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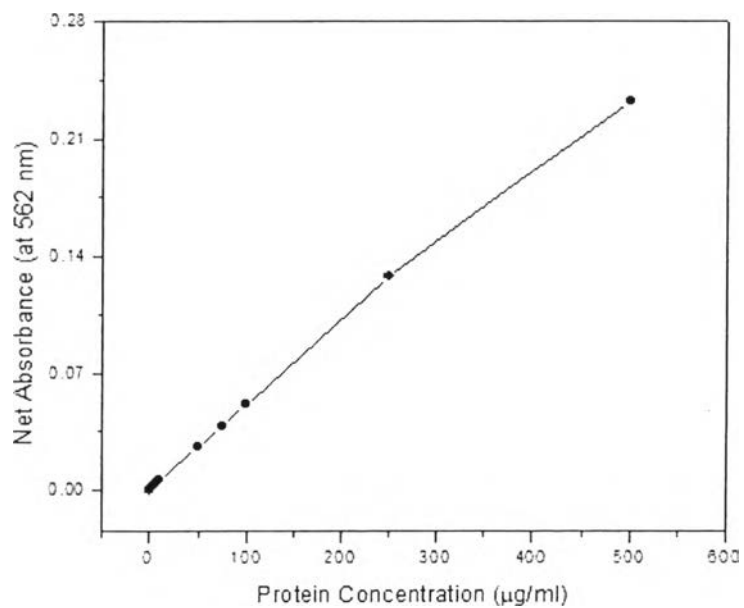
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APPENDICES

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	500	0.2323
2	250	0.1280
3	100	0.0513
4	75	0.0383
5	50	0.0261
6	10	0.006
7	5	0.003
8	1	0.001
9	0	0

Figure A1 The calibration curve for BSA using the standard.

APPENDIX B Mechanical Characterizations

Table B1 Mechanical integrity of PCL fibrous scaffolds

Aminolyzing Time (min)		Ultimate Strength (N/mm^2)	Elongation at Break (%)	Young's Modulus (N/mm^2)
0 min	1	2.2176	325.4582	4.7995
	2	2.2685	302.4799	4.5632
	3	2.1335	325.4750	4.8169
	4	2.1249	320.7846	4.6487
	5	2.2790	341.4785	4.7445
	average	2.2047	323.1352	4.7146
	SD	0.0728	13.9599	0.1071
10 min	1	1.8775	330.4476	4.5912
	2	1.9125	298.4413	4.4145
	3	2.1788	325.9530	5.0750
	4	2.4550	292.4790	4.7747
	5	1.9875	341.7370	4.6488
	average	2.0822	317.8116	4.7008
	SD	0.2387	21.3033	0.2460
30 min	1	2.1146	297.4151	4.9717
	2	1.9833	329.8646	5.1457
	3	1.8762	327.1956	4.9133
	4	2.3726	293.0474	4.9784
	5	2.1740	342.3279	4.1348
	average	2.1006	317.9701	4.6755
	SD	0.1517	19.3056	0.4694
60 min	1	1.9457	312.7746	4.4715
	2	1.9549	321.7459	4.7451
	3	2.1844	291.1260	4.5358
	4	2.0983	336.4790	4.9059
	5	1.7927	322.1650	4.5939
	average	1.9952	316.8581	4.6504
	SD	0.1512	16.7022	0.1751
120 min	1	2.0413	297.1246	4.7145
	2	1.7755	310.4154	5.1246
	3	1.8913	329.4140	4.2004
	4	2.1733	317.4156	4.5624
	5	2.0696	329.4757	4.4566
	average	1.9902	316.7690	4.6117
	SD	0.1568	13.6752	0.3425
180 min	1	1.8813	303.3194	4.5073
	2	1.7563	323.2621	4.8791
	3	2.4059	319.3610	4.4456
	4	1.8925	299.1456	4.4112
	5	1.9751	305.4159	4.7750
	average	1.9822	310.1008	4.6037
	SD	0.2494	10.5703	0.2101

APPENDIX C Degradation of PCL Fibrous Scaffolds

Weight Loss

The rate of degradation can be calculated using following equation

$$\text{Weight loss } (\%) = \frac{W_f - W_i}{W_i} \times 100$$

Where M_i is the initial weight of the scaffolds and Mf is the weight of the scaffold at the given degradation time point, immersed in 0.1 M PBS solution. The weight loss was calculated from those of five samples in each group.

Table C1 Remaining weight of degraded scaffolds in PBS solution without lipase at 37°C

Time (days)	Weight Loss (%)																					
	Neat PCL		Activated PCL[a]										BSA-immobilized PCL[b]									
			10 min		30 min		60 min		120 min		180 min		10 min		30 min		60 min		120 min		180 min	
	ave	SD	ave	SD	ave	SD	ave	SD	ave	SD	ave	SD	ave	SD	ave	SD	ave	SD	ave	SD	ave	SD
5	1.21	1.05	3.45	1.72	4.23	1.83	1.82	0.79	2.81	0.97	2.98	1.03	1.32	1.32	3.39	1.69	1.49	0.00	1.63	0.70	2.93	1.92
10	1.83	0.79	1.71	1.71	2.46	2.46	1.14	1.14	2.58	0.89	1.05	0.91	2.06	1.79	1.60	0.00	2.46	1.71	1.67	0.72	1.70	1.70
15	2.78	1.92	3.70	1.28	2.56	0.00	2.21	0.96	4.72	2.36	4.93	3.23	2.89	2.00	3.09	1.07	3.80	1.90	2.07	1.89	2.98	1.03
20	2.94	0.73	1.52	1.52	2.26	2.26	0.99	0.86	1.67	0.00	3.02	1.51	2.25	0.78	1.77	2.03	2.87	1.99	1.66	0.72	0.49	0.84
30	0.92	0.00	1.17	0.00	3.55	1.78	1.51	0.65	2.34	1.62	2.07	0.72	2.00	0.69	1.24	0.00	1.24	0.00	2.83	0.70	2.12	1.47

Table C2 Remaining weight of degraded scaffolds in lipase/PBS solution at 37°C

Time (days)	Weight Loss (%)																					
	Neat PCL		Activated PCL[a]										BSA-immobilized PCL[b]									
			10 min		30 min		60 min		120 min		180 min		10 min		30 min		60 min		120 min		180 min	
	ave	SD	ave	SD	ave	SD	ave	SD	ave	SD	ave	SD	ave	SD	ave	SD	ave	SD	ave	SD	ave	SD
5	23.71	3.25	18.97	7.90	14.81	4.58	16.36	4.09	21.91	4.46	32.14	9.45	29.96	3.33	17.51	2.59	19.90	0.86	9.35	1.41	10.88	0.72
10	27.40	2.37	32.00	3.57	31.15	1.42	22.35	7.57	40.72	4.46	32.98	8.16	47.94	5.58	36.70	8.29	32.02	9.84	22.50	2.50	19.32	5.21
15	35.00	7.63	34.07	3.40	36.75	8.24	38.12	8.77	50.39	5.46	49.30	1.22	49.13	8.20	36.42	7.48	36.09	1.90	22.73	3.98	26.79	12.50
20	36.59	3.44	34.52	5.77	48.87	3.45	35.15	1.71	51.11	5.09	47.74	0.87	53.60	6.24	39.27	2.09	49.43	8.15	34.85	6.22	35.61	10.98
30	38.65	1.84	36.02	4.09	46.15	4.70	51.32	4.57	56.07	3.71	50.21	2.59	64.40	1.39	44.63	1.24	56.85	9.30	48.58	6.43	54.66	5.54

[a]The PCL e-spun fiber mats were immersed in 0.2 g/ml 1,6-hexamethylenediamine solution.

[b]The activated PCL fibers were immersed in bovine serum albumin solution 3.0 mg/ml for 24 h followed by rinsing process.

APPENDIX D Biological Characterization

This 4 types of fibrous scaffolds were evaluated in vitro with mouse calvaria-derived pre-osteoblastic cells (MC3T3-E1), based on the initial 40,000 cells/cm^2 of cells seeded. To ensure the scaffolds were safe for cells, the cytotoxicity of the scaffolds was tested compared by TCPS. The result was shown in Table D1.

Table D1 Raw data of cytotoxic test of fibrous scaffolds which evaluated from the absorbance at 570 nm by MTT method

Time		Absorbance at 570 nm				
		TCPS (control)	Neat PCL	Aminolyzed PCL	Activated PCL	BSA-immobilized PCL
Day 1	1	0.220	0.179	0.173	0.182	0.194
	2	0.198	0.179	0.178	0.180	0.200
	3	0.203	0.196	0.189	0.194	0.195
	Average	0.207	0.185	0.180	0.185	0.196
	SD	0.012	0.010	0.008	0.008	0.003
Day 3	1	0.206	0.202	0.208	0.173	0.174
	2	0.203	0.199	0.208	0.165	0.162
	3	0.206	0.213	0.205	0.176	0.172
	Average	0.205	0.205	0.207	0.171	0.169
	SD	0.002	0.007	0.005	0.006	0.006
Day 7	1	0.185	0.180	0.175	0.178	0.207
	2	0.186	0.178	0.164	0.176	0.191
	3	0.195	0.184	0.172	0.166	0.185
	Average	0.189	0.181	0.170	0.173	0.194
	SD	0.006	0.003	0.006	0.006	0.011

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Presentations:

1. Poomsurard, P.; Supaphol, P.; and Pavasant, P. (2013, March 11-15) Degradation and biological evaluation of immobilized-electrospun polycaprolactone for bone tissue engineering. Paper presented at POLYCHAR 21 World Forum on Advance Materials, Gwangju, Republic of Korea.

2. Poomsurard, P.; Supaphol, P.; and Pavasant, P. (2013, April 23) Degradation and biological evaluation of immobilized-electrospun polycaprolactone for bone tissue engineering. Paper presented at Proceedings of the 19th PPC Symposium on Petroleum, Petrochems, and Polymers, Bangkok, Thailand.