CDCl_3$) of poly(DTE-*co*-20%DT carbonate) film

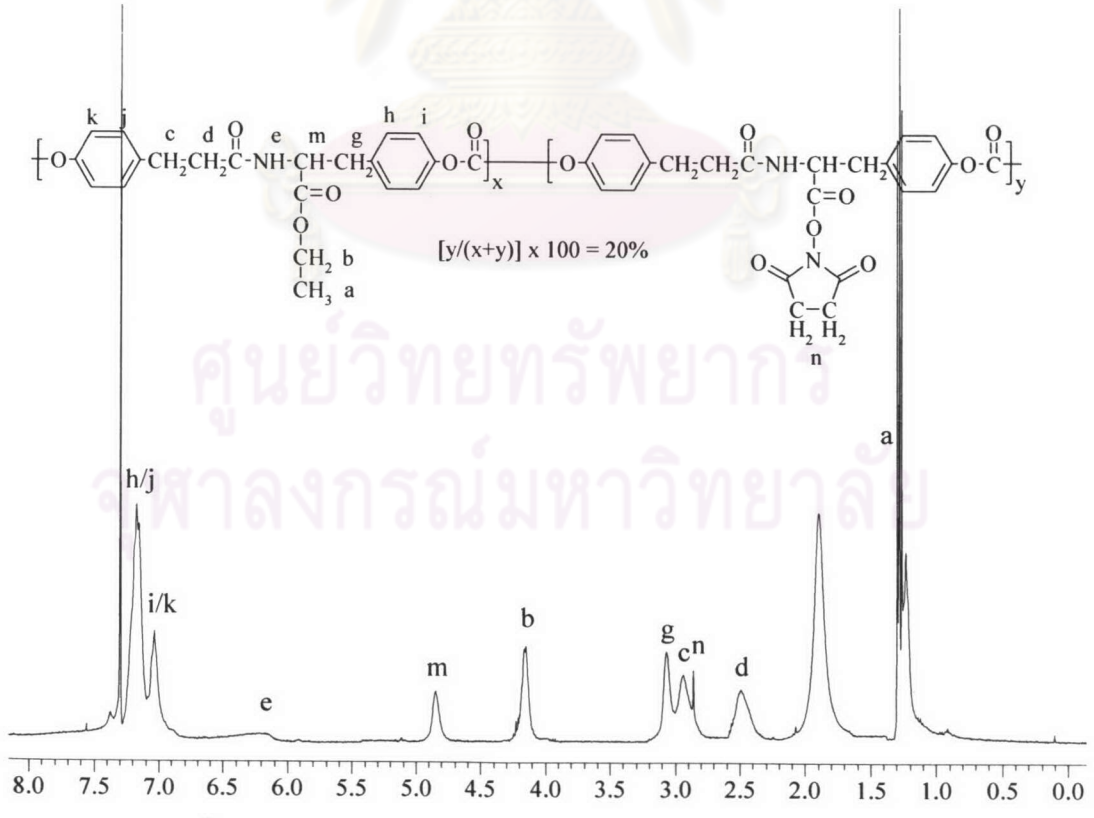

Figure A-2 The ^{1}H-NMR (400 MHz $CDCl_3$) of activated poly(DTE-*co*-20%DT carbonate) film

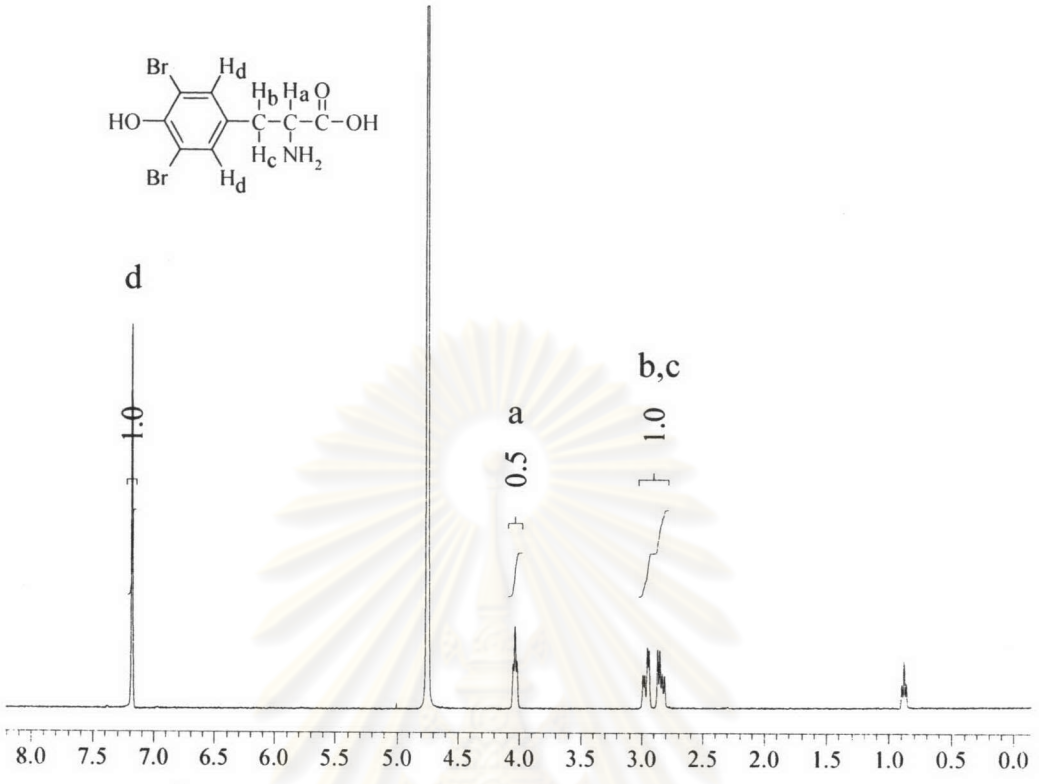

Figure A-3 The ^{1}H-NMR (400 MHz DCl) of L-3,5-dibromotyrosine

APPENDIX B

Table B-1 Water contact angle of activated poly(DTE-*co*-20%DT carbonate) films after reaction with *N*-hydroxysuccinimide in water as a function of reaction time.

Time (min)	Advancing water contact angle (θ_A) (degree)	Receding water contact angle (θ_R) (degree)
0	71.5 ± 0.77	29.8 ± 1.12
30	75.5 ± 1.18	27.0 ± 1.67
60	76.3 ± 1.79	25.9 ± 1.04
90	77.5 ± 1.28	34.0 ± 1.55
120	81.7 ± 0.90	32.6 ± 1.58
150	83.6 ± 0.49	38.6 ± 1.48
180	84.0 ± 0.77	37.8 ± 1.79
210	83.4 ± 0.92	39.0 ± 1.41
240	84.6 ± 0.92	36.1 ± 1.58

The advancing contact angles of all activated poly(DTE-*co*-20%DT carbonate) films were significantly higher than that of poly(DTE-*co*-20%DT carbonate) film ($p<0.01$).

Table B-2 %Yield of activation of poly(DTE-*co*-20%DT carbonate) with *N*-hydroxysuccinimide as a function of coupling agent concentration.

Concentration of coupling agent (M)		% yield
[EDCI]	[NHS]	
0.01	0.01	0.5
0.02	0.02	1.6
0.03	0.03	2.0
0.04	0.04	4.5
0.05	0.05	6.6
0.06	0.06	9.4
0.07	0.07	11.8
0.10	0.10	19.8

Table B-3 %Yield of activation of poly(DTE-*co*-20%DT carbonate) with *N*-hydroxysuccinimide as a function of reaction time.

Time (h)	% Yield	
	[EDCI] = [NHS] = 0.05 M	[EDCI] = [NHS] = 0.10 M
0.5	3.9	7.9
1	5.7	13.8
2	6.6	19.8
3	6.2	20.1
4	7.0	21.0

Determination of Amino Groups of Modified poly(DTE-*co*-20%DT carbonate) Films

Table B-4 UV-absorbance at λ = 538 nm of standard 1,6-hexanediamine solution for generating a calibration curve

Concentration of 1,6-hexanediamine (x 10^{-3} M)	Abs
0.001	0.012
0.005	0.025
0.010	0.052
0.030	0.164
0.050	0.290
0.100	0.600

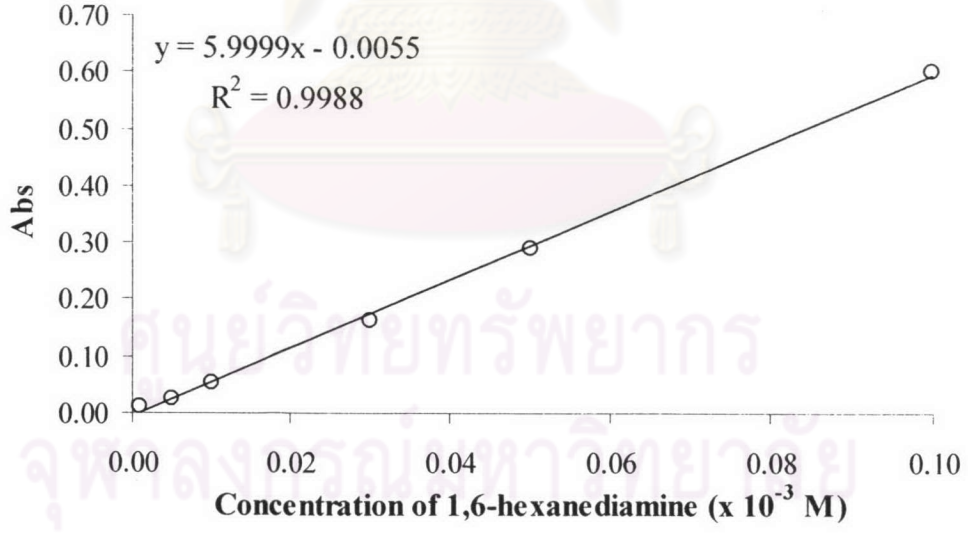

Figure B-1 Calibration curve of UV-absorbance as a function of 1,6-hexanediamine concentration analyzed by ninhydrin method.

Table B-5 Amino content of RGD-poly(DTE-*co*-20%DT carbonate) film as a function of immobilization time.

Time (h)	Abs	Concentration (x 10^{-8} mole/cm^2)	Average ± S.D. (x 10^{-8} mole/cm^2)
2	0.028	0.99	1.16 ± 0.20
	0.034	1.16	
	0.045	1.49	
	0.032	1.11	
	0.030	1.05	
6	0.110	3.40	3.35 ± 0.27
	0.099	3.08	
	0.118	3.64	
	0.115	3.55	
	0.098	3.05	
12	0.180	5.47	4.97 ± 0.39
	0.152	4.64	
	0.175	5.32	
	0.153	4.67	
	0.155	4.73	
24	0.287	8.62	8.07 ± 0.53
	0.245	7.38	
	0.264	7.94	
	0.286	8.59	
	0.260	7.83	
48	0.275	8.27	8.96 ± 0.43
	0.311	9.33	
	0.295	8.86	
	0.310	9.30	
	0.302	9.06	

The amino contents of all RGD- immobilized poly(DTE-*co*-20%DT carbonate) films were significantly higher ($p<0.01$) than that of poly(DTE-*co*-20%DT carbonate) film.

Table B-6 Amino content of RGD-poly(DTE-*co*-20%DT carbonate) film as a function of RGD concentration.

Concentration of RGD peptide (x 10^{-3} M)	Abs	Concentration (x 10^{-8} mole/cm^2)	Average ± S.D. (x 10^{-8} mole/cm^2)
1	0.042	1.40	1.20 ± 0.21
	0.040	1.34	
	0.037	1.25	
	0.034	1.16	
	0.024	0.87	
5	0.077	2.43	2.14 ± 0.22
	0.059	1.90	
	0.073	2.31	
	0.062	1.99	
	0.065	2.08	
10	0.141	4.32	4.17 ± 0.42
	0.138	4.23	
	0.122	3.76	
	0.123	3.79	
	0.157	4.79	
20	0.211	6.38	6.02 ± 0.43
	0.204	6.17	
	0.195	5.91	
	0.175	5.32	
	0.209	6.62	
50	0.287	8.62	8.07 ± 0.53
	0.245	7.38	
	0.264	7.94	
	0.286	8.59	
	0.260	7.83	

The amino contents of all RGD- immobilized poly(DTE-*co*-20%DT carbonate) films were significantly higher ($p<0.01$) than that of poly(DTE-*co*-20%DT carbonate) film.

Table B-7 Amino content of RGDS-poly(DTE-*co*-20%DT carbonate) and GRGDS-poly(DTE-*co*-20%DT carbonate) using peptide concentration of 0.05 M.

RGD-peptide	Abs	Concentration (x 10^{-8} mole /cm^2)	Average ± S.D. (x 10^{-8} mole/cm^2)
RGDS	0.222	6.71	6.51 ± 0.61
	0.216	6.53	
	0.199	6.03	
	0.194	5.88	
	0.246	7.41	
GRGDS	0.180	5.47	5.13 ± 0.60
	0.134	4.11	
	0.177	5.38	
	0.167	5.08	
	0.185	5.61	

The amino content of RGD-immobilized poly(DTE-*co*-20%DT carbonate) films was significantly higher ($p<0.01$) than those of the RGDS-immobilized poly(DTE-*co*-20%DT carbonate) film and GRGDS-immobilized poly(DTE-*co*-20%DT carbonate) film. There was significant ($p<0.05$) difference between the amino content of RGDS-immobilized poly(DTE-*co*-20%DT carbonate) film and that of GRGDS-immobilized poly(DTE-*co*-20%DT carbonate) film.

Table B-8 Number of fibroblast attachment on polymer surface after 12 h incubation.

Polymer surface	Number of fibroblast attachment (cells/cm^3)	% CAR
poly(DTE-*co*-20%DT carbonate)	2283 ± 155	92.3 ± 6.78
NHS-poly(DTE-*co*-20%DT carbonate)	799 ± 86	32.3 ± 10.80
RGD-poly(DTE-*co*-20%DT carbonate)	2479 ± 136	100.2 ± 5.48
soluble RGD + poly(DTE-co-20%DT carbonate)	2412 ± 79	97.5 ± 3.26
RGDS-poly(DTE-*co*-20%DT carbonate)	2416 ± 158	97.6 ± 6.56
GRGDS-poly(DTE-*co*-20%DT carbonate)	2896 ± 183	117.0 ± 6.31
Tissue culture polystyrene (TCPS)	2475 ± 196	100.0 ± 7.91

The number of fibroblasts attached on GRGDS-immobilized poly(DTE-*co*-20%DT carbonate) films was significantly higher ($p<0.01$) than those of the poly(DTE-*co*-20%DT carbonate) film, RGD-immobilized poly(DTE-*co*-20%DT carbonate) film, RGDS-immobilized poly(DTE-*co*-20%DT carbonate) film, soluble RGD + poly(DTE-*co*-20%DT carbonate) and TCPS. There are no significant difference of the number of fibroblast attachment among TCPS, poly(DTE-*co*-20%DT carbonate), RGD-poly(DTE-*co*-20%DT carbonate), RGDS-poly(DTE-*co*-20%DT carbonate) and soluble RGD + poly(DTE-*co*-20%DT carbonate) ($p > 0.05$).

Table B-9 Number of fibroblast proliferation on polymer surface

Polymer surface	48 h proliferation		96 h proliferation	
	Cell Number (x100 cells/cm^3)	% CPR	Cell Number (x 100 cells/cm^3)	% CPR
poly(DTE-*co*-20%DT carbonate)	193 ± 14	106.0 ± 7.2	642 ± 41	89.6 ± 6.3
RGD-poly(DTE-*co*-20%DT carbonate)	164 ± 17	90.3 ± 10.5	569 ± 29	79.4 ± 5.1
RGDS-poly(DTE-*co*-20%DT carbonate)	166 ± 26	91.3 ± 17.5	670 ± 21	93.4 ± 3.1
GRGDS-poly(DTE-*co*-20%DT carbonate)	236 ± 20	129.3 ± 8.3	879 ± 32	122.6 ± 3.6
Tissue culture polystyrene (TCPS)	182 ± 14	100.0 ± 7.7	717 ± 30	100 ± 4.0

The number of fibroblast proliferation on GRGDS-immobilized poly(DTE-*co*-20%DT carbonate) films was significantly higher ($p<0.01$) than those of the poly(DTE- *co*-20%DT carbonate) film, RGD-immobilized poly(DTE-*co*-20%DT carbonate) film, RGDS-immobilized poly(DTE-*co*-20%DT carbonate) film and TCPS.

APPENDIX C

Amino Acid Assay by Ninhydrin Colorimetric Method [27, 28]

The reaction between alpha-amino acid and ninhydrin involve the development of color which can be described by the following five mechanistic steps:

alpha-amino acid + ninhydrin ---> reduced ninhydrin + alpha-amino acid + H_2O (A-1)

alpha-amino acid + H_2O ---> alpha-keto acid + NH_3 (A-2)

alpha-keto acid + NH_3 ---> aldehyde + CO_2 (A- 3)

Step (A-1) is an oxidative deamination reaction that removes two hydrogens from the alpha-amino acid to yield an alpha-imino acid. Simultaneously, the original ninhydrin is reduced and loses an oxygen atom with the formation of a water molecule. In Step (A-2), the NH group in the alpha-amino acid is rapidly hydrolyzed to form an alpha-keto acid with the production of an ammonia molecule. This alpha-keto acid further undergoes decarboxylation reaction in Step (A-3) under heated condition to form an aldehyde that has one less carbon atom than the original amino acid. A carbon dioxide molecule is also produced. These first three steps produce the reduced ninhydrin and ammonia that are required for the production of blue color. The overall reaction for the above reactions is simply expressed in equation (A-4) as follows:

alpha-amino acid + 2 ninhydrin ---> CO_2 + aldehyde + final complex (blue) + $3H_2O$ (A-4)

In summary, ninhydrin, which is originally yellow, reacts with amino acid and turns into deep purple. It is this purple color that is detected in this method. Ninhydrin will react with a free alpha-amino group of NH_2-CHR-COOH. This group is a part of all amino acids, peptides, or proteins. Whereas, the decarboxylation reaction would proceed for a free amino acid and a free amino group at chain end or side chain of

protein or peptide, it does not occur for an amino group within peptides and proteins. Thus, theoretically only amino acids will lead to the color development. However, one should always check out the possible interference from peptides and proteins by performing blank tests especially when such solutions are readily available. For example, one can simply add the ninhydrin reagent to a solution of only proteins and see if there is any color development. There is no excuse for failing to perform such a vital test when the sample mixture contains both proteins and amino acids. There are also reports that chemical compounds other than amino acids also yield positive results.

This test can be used routinely for the detection of glycine in the absence of other interfering species. Although this is a fast and sensitive test for the presence of alpha-amino acids because of the nonselectivity, it cannot be used to analyze the relative individual contents of a mixture of different amino acids. Furthermore, the color intensity developed is dependent on the type of amino acid. Finally, it does not react with tertiary or aromatic amines.

VITAE

Mr. Adisorn Poopattanapong was born in Trat, Thailand. He received bachelor degree of Science form Department of Chemistry, Faculty of Science, Burapha University, Chonburi Thailand in 2002. In the same year he was admitted to a Master's degree in Program of Petrochemistry and Polymer science, Faculty of Science, Chulalongkorn University and completed program in 2005. His address is 77/307 Nawamin Road, Klongkum, Buengkum, Bangkok 10230.