References

[1] Wang, Y.; Kim, H.J.; Novakovic, G.V.; and Kaplan, D.L. Stem cell-based tissue engineering with silk biomaterials. Biomaterials 27 (2006): 6064–6082.

[2] Meechaisue, C.; Wutticharoenmongkol, P.; Warapyt, R.; Huangjing, T.; Ketbumrung, N.; Pavasant, P.; and Supaphol, P. Preparation of electrospun silk fibroin fiber mats as bone scaffolds: a preliminary study. Biomedical Materials 2 (2007): 181-188.

[3] Chamchongkaset, J.; Kanokpanont, S.; Kaplan, D.L.; and Damrongsakkul, S. Modification of thai silk fibroin scaffolds by gelatin conjugation for tissue engineering. Advanced Materials Research 55-57 (2008): 685-688.

[4] Tomihata, K.; and Ikada, Y. Cross-linking of gelatin with carbodiimides. Tissue engineering 2 (1996): 307-313.

[5] Gil, E.S.; Spontak, R.J.; and Hudson, S.M. Effect of β-sheet crystals on the thermal and rheological behavior of protein-based hydrogels derived from gelatin and silk fibroin. Macromolecular bioscience 5 (2005): 702-709.

[6] Lv, Q.; Hu, K.; Feng, Q.L.; and Cui, F. Fibroin/collagen hybrid hydrogels with crosslinking method: preparation, properties, and cytocompatibility. J Biomed Mater Res 84A (2007): 198-207.

[7] Vepari, C.; and Kaplan, D.L. Silk as a biomaterial. Progress in Polymer Science 32 (2007): 991–1007.

[8] Gil, E.S.; Frankowski, D.J.; Bowman, M.K.; Gozen, A.O.; Hudson, S.M.; and Spontak, R.J. Mixed protein blends composed of gelatin and bombyx mori silk fibroin: effects of solvent-induced crystallization and composition. Biomacromolecules 7 (2006): 728-735.

[9] Kim, U.J.; Park, J.; Kim, H.J.; Wada, M.; and Kaplan, D.L. Three-dimensional aqueous-derived biomaterial scaffolds from silk fibroin. Biomaterials 26 (2005): 2775-2785.

[10] Sarovart, S.; Sudatis, B.; Meesilpa, P.; Grady, B.P.; and Magaraphan R. The use of sericin as an antioxidant and antimicrobial for polluted air treatment. Adv.Mater.Sci. 5 (2003): 193-198.

[11] http://www.en.rmut.ac.th/prd/Journal/Silk_with_figuresnew.pdf (Mar 20, 2008)

[12] http://www.concord.org/~barbara/workbench_web/unitV_revised/silk/images/silk_beta_sheets.jpg (Mar 20, 2008)

[13] Sofia, S.; McCarthy, M.B.; Gronowicz, G.; and Kaplan, D.L. Functionalized silk-based biomaterials for bone formation. Journal of Biomedical Materials Research 54 (2001): 139–148.

[14] http://dspace.library.drexel.edu/retrieve/4251/CHAPTER+2.pdf (Mar 20, 2008)

[15] http://www.moac.go.th/builder/mu/index.php?page=415&clicksub=415&sub=12 6 (Mar 20, 2008)

[16] http://www.sumitomo.gr.jp/english/discoveries/special/images/silk_p16.jpg (Mar 20, 2008)

[17] http://www.gelatin-gmia.com/ (Mar 13, 2008)

[18] http://en.wikipedia.org/wiki/Gelatin (Mar 13, 2008)

[19] http://www.lsbu.ac.uk/water/hygel.html (Mar 13, 2008)

[20] Gil, E.S.; Frankowski, D.J.; Spontak, R.J.; and Hudson, S.M. Swelling behavior and morphological evolution of mixed gelatin/silk fibroin hydrogels. Biomacromolecules 6 (2005): 3079-3087.

[21] Tabata, Y.; and Ikada, Y. Protein release from gelatin matrices. Advance drug delivery reviews 31 (1998): 287-301.

[22] http://www.gelatin-gmia.com/html/rawmaterials_app.html (Mar 13, 2008)

[23] http://www.gelatin-gmia.com/html/gelatine_health.html (Mar 13, 2008)

[24] Wahl, D.A.; and Czernuszka, J.T. Collagen-hydroxyapatite composites for hard tissue repair. European cells and materials 11 (2006): 43-56.

[25] http://www.azom.com/details.asp?ArticleID=107 (July 8, 2008)

[26] http://en.wikipedia.org/wiki/Hydroxyapatite (July 8, 2008)

[27] http://www.chemistry.upatras.gr/studs/sotk/hap.htm (July 8, 2008)

[28] http://www.pentax.co.jp (July 8, 2008)

[29] Ma, P.X. Scaffolds for tissue fabrication. Materialstoday (2004).

[30] Sachlos, E.; and Czernuszka, J.T. Making tissue engineering scaffolds work. European cells and materials 5 (2003): 29-40.

[31] Snowman, John W. Downstream processes: equipment and techniques. Alan R. Liss, Inc. (1998): 315-351.

[32] http://www.labconco.com/pdf/freeze_dry/guide_fd.pdf (June 7, 2008)

[33] Lee, J.M.; Edwards, H.H.L.; Pereira,C.A.; and Samii, S.I. Crosslinking of tissue-derived biomaterials in 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC). Journal of materials science 7 (1996):531-541.

[34] Barnes, C.P.; Pemble, C.W.; Brand, D.D.; Simpson, D.G.; and Bowlin, G.L. Cross-linking electrospun type II collagen tissue engineering scaffolds with carbodiimide in ethanol. Tissue engineering 13 (2007):1593-1605.

[35] http://www.piercenet.com/Objects/View.cfm?type=ProductFamily&ID= 02030312 (June 18, 2008)

[36] Grabarek, Z.; and Gergely, J. Zero-length crosslinking procedure with the use of active esters. Anal Biochem 185 (1990):131-135.

[37] http://www.interchim.com/interchim/bio/produits_uptima/tech_sheet/FT-UP52005(EDC).pdf (June 18, 2008)

[38] Damink, L.H.H.; Dijkstra, P.J.; Feijen, J.; Luyn, M.J.A.; Wachem, P.B.; and Nieuwenhuis, P. Cross-linking of dermal sheep collagen using a water-soluble carbodiimide. Biomaterials 17 (1996): 765–773.

[39] Davey, J.; and Lord, M. Essential cell biology Volume 1: Cell structure A practical approach. Oxford University Press, (2003).

[40] http://www.ljm.org.ly/articles/AOP/AOP070705/fig1.jpg (Aug 18, 2009)

[41] http://www.brc.riken.jp/lab/cell/images/photo/RCB1451.jpg (Aug 18, 2009)

[42] Kundu, J.; Dewan, M.; Ghoshal, S.; and Kundu, S.C. Mulberry non-engineered silk gland protein vis-a-vis silk cocoon protein engineered by silkworms as biomaterial matrices. J Master Sci (2008), in press.

[43] Meinel, L.; Karageorgiou, V.; Hofmann, S.; Fajardo, R.; Snyder, B.; Li, C.; Zichner, L.; Langer, R.; Vanjak-Novakovic, G.; and Kaplan, D.L. Engineering bone-like tissue in vitro using human bone marrow stem cells and silk scaffolds. J Biomed Mater Res 71A (2004): 25-34.

[44] Chang, M.C.; Koa, C.C.; and Douglas, W.H. Preparation of hydroxyapatite-gelatin nanocomposite. Biomaterials 24 (2003): 2853–2862.

[45] Wang, L.; Ning, G.L.; and Senna, M. Microstructure and gelation behavior of hydroxyapatite-based nanocomposite sol containing chemically modified silk fibroin. Colloids and surfaces 254 (2005): 159–164.

[46] Takahashi, Y.; Yamamoto, M.; and Tabata, Y. Osteogenic differentiation of mesenchymal stem cells in biodegradable sponges composed of gelatin and β-tricalcium phosphate. Biomaterials 26 (2005): 3587–3596.

[47] Tanaka, T.; Hirose, M.; Kotobuki, N.; Ohgushi, H.; Furuzono, T.; and Soto, J. Nano-scaled hydroxyapatite/silk fibroin sheets support osteogenic differentiation of rat bone marrow mesenchymal cells. Materials Science and Engineering C27 (2007): 817-823.

[48] Kino, R.; Ikoma, T.; Yunoki, S.; Nagai N.; Tanaka, J.; Asakura, T.; and Munekata, M. Preparation and Characterization of Multilayered Hydroxyapatite/Silk Fibroin Film. Journal of Bioscience and Bioengineering 6 (2007): 514-520.

[49] Du, C.; Jin, J.; Li, Y.; Kong, X.; Wei, K.; and Yao, J. Novel silk fibroin/hydroxyapatite composite films: Structure and properties. Materials Science and Engineering C (2008), in press.

[50] Venugopal, J.; Low, S.; Choon, A.T.; Kumar, T.S.; and Ramakrishna, S. Mineralization of osteoblasts with electrospun collagen/hydroxyapatite nanofibers. J Mater Sci: Mater Med 19 (2008): 2039–2046.

[51] Pek, Y.S.; Gao, S.; Arshad, M.; Leck, K.J; and Ying, J.Y. Porous collagen-apatite nanocomposite foams as bone regeneration scaffolds. Biomaterials 29 (2008): 4300-4305.

[52] Li, M.; Ogiso, M.; and Minoura, N. Enzymatic degradation behavior of porous silk fibroin sheets. Biomaterials 24 (2003): 357–365.

[53] Petrini, P.; Parolari, C.; and Tanzi, M.C. Silk fibroin-polyurethane scaffolds for tissue engineering. J. of materials science: materials in medicine 12 (2001): 849-853.

[54] Marolt, D.; Augst, A.; Freed, L. E.; Vepari, C.; Fajardo, R.; Patel, N.; Gray, M.; Farley, M.; Kaplan, D.; and Vunjak-Novakovic, G. Bone and cartilage tissue constructs grown using human bone marrow stromal cells, silk scaffolds and rotating bioreactors. Biomaterials 27 (2006): 6138–6149.

[55] Hofmann, S.; Hagenmullar, H.; Koch, A.M.; Mullar, R.; Novakovic, G.V.; Kaplan, D.L.; Merkle, H.P.; and Meinel, L. Control of in vitro tissue-engineered bone-like structures using human mesenchymal stem cells and porous silk scaffolds. Biomaterials 28 (2007): 1152-1162.

[56] Li, M.; Wu, Z.; Zhang, C.; Lu, S.; Yan, H.; Huang, D.; and Ye, H. Study on porous silk fibroin materials. II. Preparation and characteristics of spongy porous silk fibroin material. Journal of Applied Polymer Science 79 (2001): 2192-2199.

[57] Mandal, B.B.; Mann, J.K.; and Kundu, S.C. Silk fibroin/gelatin multilayered films as a model system for controlled drug release. European Journal of Pharmaceutical Sciences 37 (2009): 160–171.

[58] Ulubayram, K.;Cakar, N.;Korkusuz, P.;Ertan, C.; and Hasirci, N. EGF containing gelatin-based wound dressings. Biomaterials 22 (2001): 1345-1356.

[59] Kim, U.J.; Park, J.; Li, C.; Jin, H.J.; Valluzzi, R.; and Kaplan, D.L. Structure and properties of silk hydrogels. Biomacromolecules 5 (2004): 786-792.

[60] She, Z.; Zhang, B.; Jin, C.; Feng, Q.; and Xu, Y. Preparation and in vitro degradation of porous three-dimensional silk fibroin/chitosan scaffold. Polymer Degradation and Stability (2008): 1-7.

[61] Kishida, A.; Iwata, H.; Tamada, Y.; and Ikada, Y. Cell behaviour on polymer surfaces grafted with non-ionic and ionic monomers. Biomaterials 12 (1991): 786-792.

[62] Calvert, J.W.; Marra, K.G.; Cook, L.; Kumta, P.N.; Dimilla, P.A.; and Weiss, L.E. Characterization of osteoblast-like behavior of cultured bone marrow stromal cells on various polymer surfaces. Journal of Biomedical Materials Research (2000) 52:279.

[63] Han, T.; and Misra, R.D.K. Biomimetic chitosan nanohydroxyapatite composite scaffolds for bone tissue engineering. Acta Biomaterialia 5 (2009): 1182-1197.

[64] Hiraoka, Y.; Kimura, Y.; Ueda, H.; and Tabata, Y. Fabrication and biocompatibility of collagen sponge reinforced with poly(glycolic acid) fiber. Tissue Engineering 9 (2003): 1101-1112.

[65] Arpornmaeklong, P.; Suwatwirote, N.; Pripatnanont, P.; and Oungbho, K. Growth and differentiation of mouse osteoblasts on chitosan–collagen sponges. International Journal of Oral and Maxillofacial Surgery 36 (2007): 328–337.

APPENDICES

APPENDIX A

Raw data of weight loss (%)

Table A-1 Mean and SD of weight loss (%) of Thai silk fibroin/gelatin scaffolds (SF/G).

Weight blending ratio of Thai silk fibroin/gelatin	Weight loss (%)			
	Non-crosslinked scaffolds		Crosslinked scaffolds	
	mean	SD	mean	SD
0/100	93.94	1.56	24.21	0.36
20/80	45.38	0.95	24.14	1.99
40/60	20.27	1.34	11.99	0.70
50/50	15.50	1.15	14.05	0.37
60/40	17.78	1.50	11.39	0.34
80/20	33.51	2.40	10.39	0.70
100/0	58.84	5.94	11.95	3.33

APPENDIX B

Raw data of compressive modulus

Table B-1 Mean and SD of compressive modulus of Thai silk fibroin/gelatin scaffolds (SF/G) in dry condition.

Weight blending ratio of Thai silk fibroin/gelatin	Compressive modulus (kPa)			
	Non-crosslinked scaffolds		Crosslinked scaffolds	
	mean	SD	mean	SD
0/100	140.32	12.26	340.00	83.85
20/80	230.67	12.30	450.00	34.06
40/60	552.50	59.35	746.67	55.07
50/50	482.50	45.12	680.00	88.32
60/40	464.33	47.62	751.67	38.86
80/20	240.00	28.28	562.50	36.86
100/0	206.67	50.33	615.00	64.03

Table B-2 Mean and SD of compressive modulus of Thai silk fibroin/gelatin scaffolds (SF/G) in wet condition.

Weight blending ratio of Thai silk fibroin/gelatin	Compressive modulus (kPa)			
	Non-crosslinked scaffolds		Crosslinked scaffolds	
	mean	SD	mean	SD
0/100	-	-	-	-
20/80	38.67	5.77	45.00	5.77
40/60	93.33	5.28	76.50	5.00
50/50	97.50	6.30	90.00	7.89
60/40	82.50	7.08	75.00	8.25
80/20	47.50	5.00	32.86	4.88
100/0	-	-	34.29	5.35

APPENDIX C

Raw data of physical properties at various contents of EDC/NHS

Table C-1 Mean and SD of weight loss (%) of Thai silk fibroin/gelatin scaffolds (SF/G) at various contents of EDC/NHS.

EDC/NHS contents (wt/wt) (base on 1 ml of 4 wt% protein solution)	Weight loss (%)					
	SF/G 60/40		SF/G 80/20		SF/G 100/0	
	mean	SD	mean	SD	mean	SD
0	17.78	1.50	33.51	2.40	58.84	5.94
0.5/0.7	11.39	0.34	10.39	0.70	11.95	3.33
1.0/1.4	10.31	0.59	12.71	0.96	10.41	0.54
1.5/2.1	12.23	0.92	12.59	0.39	14.35	0.86

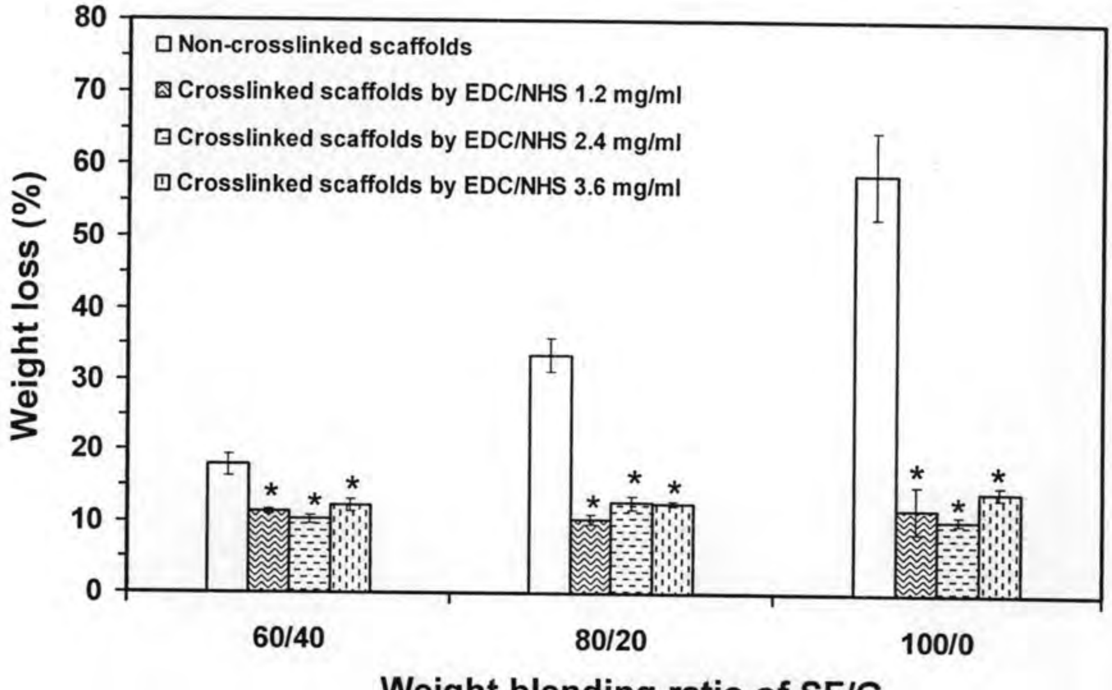

Figure C-1 Weight loss (%) of Thai silk fibroin/gelatin scaffolds (SF/G) at various contents of EDC/NHS.

* represent the significant difference (p<0.05) relative to non-crosslinked scaffolds at each weight blending ratio of SF/G.

Table C-2 Mean and SD of compressive modulus in dry condition of Thai silk fibroin/gelatin scaffolds (SF/G) at various contents of EDC/NHS.

EDC/NHS contents (wt/wt) (base on 1 ml of 4 wt% protein solution)	Compressive modulus (kPa) (Dry condition)					
	SF/G 60/40		SF/G 80/20		SF/G 100/0	
	mean	SD	mean	SD	mean	SD
0	464.33	47.62	240.00	28.28	206.67	50.33
0.5/0.7	751.67	38.86	562.50	36.86	615.00	64.03
1.0/1.4	810	113.14	594	26.08	542.5	71.36
1.5/2.1	705	91.92	755	66.58	358	62.21

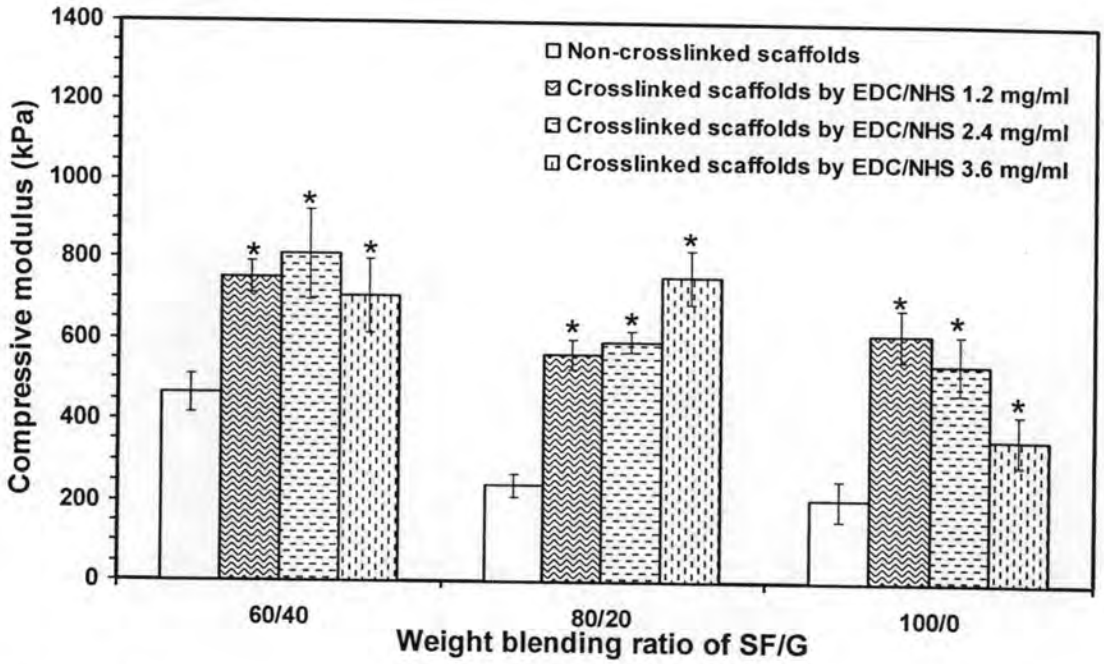

Figure C-2 Compressive modulus in dry condition of Thai silk fibroin/gelatin scaffolds (SF/G) at various contents of EDC/NHS.

* represent the significant difference ($p<0.05$) relative to non-crosslinked scaffolds at each weight blending ratio of SF/G.

Table C-3 Mean and SD of compressive modulus in wet condition of Thai silk fibroin/gelatin scaffolds (SF/G) at various contents of EDC/NHS.

EDC/NHS contents (wt/wt) (base on 1 ml of 4 wt% protein solution)	Compressive modulus (kPa) (Wet condition)					
	SF/G 60/40		SF/G 80/20		SF/G 100/0	
	mean	SD	mean	SD	mean	SD
0	82.5	7.08	47.5	5	-	-
0.5/0.7	75	8.25	32.86	4.88	34.29	5.35
1.0/1.4	45	5.48	23.33	5.16	34	5.48
1.5/2.1	46.67	5.16	37.14	8.81	24	548

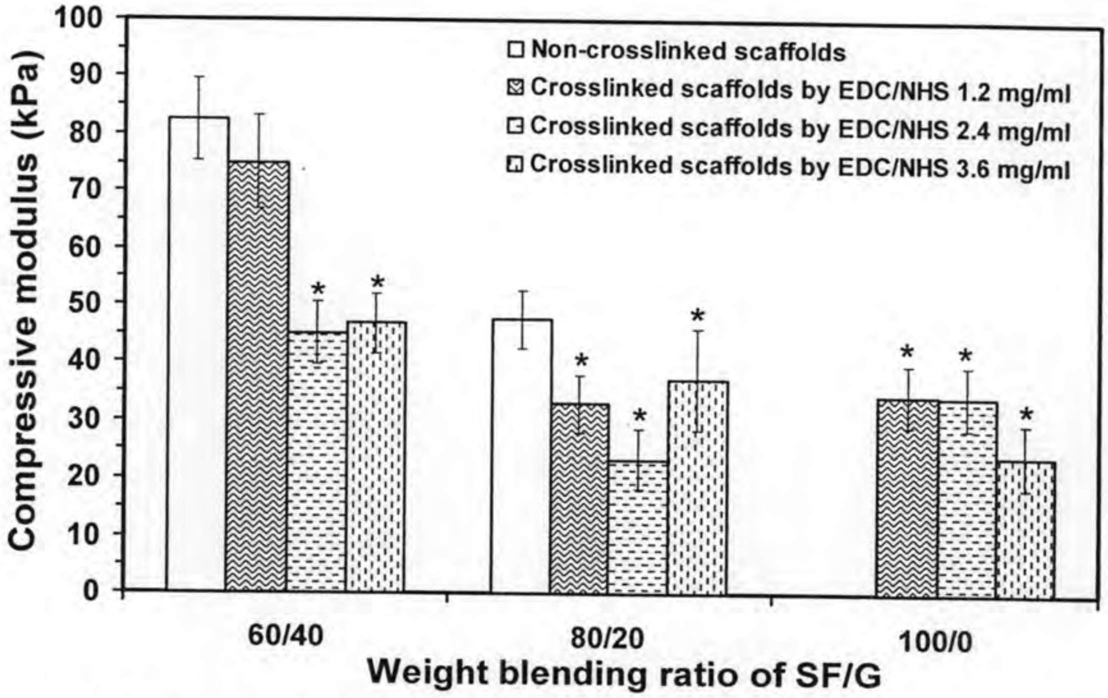

Figure C-3 Compressive modulus in wet condition of Thai silk fibroin/gelatin scaffolds (SF/G) at various contents of EDC/NHS.

* represent the significant difference ($p<0.05$) relative to non-crosslinked scaffolds at each weight blending ratio of SF/G.

APPENDIX D

Raw data of remaining weight (%)

Table D-1 Mean and SD of remaining weight (%) of crosslinked Thai silk fibroin/gelatin scaffolds at the weight blending ratios of 0/100, 20/80, and 40/60 (SF/G) during the enzymatic degradation for 168 h.

Degradation time	Remaining weight (%)					
	C 0/100		C 20/80		C 40/60	
	mean	SD	mean	SD	mean	SD
15 min	10.41	2.55	51.10	4.49	75.05	3.43
30 min	7.64	1.10	45.25	1.60	71.28	1.52
1 h	0	0	35.35	3.52	56.86	2.77
6 h	0	0	18.09	1.94	49.28	2.42
12 h	0	0	13.89	1.12	43.03	4.36
24 h	0	0	12.83	1.88	34.00	3.44
72 h	0	0	4.46	0.72	23.68	2.23
120 h	0	0	0.67	1.15	21.18	1.31
168 h	0	0	0	0	10.12	1.16

Table D-2 Mean and SD of remaining weight (%) of crosslinked Thai silk fibroin/gelatin scaffolds at the weight blending ratios of 50/50, 60/40, 80/20, and 100/0 (SF/G) during the enzymatic degradation for 168 h.

Degradation time	Remaining weight (%)							
	C 50/50		C 60/40		C 80/20		C 100/0	
	mean	SD	mean	SD	mean	SD	mean	SD
15 min	86.37	3.38	89.19	3.88	98.84	1.00	99.98	0.03
30 min	77.25	6.88	81.14	1.65	99.42	1.01	99.69	0.53
1 h	79.22	2.04	78.61	1.34	96.26	3.65	99.37	0.56
6 h	59.73	2.52	61.68	3.60	91.80	5.44	99.11	0.92
12 h	55.86	3.48	59.41	2.80	87.98	2.49	99.68	0.55
24 h	46.25	2.56	58.66	0.56	73.87	3.07	98.18	1.07
72 h	33.70	2.43	48.79	0.69	68.64	0.59	95.79	4.11
120 h	24.39	1.13	46.22	0.63	65.56	2.17	94.35	3.22
168 h	17.55	0.48	41.55	0.90	56.79	0.80	91.01	1.04

Table D-3 Mean and SD of remaining weight (%) of non-crosslinked Thai silk fibroin/gelatin scaffolds at the weight blending ratios of 40/60, 50/50, and 60/40 (SF/G) during the enzymatic degradation for 168 h.

Degradation time	Remaining weight (%)					
	NC 40/60		NC 50/50		NC 60/40	
	mean	SD	mean	SD	mean	SD
15 min	56.52	2.22	77.42	0.02	80.43	1.11
30 min	40.44	1.21	69.68	2.16	79.13	1.10
1 h	29.32	3.74	50.25	2.03	57.26	3.54
6 h	19.97	2.33	24.46	3.20	47.06	1.81
12 h	12.20	0.35	21.26	2.11	27.06	1.52
24 h	4.10	0.08	6.70	1.88	8.29	1.51
72 h	0	0	0	0	5.61	4.91
120 h	0	0	0	0	3.82	2.21
168 h	0	0	0	0	1.96	3.40

Table D-4 Mean and SD of remaining weight (%) of crosslinked Thai silk fibroin/gelatin scaffolds at the weight blending ratios of 80/20 and 100/0 (SF/G) during the enzymatic degradation for 14, 21, and 28 days.

Degradation time	Remaining weight (%)			
	C 80/20		C 100/0	
	mean	SD	mean	SD
14 days	44.61	4.75	81.79	2.33
21 days	32.54	1.34	76.89	1.04
28 days	25.32	2.11	66.03	2.57

APPENDIX E

Standard curve of *in vitro* cell culture test

Table E-1 Fluorescence intensity at the excitation and emission wavelengths of 355 and 460 nm, respectively from DNA assay for standard curve of number of cells.

Replication no.	Number of cells			
	500,000	250,000	125,000	62,500
1	41425	18631	7935	2708
2	43006	20691	8978	1901
3	45163	20511	8639	1838
mean	43198	19944.33	8517.33	2149
SD	1876.38	1140.94	532.04	485.13

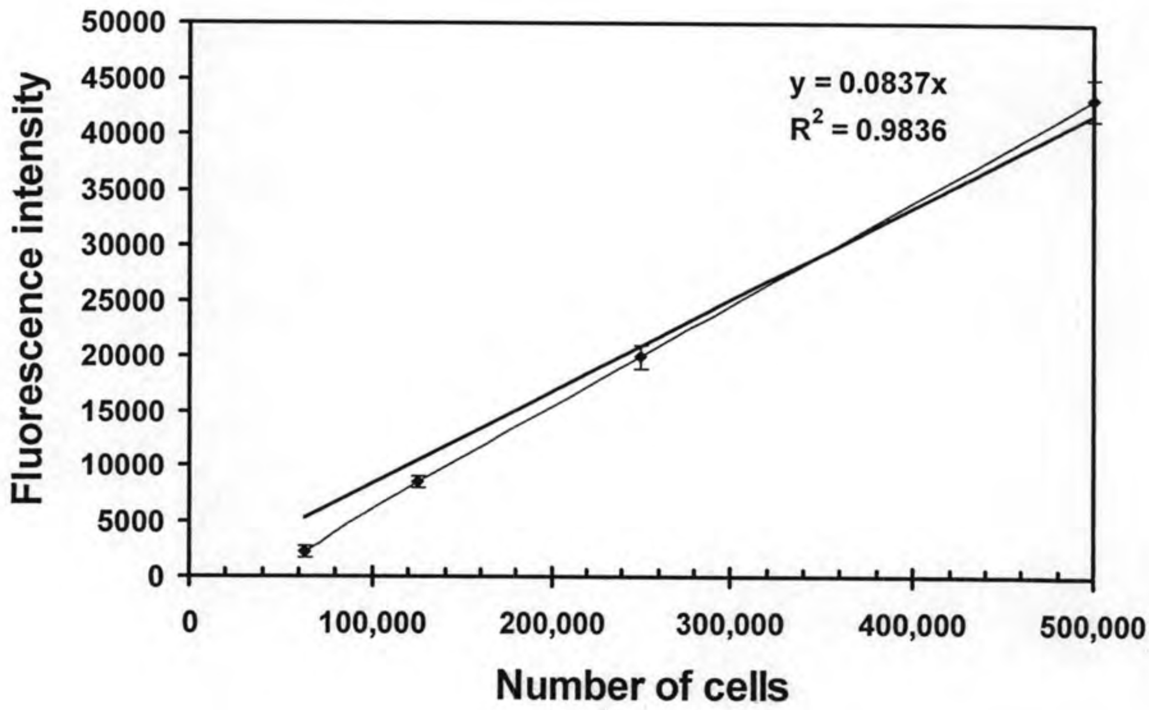

Figure E-1 Standard curve of number of cells for DNA assay.

Table E-2 Absorbance at 405 nm from ALP activity for standard curve of p-nitrophenol standard solution.

Replication no.	Standard solution (mM)						
	5	2.5	1.25	0.625	0.3125	0.15625	0.078125
1	3.940	2.299	1.171	0.574	0.294	0.147	0.082
2	3.674	2.260	1.171	0.583	0.292	0.148	0.074
3	3.499	2.290	1.183	0.580	0.292	0.146	0.075
mean	3.704	2.282	1.175	0.579	0.292	0.147	0.077
SD	0.222	0.020	0.007	0.005	0.001	0.001	0.004

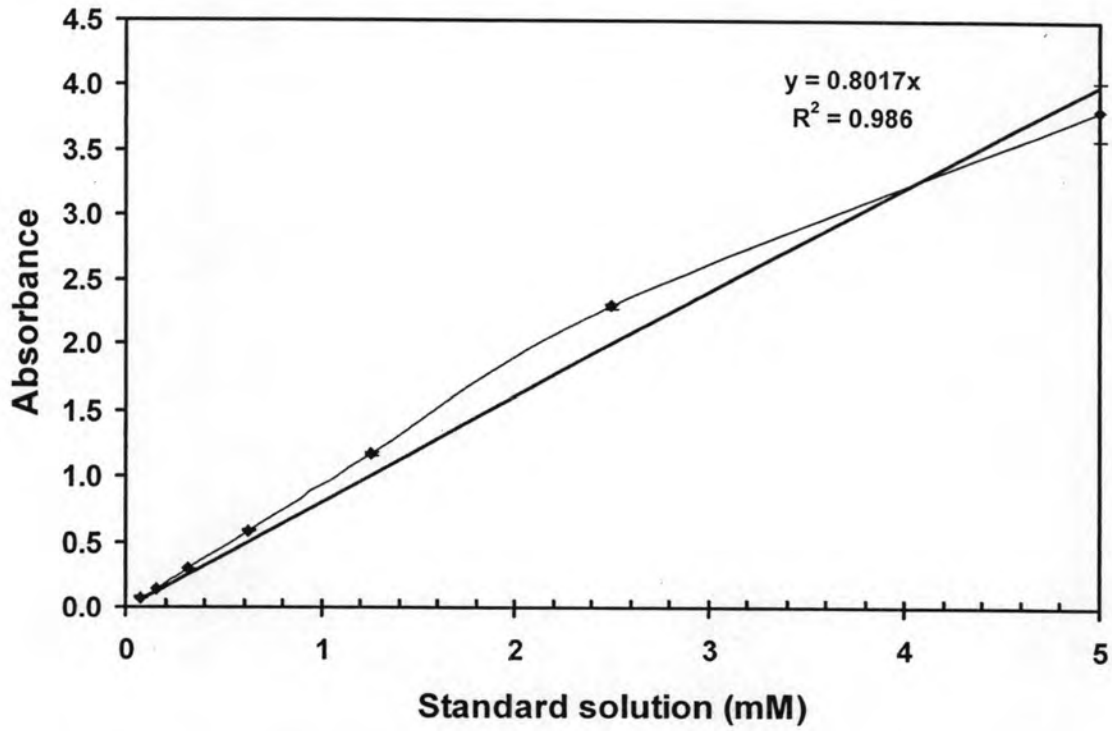

Figure E-2 Standard curve of p-nitrophenol standard solution for ALP activity.

Table E-3 Absorbance at 570 nm from calcium content for standard curve of $CaCO_3$ standard solution.

Replication no.	Standard solution (mg/ml Ca)				
	2.5	1.25	0.625	0.3125	0.15625
1	0.521	0.278	0.185	0.082	0.031
2	0.612	0.310	0.126	0.111	0.050
3	0.591	0.292	0.167	0.095	0.045
mean	0.575	0.293	0.159	0.096	0.042
SD	0.047	0.016	0.030	0.014	0.009

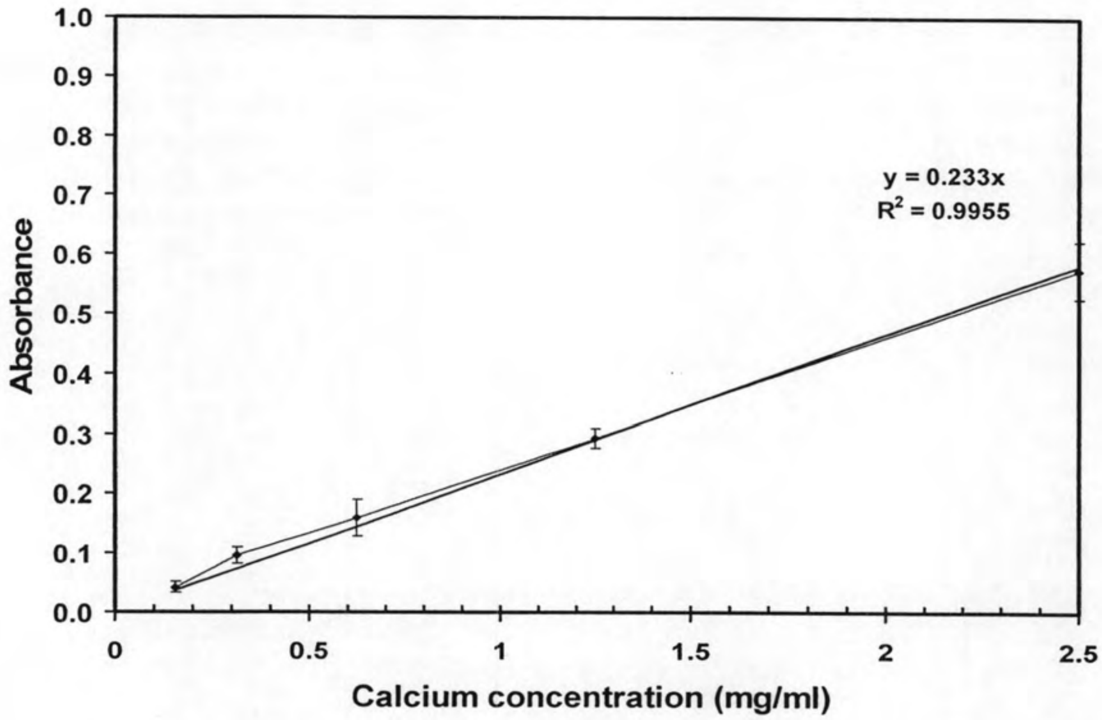

Figure E-3 Standard curve of $CaCO_3$ standard solution for calcium content.

Biography

Miss Panida Jetbumpenkul was born in Nakhonratchasima, Thailand on September 3, 1985. She finished the high school education in 2002 from Suranaree Withaya School. In 2006, she received her Bachelor Degree of Engineering with a major of Chemical Engineering from Faculty of Engineering, Khon Kaen University. After the graduation, she pursued her graduate study in Master of Engineering (Chemical Engineering), The Faculty of Engineering, Chulalongkorn University.

A part of this work was presented at the interconference as follow;

- Jetbumpenkul, P.; and Damrongsakkul, S. Chemical crosslinking of Thai silk fibroin/gelatin scaffolds using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC). Oral presentation in polymer chemistry at PACCON2009: Pure and Applied Chemistry International Conference, 14-16 January 2009, Narasuan University, Phitsanulok, Thailand (The outstanding oral presentation award).