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APPENDICES

Appendix A Experimental Data of Density, Porosity and Pore Volume

The density of the scaffolds ($\rho_{scaffolds}$) can be calculated using the following equation

$$\textbf{Apparent density } (\rho_{scaffold}, \textbf{g/cm}^3) = \frac{m}{txA}$$

where m is the mass of the scaffold (g), t is the thickness of the scaffold (cm) and A is the area of the scaffold (cm^2)

The porosity and pore volume of the scaffolds can be calculated using the following equation (Hou *et al.* 2003)

$$\textbf{Porosity (\%)} = \left(1 - \frac{\rho_{scaffold}}{\rho_{polymer}}\right) x100$$

$$\textbf{Pore volume} = \left(\frac{1}{\rho_{scaffold}} - \frac{1}{\rho_{polymer}}\right)$$

where $\rho_{scaffold}$ is the apparent density of the fibrous scaffolds(g/cm^3) and $\rho_{polymer}$ is the density of the non-fibrous polymer ($\rho_{polymer}$ of PCL is 1.145 g/cm^3).

Table A1 Raw data of the density, porosity and pore volume of the unmodified PCL scaffolds and modified PCL scaffolds

Material	weight(g)	thickness(mm)					density ($x10^{-2}g/cm^3$)	porosity (%)	pore volume (cm^3/g)
		1	2	3	avg	SD			
Neat PCL (control)	0.006	0.077	0.073	0.080	0.077	0.004	5.00	95.63	19.13
	0.004	0.075	0.056	0.056	0.062	0.011	4.27	96.27	22.53
	0.004	0.052	0.052	0.046	0.050	0.003	4.94	95.69	19.38
	0.007	0.110	0.116	0.121	0.116	0.006	4.16	96.37	23.19
	0.004	0.056	0.045	0.049	0.050	0.006	5.07	95.57	18.86
Aminolyzed PCL	0.005	0.054	0.063	0.051	0.056	0.006	5.22	95.44	18.28
	0.006	0.072	0.071	0.072	0.072	0.001	5.36	95.32	17.78
	0.007	0.121	0.107	0.116	0.115	0.007	3.80	96.68	25.47
	0.006	0.118	0.119	0.110	0.116	0.005	3.54	96.91	27.39
	0.007	0.121	0.127	0.139	0.129	0.009	3.32	97.10	29.21
Activated PCL	0.009	0.148	0.148	0.148	0.148	0.000	3.91	96.59	24.73
	0.009	0.160	0.140	0.150	0.150	0.010	3.98	96.52	24.23
	0.007	0.108	0.100	0.104	0.104	0.004	4.12	96.40	23.38
	0.008	0.163	0.139	0.151	0.151	0.012	3.57	96.88	27.13
	0.009	0.177	0.158	0.017	0.117	0.088	5.10	95.55	18.75
gelatin A immobilized PCL	0.007	0.144	0.148	0.139	0.144	0.005	2.94	97.43	33.15
	0.005	0.077	0.080	0.077	0.078	0.002	4.41	96.14	21.78
	0.007	0.134	0.132	0.119	0.128	0.008	3.49	96.95	27.76
	0.008	0.178	0.178	0.018	0.125	0.092	4.22	96.31	22.81
	0.005	0.052	0.036	0.149	0.079	0.061	3.70	96.77	26.15
gelatin B immobilized PCL	0.007	0.133	0.143	0.014	0.097	0.072	4.98	95.65	19.22
	0.008	0.173	0.171	0.176	0.173	0.003	2.85	97.51	34.24
	0.007	0.136	0.149	0.121	0.135	0.014	3.12	97.28	31.18
	0.009	0.199	0.185	0.187	0.190	0.008	3.07	97.32	31.68
	0.006	0.110	0.102	0.094	0.102	0.008	3.95	96.55	24.45
BSA immobilized PCL	0.008	0.143	0.145	0.014	0.101	0.075	4.90	95.72	19.54
	0.003	0.055	0.047	0.044	0.049	0.006	3.60	96.85	26.87
	0.007	0.149	0.158	0.145	0.151	0.007	3.02	97.36	32.26
	0.007	0.132	0.143	0.139	0.138	0.006	3.44	97.00	28.23
	0.005	0.097	0.054	0.086	0.079	0.022	4.28	96.27	22.51
CBP immobilized PCL	0.0044	0.053	0.064	0.049	0.055	0.008	5.17	95.49	18.49
	0.0072	0.141	0.12	0.128	0.130	0.011	3.61	96.85	26.85
	0.0049	0.072	0.084	0.085	0.080	0.007	3.96	96.54	24.36
	0.0029	0.037	0.028	0.031	0.032	0.005	5.89	94.86	16.11
	0.0056	0.082	0.087	0.085	0.085	0.003	4.30	96.25	22.40

APPENDICES

Appendix B Experimental Data of Water Retention Capacity and Weight Loss of Polycaprolactone Electrospun Fibrous Scaffolds

1. Water Retention Capacity

The water retention was calculated by using the following equation. (Kothapalli *et al.*, 2005)

$$\textbf{Water Retention (\%)} = \left(\frac{M_{wet} - M_{dry}}{M_{dry}}\right) x100$$

where M_{dry} and M_{wet} are the weight of the scaffold before and after immersion in 0.1 M PBS solution respectively. Five measurements were performed for the calculation of an average water retention value.

2. Degradation Study of Fibrous Scaffolds

The rate of degradation can be calculated using the following equation.

$$\textbf{Weight loss (\%)} = \left(\frac{Mf - Mi}{Mi}\right) x100$$

where M_i is the initial weight of the scaffolds and M_f and is the weight of the scaffold at the given degradation time point, immersed in 0.1 M PBS solution. Five measurements were performed for the calculation of an average water degradation rate value.

Table B1 Raw data of water retention capacity of aminolyzed PCL electrospun fibrous scaffolds at various HMD treatments

Time(h)	water retention (%)																			
	0.25		0.5		1		2		4		6		12		18		24		48	
Sample	avg	SD	avg	SD	avg	SD	avg	SD	avg	SD	Avg	SD	avg	SD	avg	SD	avg	SD	avg	SD
Neat PCL	56.93	0.4	61.31	0.16	66.64	0.26	82.66	1.11	119.56	0.69	125.96	0.34	133.14	0.76	136.99	0.94	140.00	0.45	140.50	0.28
0.04 g/ml HMD treated PCL	81.36	0.59	-	-	89.44	0.57	97.10	0.62	153.00	0.49	182.00	0.75	-	-	200.00	0.66	-	-	212.29	1.39
0.06 g/ml HMD treated PCL	93.29	1.41	-	-	95.27	1.74	101.46	0.93	204.13	0.62	225.18	1.42	-	-	236.16	1.23	-	-	263.38	1.44
0.08 g/ml HMD treated PCL	183.49	0.91	-	-	217.04	0.91	231.98	0.55	234.71	0.3	235.35	1.42	-	-	255.51	0.83	-	-	273.04	0.49
0.10 g/ml HMD treated PCL	200.00	0.61	-	-	228.13	0.41	235.60	0.35	245.98	1.23	254.69	1.27	-	-	273.09	1.06	-	-	278.39	0.7
0.20 g/ml HMD treated PCL	228.45	0.9	-	-	235.57	0.76	244.80	1.4	247.53	0.98	259.86	0.76	-	-	277.39	0.95	-	-	289.74	0.93
0.40 g/ml HMD treated PCL	196.26	0.47	213.24	0.7	225.87	0.77	242.60	0.31	254.61	0.17	257.69	0.53	261.33	0.81	271.98	0.25	280.00	0.78	300.42	1.3

Table B2 Raw data of water retention capacity of aminolyzed PCL electrospun fibrous scaffolds at various HMD treatments

Time(h)	weight loss (%)																			
	0.25		0.5		1		2		4		6		12		18		24		48	
Sample	avg	SD	avg	SD	avg	SD	avg	SD	avg	SD	Avg	SD	avg	SD	avg	SD	avg	SD	avg	SD
Neat PCL	0.00	0.35	0.30	0.45	1.06	0.12	1.08	0.09	1.20	0.14	1.80	0.15	3.18	0.05	3.23	0.38	4.42	0.40	4.47	0.15
0.04 g/ml HMD treated PCL	1.89	0.13	-	-	2.62	0.13	2.63	0.23	2.75	0.24	2.94	0.19	-	-	3.03	0.22	-	-	3.99	0.33
0.06 g/ml HMD treated PCL	0.48	0.46	-	-	0.74	0.47	0.96	0.27	1.17	0.18	1.41	0.43	-	-	1.79	0.32	-	-	3.04	0.34
0.08 g/ml HMD treated PCL	0.55	0.79	-	-	1.43	0.81	1.51	0.48	1.79	0.30	2.73	0.89	-	-	3.45	0.59	-	-	4.84	0.33
0.10 g/ml HMD treated PCL	0.00	0.49	-	-	0.74	0.31	1.23	0.25	2.13	0.41	3.58	0.57	-	-	3.61	0.29	-	-	3.68	0.28
0.20 g/ml HMD treated PCL	1.14	0.47	-	-	2.50	0.25	3.03	0.40	3.71	0.31	4.26	0.15	-	-	4.76	0.23	-	-	5.56	0.29
0.40 g/ml HMD treated PCL	0.49	0.16	0.68	0.07	0.68	0.21	0.93	0.07	2.72	0.06	3.64	0.14	4.47	0.11	5.24	0.05	6.80	0.21	7.09	0.42

APPENDICES

Appendix C Experimental Data of NH_2 Density, Elemental Composition of the Surface and Water Contact Angle Measurement

1. NH_2 Density on the Modified PCL Scaffold

The ninhydrin analysis method was carried out to quantitatively determine the amount of NH_2 groups on the aminolysed PCL and biomolecule-immobilized PCL scaffolds. The experiment measured the absorbance at the wavelength of 538 nm using a UV-vis spectrophotometer. A calibration curve was obtained with known concentration of 1,6-hexamethylenediamine in 1,4-dioxane/IPA (1:1, v/v) solution as shown in Figure C1.

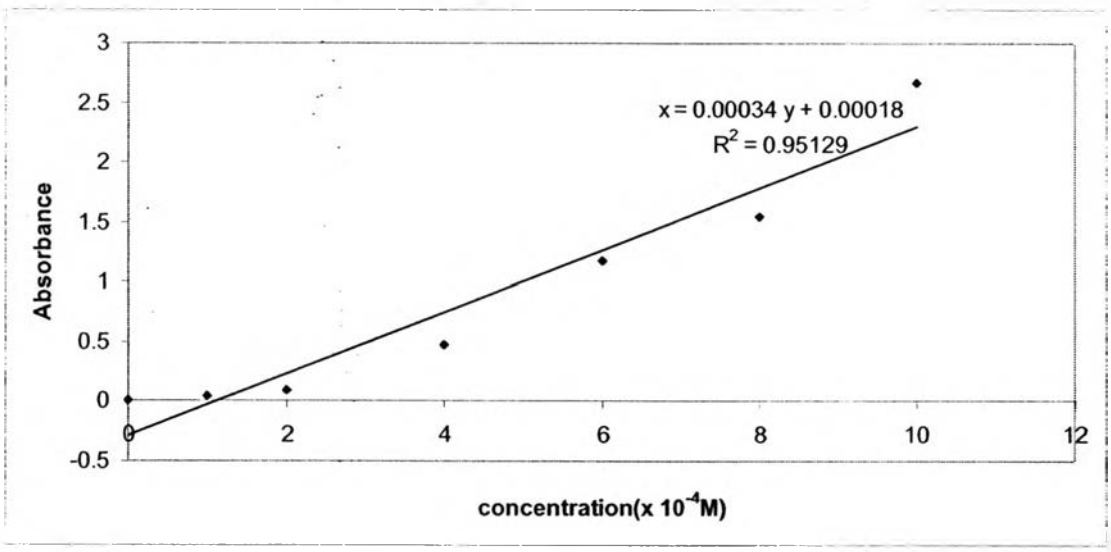

Figure C1 Calibration curve of UV absorbance as a function of 1,6-hexamethylenediamine (HMD) concentration analyzed by ninhydrin assay method

Table C1 NH_2 concentration and density as a function of 1,6-hexamethylenediamine

1,6-hexamethylenediamine concentration(g/ml)	Absorbance	NH_2 concentration (x 10^{-4}M)	NH_2 density (x10^{-7} mol/cm^2)
0.04	0.0085	1.84	1.20
0.06	0.012	1.87	1.21
0.08	0.02	1.88	1.22
0.1	0.0249	2.00	1.30
0.2	0.0598	2.10	1.37
0.4	0.0896	2.12	1.38

2. Elemental Composition of the Surface

The surface of neat and modified PCL fibrous scaffolds were further determined the elemental composition by using X-ray Photoelectron Spectrometer (XPS).

Table C2 Elemental compositions of C_{1s}, N_{1s}, O_{1s} and N_{1s}/C_{1s} ratio on the surface of aminolyzed PCL fibrous scaffolds as a function of 1,6-hexamethylenediamine

Diamine concentration (g/ml)	C_{1s}	N_{1s}	O_{1s}	N_{1s}/C_{1s} ratio
0.04	76.52	0.48	23.01	0.0063
0.06	77.13	0.61	22.25	0.0079
0.08	75.93	1.24	22.84	0.0163
0.10	75.24	1.27	23.49	0.0169
0.20	76.3	1.3	22.4	0.0170
0.40	75.2	1.28	23.9	0.0170

Table C3 Elemental compositions of C_{1s}, N_{1s}, O_{1s} and N_{1s}/C_{1s} ratio on the surface of the neat, aminolyzed ,and all types of immobilized PCL scaffolds

Sample	C_{1s}	N_{1s}	O_{1s}	N_{1s}/C_{1s} ratio
Control PCL	78.64	0.37	20.98	0.0047
Aminolyzed PCL	76.3	1.3	22.4	0.0170
Activated PCL	75.2	0.91	23.9	0.0121
Gelatin type-A Immobilized PCL	73.2	4.76	22.04	0.0650
Gelatin type-B Immobilized PCL	73.98	4.56	21.46	0.0616
BSA Immobilized PCL	73.4	3.5	23.1	0.0477
CBP Immobilized PCL	73.0	5.0	22.0	0.0685

APPENDICES

Appendix D Experimental Data of Cell Cuture Studies

The potential use as bone scaffolds of PCL electrospun fibrous materials was evaluated using MC3T3-E1 cell culture studies, including direct cytotoxicity test, indirect cytotoxicity test, cell attachment, cell proliferation, ALP activity at 3 days and 7 days and mineralization as shown in Table D1, D2, D3, D4, D5, D6, and D7, respectively.

Table D1 Raw data of direct cytotoxicity test of proteins, which were immobilized onto the PCL fibrous scaffolds, determined the viability of cells by MTT assay method at 570 nm

Time(day)	% viability of MC3T3-E1 cells (relative to 2%MEM at 1 day)					
	1		2		3	
Material	**avg**	**SD**	**avg**	**SD**	**avg**	**SD**
2%MEM(control)	100.00	0.22	140.15	8.77	182.45	0.35
gelatin	95.78	4.06	110.17	5.57	169.02	7.43
gelatin	103.02	5.34	134.65	8.26	168.01	6.62
BSA	100.64	2.73	137.67	8.32	172.41	14.78
CBP	83.50	3.82	128.14	5.27	148.90	8.75

Table D2 Raw data of indirect cytotoxicity test of all types of PCL fibrous scaffolds, determined the viability of cells by MTT assay method at 570 nm

Time(day)	% viability of MC3T3-E1 cells (relative to TCPs at 1 day)					
	1		2		3	
Material	**avg**	**SD**	**avg**	**SD**	**avg**	**SD**
TCPs (control)	100.00	0.22	140.15	8.77	182.45	0.35
neat PCL	94.78	4.57	109.62	1.16	150.14	0.24
aminolysed PCL	100.00	2.00	113.29	1.81	157.42	4.48
activated PCL	104.86	4.56	121.54	5.16	159.49	10.11
gelatinA immobilized PCL	103.57	0.89	119.71	1.81	152.06	1.19
gelatinB immobilized PCL	104.40	2.76	129.42	8.96	156.32	7.98
BSA immobilized PCL	102.57	2.26	143.08	11.55	157.84	1.19
CBP immobilized PCL						

Table D3 Raw data of cell attachment of MC3T3-E1 onto all types of PCL fibrous scaffolds at 2, 4, and 6 hours, determined the viability of cells by MTT assay method at 570 nm

Time(h)	% viability of MC3T3-E1 cells (relative to TCPs at 2 h)					
	2		4		6	
Material	**avg**	**SD**	**avg**	**SD**	**avg**	**SD**
TCPs	100.00	18.03	170.08	23.37	256.97	39.81
neat PCL	154.92	24.16	154.10	25.48	136.48	17.41
aminolysed PCL	113.93	19.33	110.66	33.28	131.56	17.26
activated PCL	112.70	43.59	103.69	13.23	137.70	42.09
gelatinA immobilized PCL	101.23	12.92	108.20	21.30	131.56	18.39
gelatinB immobilized PCL	118.44	47.12	108.20	18.02	131.56	20.73
BSA immobilized PCL	118.03	23.89	118.85	18.40	143.44	34.27
CBP immobilized PCL	143.59	18.48	147.44	22.00	175.64	22.28

Table D4 Raw data of cell proliferation of MC3T3-E1 onto all types of PCL fibrous scaffolds at 1, 2, and 3 days, determined the viability of cells by MTT assay method at 570 nm

time(day)	% viability of MC3T3-E1 cells (relative to TCPs at 1 day)					
	1		2		3	
Material	**avg**	**SD**	**avg**	**SD**	**avg**	**SD**
TCPs (control)	100.00	8.83	126.80	13.27	132.91	8.82
neat PCL	117.05	7.33	240.03	61.14	292.20	52.33
aminolysed PCL	116.95	55.56	321.13	79.59	450.10	85.67
activated PCL	106.70	12.84	426.90	71.86	471.00	76.92
gelatinA immobilized PCL	80.97	12.68	332.06	84.07	805.36	59.13
gelatinB immobilized PCL	78.34	11.32	392.32	62.06	804.94	183.19
BSA immobilized PCL	144.90	10.42	498.09	43.30	840.39	62.77
CBP immobilized PCL	221.08	7.38	450.98	17.53	789.22	57.29

Table D5 Raw data of ALP Activity of MC3T3-E1 seeded onto all types of PCL fibrous scaffolds at 3 days

Time(h)	ALP activity test (3 days)							
	1			2			ALP activity	
Material	ALP assay	protein assay	ALP activity	ALP assay	protein assay	ALP activity	avg	SD
TCPs	2.73550	13.97004	0.19581	2.33871	9.03533	0.26	0.23	0.04
Neat PCL	6.81594	28.72645	0.23727	6.07297	40.78989	0.15	0.19	0.06
Aminolysed PCL	5.42109	26.95169	0.20114	5.67283	48.05050	0.12	0.16	0.06
Activated PCL	5.52195	34.51732	0.15998	7.30696	50.52417	0.14	0.15	0.01
GelatinA immobilized PCL	5.97322	27.07740	0.22060	6.81594	34.78842	0.20	0.21	0.02
GelatinB immobilized PCL	7.06182	28.47695	0.24798	7.11091	36.98263	0.19	0.22	0.04
BSA immobilized PCL	3.76878	21.10507	0.17857	7.79525	19.12051	0.41	0.29	0.16
CBP immobilized PCL	11.54670	54.51166	0.21182	10.99686	56.78298	0.19	0.20	0.01

Table D6 Raw data of ALP Activity of MC3T3-E1 seeded onto all types of PCL fibrous scaffolds at 7 days

Time(h)	ALP activity test (3 days)							
	1			2			ALP activity	
Material	ALP assay	protein assay	ALP activity	ALP assay	protein assay	ALP activity	avg	SD
TCPs	4.137722	1.987754	2.08	3.340162	1.29369	2.58	2.33	0.35
Neat PCL	4.398301	2.164105	2.03	4.810824	3.333577	1.44	1.74	0.42
Aminolysed PCL	9.826533	5.966295	1.65	6.222275	4.632124	1.34	1.50	0.21
Activated PCL	8.765606	2.969726	2.95	9.200245	5.199743	1.77	2.36	0.84
GelatinA immobilized PCL	5.622587	4.257312	1.32	6.668014	4.915135	1.36	1.34	0.03
GelatinB immobilized PCL	9.682136	4.915135	1.97	7.6977883	4.538146	1.70	1.83	0.19
BSA immobilized PCL	10.83588	4.915135	2.20	10.06705	3.333577	3.02	2.61	0.58
CBP immobilized PCL	109.53133	56.87312085	1.93	104.31555	59.24283422	1.76	1.84	0.12

Table D7 Raw data of quantity of mineral deposition on all types of PCL fibrous scaffolds using Alizarin Red-S method at 570 nm

Materials	1	2	avg	SD
TCPS	0.025	0.03	0.0275	0.003536
Neat PCL	0.393	0.401	0.397	0.005657
Aminolyzed PCL	0.394	0.392	0.393	0.001414
Activated PCL	1.347	1.179	1.263	0.118794
Gelatin type-A immobilized PCL	1.841	1.886	1.8635	0.03182
Gelatin type-B immobilized PCL	1.839	1.389	1.614	0.318198
BSA immobilized PCL	1.44	1.864	1.652	0.299813
CBP immobilized PCL	3.61	2.517	3.0635	0.772868

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Presentations:

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