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APPENDICES

Appendix A Bicinchoninic Acid Assay (BCA assay)

Table A1 The absorbance of Bovine Serum Albumin (BSA) standard in PBS

Standard no.	**BSA concentration (μg/ml)**	**Average net absorbance**
1	0	0.0000
2	2.5	0.0070
3	5	0.0053
4	10	0.0250
5	25	0.0622
6	50	0.1292
7	100	0.2790

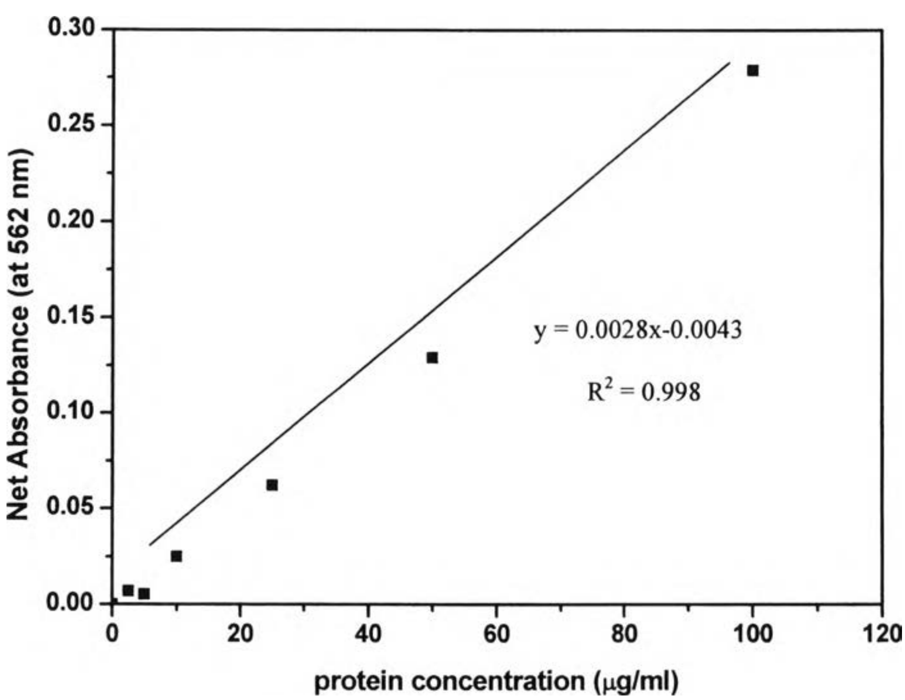

Figure A1 The calibration curve of BSA standard in PBS.

Table A2 The adsorption isotherm of the adsorbed bovine serum albumin on the neat PCL films (diameter = 1.5 cm) casted from chloroform at equilibrium protein concentration

Equilibrium protein concentration (μg/ml)	Neat PCL film casted from chloroform
	Amount of protein adsorbed (μg/cm^2)
198	1.23 $\pm$ 0.23
397	1.58 $\pm$ 0.12
597	2.31 $\pm$ 0.67
797	2.08 $\pm$ 0.27
995	3.86 $\pm$ 0.27
1492	5.33 $\pm$ 0.27
1990	6.18 $\pm$ 0.27
2988	7.72 $\pm$ 0.61

Table A3 The adsorption isotherm of the adsorbed bovine serum albumin on the neat PCL films (diameter = 1.5 cm) casted from 40:60 (v/v) EtOH/THF at equilibrium protein concentration

Equilibrium protein concentration (μg/ml)	Neat PCL film casted from 40:60 (v/v) EtOH/THF
	Amount of protein adsorbed (μg/cm^2)
195	3.78 $\pm$ 0.40
395	3.70 $\pm$ 0.58
593	4.55 $\pm$ 0.35
791	5.56 $\pm$ 0.54
990	6.33 $\pm$ 0.46
1489	7.03 $\pm$ 0.23
1987	8.73 $\pm$ 0.75
2987	8.88 $\pm$ 0.84

Table A4 The adsorption isotherm of the adsorbed bovine serum albumin on the modified PCL films (diameter = 1.5 cm) casted from chloroform at equilibrium protein concentration

Equilibrium protein concentration (μg/ml)	**Modified PCL film casted from chloroform**
	Amount of protein adsorbed (μg/cm^2)
191	5.87 $\pm$ 1.57
391	5.67 $\pm$ 1.01
590	6.67 $\pm$ 0.27
789	7.41 $\pm$ 0.58
986	9.35 $\pm$ 1.61
1486	8.98 $\pm$ 0.88
1982	11.38 $\pm$ 2.07
2984	10.51 $\pm$ 1.16

Table A5 The adsorption isotherm of the adsorbed bovine serum albumin on the modified PCL films (diameter = 1.5 cm) casted from 40:60 (v/v) EtOH/THF at equilibrium protein concentration

Equilibrium protein concentration (μg/ml)	**Modified PCL film casted from 40:60 (v/v) EtOH/THF**
	Amount of protein adsorbed (μg/cm^2)
190	6.25 $\pm$ 1.42
391	6.10 $\pm$ 1.16
589	7.18 $\pm$ 1.23
787	8.58 $\pm$ 1.75
987	8.60 $\pm$ 0.23
1484	10.66 $\pm$ 1.11
1982	11.88 $\pm$ 1.32
2958	27.51 $\pm$ 1.14

Appendix B Experimental Data of Biological Characterizations

Table B1 Indirect cytotoxicity evaluation of the materials casted from chloroform shown by the percent viability of cells by MTT assay

Materials	% viability of MC3T3-E1 cells (relative to TCPS)		
	1 d	3 d	7 d
Control	100 ± 6	100 ± 4	100 ± 13
Neat	112 ± 2	96 ± 3	106 ± 13
Adsorbed collagen (100 μg/mL)	84 ± 11	85 ± 6	96 ± 8
Pre-adsorbed BSA (200 μg/mL)	102 ± 2	99 ± 2	87 ± 11
Pre-adsorbed BSA (1500 μg/mL)	96 ± 15	96 ± 6	100 ± 14
Pre-adsorbed BSA (3000 μg/mL)	111 ± 20	97 ± 2	95 ± 4
Adsorbed BSA (3000 μg/mL)	117 ± 6	110 ± 6	102 ± 5

Table B2 Indirect cytotoxicity evaluation of the materials casted from 40:60 (v/v) EtOH/THF shown by the percent viability of cells by MTT assay

Materials	% viability of MC3T3-E1 cells (relative to TCPS)		
	1 d	3 d	7 d
Control	100 ± 6	100 ± 4	100 ± 13
Neat	106 ± 9	112 ± 3	86 ± 9
Adsorbed collagen (100 μg/mL)	92 ± 10	115 ± 6	84 ± 2
Pre-adsorbed BSA (200 μg/mL)	87 ± 8	97 ± 5	91 ± 10
Pre-adsorbed BSA (1500 μg/mL)	89 ± 3	106 ± 3	91 ± 6
Pre-adsorbed BSA (3000 μg/mL)	86 ± 7	103 ± 11	109 ± 7
Adsorbed BSA (3000 μg/mL)	100 ± 6	100 ± 4	100 ± 13

Table B3 Attachment and proliferation of MC3T3-E1 that were cultured on the materials casted from chloroform at seeding time for 4 hrs, 1 d and 3 d.

Materials	**% viability of MC3T3-E1 cells (relative to TCPS at 1 d)**		
	4 hrs	**4 hrs**	**4 hrs**
Control	69 ± 4	100 ± 5	154 ± 12
Neat	27 ± 3	71 ± 4	110 ± 7
Adsorbed collagen (100 μg/mL)	45 ± 5	69 ± 2	120 ± 6
Pre-adsorbed BSA (200 μg/mL)	30 ± 12	99 ± 4	128 ± 3
Pre-adsorbed BSA (1500 μg/mL)	43 ± 9	94 ± 6	127 ± 14
Pre-adsorbed BSA (3000 μg/mL)	41 ± 11	93 ± 2	125 ± 6
Adsorbed BSA (3000 μg/mL)	67 ± 9	91 ± 9	129 ± 11

Table B4 Attachment and proliferation of MC3T3-E1 that were cultured on the materials casted from 40:60 EtOH/THF at seeding time for 4 hrs, 1 d and 3 d.

Materials	% viability of MC3T3-E1 cells (relative to TCPS at 1 d)		
	4 hrs	**4 hrs**	**4 hrs**
Control	69 ± 5	100 ± 5	154 ± 12
Neat	45 ± 2	88 ± 5	242 ± 22
Adsorbed collagen (100 μg/mL)	58 ± 7	105 ± 9	233 ± 21
Pre-adsorbed BSA (200 μg/mL)	63 ± 15	101 ± 6	209 ± 9
Pre-adsorbed BSA (1500 μg/mL)	62 ± 10	101 ± 8	227 ± 20
Pre-adsorbed BSA (3000 μg/mL)	61 ± 10	108 ± 8	244 ± 32
Adsorbed BSA (3000 μg/mL)	73 ± 11	109 ± 6	365 ± 22

Table B5 ALP activities of MC3T3-E1 that were cultured on the materials casted from chloroform and 40:60 EtOH/THF at seeding time for 7 d.

Surface	ALP activity (μmole/μg.min)	
	Chloroform	**40:60 (v/v) EtOH/THF**
TCPS	1.94 ± 0.14	1.94 ± 0.14
Neat PCL	0.71 ± 0.02	0.78 ± 0.01
Adsorbed collagen (100 μg/mL)	0.72 ± 0.03	0.82 ± 0.02
Pre-adsorbed BSA (200 μg/mL)	0.74 ± 0.03	0.80 ± 0.01
Pre-adsorbed BSA (1500 μg/mL)	0.75 ± 0.03	0.81 ± 0.01
Pre-adsorbed BSA (3000 μg/mL)	0.78 ± 0.01	0.79 ± 0.03
Adsorbed BSA (3000 μg/mL)	0.78 ± 0.02	0.84 ± 0.00

CURRICULUM VITAE

Name: Ms. Khwanjai Anujarawat

Date of Birth: March 24, 1984

Nationality: Thai

University Education:

2001–2004 Bachelor Degree of Chemistry, Faculty of Science, Burapha University, Bangkok, Thailand

Work Experience:

2005-Present Position: Scientist

Company name: Research and Development Centre Thai Rubber Industry at Mahidol University

Proceeding:

1. Anujarawat, K.; and Supaphol, P. (2013, April 23) Role of Surface Topography and Protein Adsorption on Film Scaffolds on Bone Cells Behavior. Proceedings of the 19th PPC Symposium on Petroleum, Petrochemicals, and Polymers, Bangkok, Thailand.

Presentations:

1. Anujarawat, K.; and Supaphol, P. (2013, March 11-15) Role of Surface Topography and Protein Adsorption on Polycaprolactone Scaffolds on Bone Cells Behavior. Paper presented at POLYCHAR 21 World Forum on Advance Materials, Gwangju, Republic of Korea.
2. Anujarawat, K. and Supaphol, P. (2013, April 23) Role of Surface Topography and Protein Adsorption on Film Scaffolds on Bone Cells Behavior. Paper presented at Proceedings of the 19th PPC Symposium on Petroleum, Petrochemicals, and Polymers, Bangkok, Thailand.