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APPENDICES

Appendix A Bicinchoninic Acid Protein Assay (BCA Analysis)

Table A1 Bovine Serum Albumin (BSA) standards and their net absorbance

Standard No.	Standard BSA Concentration (μg/ml)	Average Net Absorbance
1	100	0.3076
2	50	0.1566
3	25	0.0783
4	10	0.0326
5	5	0.0083
6	2.5	0.0094
7	1	0.0084
8	0.5	0.0006
9	0	0

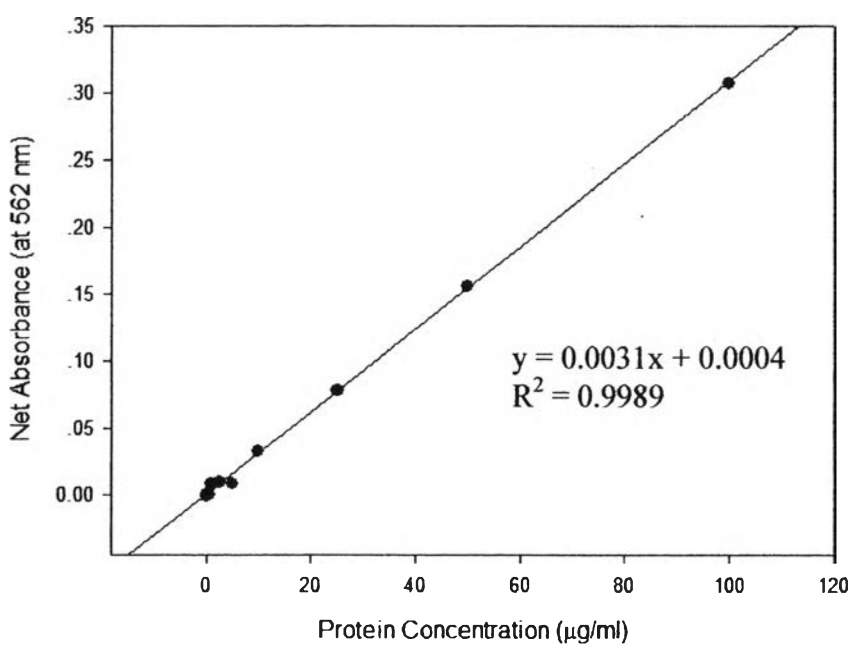

Figure A1 The calibration curve for BSA using the standards.

Table A2 The amount of protein adsorbed on the surface of PCL film (diameter = 1.5 cm) at various annealing time

Annealing Time (h)	Amount of Protein Adsorbed ($\mu g/cm^2$)
0	0.03122 ± 0.00287
1	0.03241 ± 0.00148
2	0.03293 ± 0.00291
3	0.03320 ± 0.00082
4	0.03556 ± 0.00132
5	0.03403 ± 0.00301
6	0.03451 ± 0.00279
12	0.03269 ± 0.00204
24	0.03323 ± 0.00404

Table A3 The adsorption isotherm of the adsorbed bovine serum albumin on the neat PCL films (diameter = 1.5 cm) casted from different solvent systems at various initial protein concentration

Initial Protein Concentration ($\mu g/ml$)	Amount of Protein Adsorbed ($\mu g/cm^2$)	
	Chloroform	Acetone
200	0.03232 ± 0.00011	0.03225 ± 0.00015
400	0.03282 ± 0.00004	0.03237 ± 0.00030
600	0.03382 ± 0.00010	0.03278 ± 0.00006
800	0.03371 ± 0.00008	0.03288 ± 0.00009
1000	0.03387 ± 0.00014	0.03295 ± 0.00014
1400	0.03416 ± 0.00009	0.03365 ± 0.00050
2000	0.03416 ± 0.00006	0.03467 ± 0.00008
3000	0.03446 ± 0.00011	0.03495 ± 0.00032

Initial Protein Concentration (μg/ml)	Amount of Protein Adsorbed (μg/cm^2)	
	THF	20:80 (v/v) EtOH:THF
200	0.03221 ± 0.00012	0.03305 ± 0.00024
400	0.03290 ± 0.00004	0.03326 ± 0.00010
600	0.03336 ± 0.00013	0.03321 ± 0.00004
800	0.03375 ± 0.00008	0.03336 ± 0.00002
1000	0.03399 ± 0.00006	0.03372 ± 0.00001
1400	0.03405 ± 0.00019	0.03392 ± 0.00007
2000	0.03422 ± 0.00022	0.03511 ± 0.00008
3000	0.03432 ± 0.00011	0.03511 ± 0.00003
Initial Protein Concentration (μg/ml)	**Amount of Protein Adsorbed (μg/cm^2)**	
	30:70 (v/v) EtOH:THF	**40:60 (v/v) EtOH:THF**
200	0.03319 ± 0.00009	0.03320 ± 0.00011
400	0.03337 ± 0.00009	0.03314 ± 0.00006
600	0.03362 ± 0.00015	0.03340 ± 0.00011
800	0.03363 ± 0.00011	0.03357 ± 0.00006
1000	0.03393 ± 0.00008	0.03473 ± 0.00037
1400	0.03458 ± 0.00009	0.03749 ± 0.00033
2000	0.03521 ± 0.00008	0.03755 ± 0.00013
3000	0.03530 ± 0.00007	0.03852 ± 0.00013
Initial Protein Concentration (μg/ml)	**Amount of Protein Adsorbed (μg/cm^2)**	
	1 M NaOH	**5 M NaOH**
200	0.03233 ± 0.00006	0.03270 ± 0.00005
400	0.03279 ± 0.00006	0.03296 ± 0.00012
600	0.03355 ± 0.00021	0.03322 ± 0.00008
800	0.03409 ± 0.00008	0.03415 ± 0.00011
1000	0.03411 ± 0.00008	0.03483 ± 0.00008
1400	0.03419 ± 0.00006	0.03480 ± 0.00010
2000	0.03411 ± 0.00006	0.03519 ± 0.00006
3000	0.03426 ± 0.00005	0.03545 ± 0.00006

Table A4 The adsorption isotherm of the adsorbed bovine serum albumin on the neat and modified PCL films (diameter = 1.5 cm) casted from 40:60 EtOH:THF

Initial Protein Concentration (μg/ml)	**Amount of Protein Adsorbed ($\mu g/cm^2$)**		
	Neat PCL	**Aminolyzed PCL**	**Activated PCL**
200	0.03320 ± 0.00011	0.03189 ± 0.00015	0.03322 ± 0.00012
400	0.03314 ± 0.00006	0.03197 ± 0.00012	0.03350 ± 0.00010
600	0.03340 ± 0.00011	0.03231 ± 0.00017	0.03360 ± 0.00015
800	0.03357 ± 0.00006	0.03247 ± 0.00007	0.03438 ± 0.00016
1000	0.03473 ± 0.00037	0.03246 ± 0.00007	0.03531 ± 0.00016
1400	0.03749 ± 0.00033	0.03277 ± 0.00009	0.04080 ± 0.00018
2000	0.03755 ± 0.00013	0.03305 ± 0.00012	0.04360 ± 0.00018
3000	0.03852 ± 0.00013	0.03309 ± 0.00016	0.04401 ± 0.00029

Appendix B Atomic Force Microscopy (AFM)

Table B1 The variation of roughness parameters (R_a, R_q, and R_z) of the films casted from different solvents

Roughness / Solvent	Arithmetic Average Roughness (R_a, μm)	Root Mean Square Roughness (R_q, μm)	Absolute Height (R_z, μm)
TCPS	0.00436 ± 0.00074	0.00494 ±0.00078	0.01671 ±0.00138
Chloroform	0.01564 ± 0.00093	0.01992 ± 0.00157	0.09610 ± 0.00546
THF	0.22100 ± 0.00608	0.28900 ± 0.01682	1.35633 ± 0.12208
1 M NaOH	0.32400 ± 0.01200	0.41433 ± 0.02350	1.07933 ± 0.11625
5 M NaOH	0.37833 ± 0.00945	0.48033 ± 0.01721	1.15700 ± 0.10392
Acetone	0.40667 ± 0.03803	0.49233 ± 0.03573	1.42867 ± 0.07463
20:80 EtOH:THF	0.53667 ± 0.01168	0.66300 ± 0.05231	1.62500 ± 0.29091
30:70 EtOH:THF	0.59200 ± 0.00400	0.68400 ± 0.00361	1.93633 ± 0.08105
40:60 EtOH:THF	0.97367 ± 0.07849	1.19933 ± 0.01531	2.48967 ± 0.87032

Appendix C Biological Characterization

Table C1 Indirect cytotoxicity evaluation of protein adsorbed films 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**
TCPS (control)	100.00 ± 2.87	100.00 ± 6.47	100.00 ± 1.75
Chloroform	89.73 ± 4.75	95.74 ± 9.03	111.50 ± 7.34
Acetone	97.34 ± 7.60	88.76 ± 8.57	94.77 ± 15.52
THF	87.45 ± 2.63	88.37 ± 10.03	83.62 ± 5.53
20:80 EtOH:THF	105.32 ± 7.42	93.41 ± 5.97	94.77 ± 6.12
30:70 EtOH:THF	103.42 ± 5.85	102.33 ± 6.47	106.97 ± 13.60
40:60 EtOH:THF	89.35 ± 6.59	92.64 ± 5.97	85.71 ± 6.27
1 M NaOH	93.16 ± 2.37	85.66 ± 6.40	84.67 ± 8.16
5 M NaOH	114.45 ± 2.37	95.74 ± 3.55	100.70 ± 8.89

Table C2 Indirect cytotoxicity evaluation of surface-modified films 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**
TCPS (control)	100.00 ± 2.87	100.00 ± 6.47	100.00 ± 1.75
Neat Chloroform	92.78 ± 0.66	98.84 ± 8.14	85.02 ± 1.60
Aminolyzed Chloroform	126.62 ± 10.28	93.80 ± 2.93	103.48 ± 6.96
Activated Chloroform	112.55 ± 8.86	90.31 ± 9.47	96.52 ± 11.47
Adsorbed Chloroform	128.14 ± 7.03	105.43 ± 3.48	112.20 ± 4.94
Neat 40:60 EtOH:THF	115.59 ± 5.85	102.33 ± 3.49	97.91 ± 4.35
Aminolyzed40:60EtOH:THF	116.73 ± 11.87	103.49 ± 10.34	114.63 ± 10.15
Activated 40:60 EtOH:THF	116.35 ± 10.14	103.88 ± 9.03	101.05 ± 3.67
Adsorbed 40:60 EtOH:THF	133.61 ± 12.82	123.26 ± 7.26	119.86 ± 6.39

Table C3 Cell attachment on protein adsorbed films shown by the percent viability of cells by MTT assay

Materials	% viability of MC3T3-E1 cells (relative to TCPS at 24 h)		
	4 h	**16 h**	**24 h**
TCPS (control)	63.21 ± 1.63	80.66 ± 1.42	100.00 ± 6.38
Chloroform	58.49 ± 7.79	74.06 ± 1.63	93.40 ± 6.17
THF	66.04 ± 6.38	89.15 ± 5.66	89.62 ± 2.16
Acetone	52.83 ± 4.97	78.30 ± 6.69	97.17 ± 7.12
1 M NaOH	59.43 ± 12.58	88.68 ± 4.09	109.91 ± 5.36
5 M NaOH	82.55 ± 7.26	94.81 ± 5.66	110.38 ± 6.48
20:80 EtOH:THF	71.70 ± 8.99	90.57 ± 3.74	113.21 ± 3.74
30:70 EtOH:THF	83.96 ± 7.26	93.87 ± 1.63	108.49 ± 7.79
40:60 EtOH:THF	85.85 ± 0.82	102.83 ± 1.63	125.47 ± 8.17

Table C4 Cell proliferation on protein adsorbed films shown by the percent viability of cells by MTT assay

Materials	% viability of MC3T3-E1 cells (relative to TCPS at 1 d)		
	1 d	**2 d**	**3 d**
TCPS (control)	100.00 ± 6.38	129.39 ± 6.13	192.45 ± 3.74
Chloroform	93.40 ± 6.17	99.53 ± 11.35	136.32 ± 5.89
THF	89.62 ± 2.16	114.62 ± 6.17	143.40 ± 12.04
Acetone	97.17 ± 7.12	119.34 ± 7.26	161.79 ± 15.59
1 M NaOH	109.91 ± 5.36	117.45 ± 11.23	155.19 ± 9.21
5 M NaOH	110.38 ± 6.48	140.09 ± 5.10	183.49 ± 10.99
20:80 EtOH:THF	113.21 ± 3.74	121.23 ± 5.72	158.49 ± 16.32
30:70 EtOH:THF	108.49 ± 7.79	135.38 ± 12.76	198.58 ± 16.40
40:60 EtOH:THF	125.47 ± 8.17	158.96 ± 6.98	212.26 ± 13.65

Table C5 Cell attachment on surface-modified films casted from chloroform and 40:60 (v/v) EtOH:THF shown by the percent viability of cells by MTT assay

Materials	**%viability of MC3T3-E1(relative to TCPS at 24h)**		
	4 h	**16 h**	**24 h**
TCPS (control)	63.21 ± 1.63	80.66 ± 1.42	100.00 ± 6.38
Neat Chloroform	50.00 ± 4.09	61.79 ± 2.95	88.21 ± 2.16
Aminolyzed Chloroform	62.26 ± 4.90	83.49 ± 2.38	104.01 ± 3.74
Activated Chloroform	55.19 ± 2.31	84.20 ± 3.65	102.59 ± 5.28
Adsorbed Chloroform	62.74 ± 4.97	87.74 ± 2.45	106.13 ± 2.45
Neat 40:60 EtOH:THF	68.87 ± 8.65	83.02 ± 10.81	96.23 ± 5.10
Aminolyzed40:60EtOH:THF	80.66 ± 2.65	99.06 ± 3.75	104.72 ± 8.01
Activated 40:60 EtOH:THF	76.89 ± 6.38	97.64 ± 3.74	112.74 ± 6.54
Adsorbed 40:60 EtOH:THF	82.08 ± 4.25	106.13 ± 7.35	148.11 ± 5.36

Table C6 Cell proliferation on surface-modified films casted from chloroform and 40:60 (v/v) EtOH:THF shown by the percent viability of cells by MTT assay

Materials	**%viability of MC3T3-E1 (relative to TCPS at 1 d)**		
	1 d	**2 d**	**3 d**
TCPS (control)	100.00 ± 6.38	129.39 ± 6.13	192.45 ± 3.74
Neat Chloroform	88.21 ± 2.16	99.06 ± 3.74	125.00 ± 3.27
Aminolyzed Chloroform	104.01 ± 3.23	109.67 ± 7.60	121.70 ± 4.15
Activated Chloroform	102.59 ± 5.94	112.50 ± 7.34	135.14 ± 3.34
Adsorbed Chloroform	106.13 ± 2.45	126.89 ± 9.21	166.98 ± 3.74
Neat 40:60 EtOH:THF	96.23 ± 5.10	109.91 ± 4.09	131.60 ± 3.74
Aminolyzed40:60EtOH:THF	104.72 ± 8.01	114.62 ± 6.83	148.58 ± 1.23
Activated 40:60 EtOH:THF	112.74 ± 6.54	141.98 ± 7.12	157.08 ± 14.91
Adsorbed 40:60 EtOH:THF	148.11 ± 5.36	167.92 ± 4.55	243.87 ± 7.79

Table C7 The average absorbance of Alizarin Red-S staining on day 21 by mineralization analysis

Materials	**Average absorbance at 570 nm**	
	Neat PCL film	**Protein-adsorbed PCL film**
TCPS (control)	0.13067 ± 0.00907	0.13067 ± 0.00907
Chloroform	0.14867 ± 0.00681	0.19067 ± 0.01201
THF	0.16367 ± 0.00681	0.19800 ± 0.00917
Acetone	0.19200 ± 0.00361	0.20867 ± 0.00252
1 M NaOH	0.21067 ± 0.00651	0.21433 ± 0.00451
5 M NaOH	0.21000 ± 0.00954	0.21933 ± 0.00153
20:80 EtOH:THF	0.22767 ± 0.00651	0.24900 ± 0.00265
30:70 EtOH:THF	0.25533 ± 0.01301	0.28233 ± 0.01365
40:60 EtOH:THF	0.30900 ± 0.00721	0.43967 ± 0.00603

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