References

[1] 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.

[2] Lv, Q.; Feng, Q.; Hu, K.; and Cui F. Three-dimensional fibroin/collagen scaffolds derived from aqueous solution and the use for HepG2 culture. Polymer 46 (2005): 12662–12669.

[3] Cai, K.; Yao, K.; Lin, S.; Yang, Z.; Li, X.; Xie, H.; Qing, T.; and Gao, L. Poly(D,L-lactic acid) surfaces modified by silk fibroin: effects on the culture of osteoblast *in vitro*. Biomaterials 23 (2002): 1153–1160.

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

[5] 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.

[6] http://www.en.rmut.ac.th/prd/Journal/Silk_with_figuresnew.pdf (October 8, 2005).

[7] Huang, Y.; Onyeri, S.; Siewe, M.; Moshfeghian, A.; and Madihally, S.V. *In vitro* characterization of chitosan–gelatin scaffolds for tissue engineering. Biomaterials 26 (2005): 7616–7627.

[8] Ratanavaraporn, J. Physical and biological properties of collagen/gelatin scaffolds. Master thesis Department of Chemical Engineering Chulalongkorn University, 2005.

[9] Wang, L.; Nemoto, R.; and Senna, M. Changes in microstructure and physico-chemical properties of hydroxyapatite–silk fibroin nanocomposite with varying silk fibroin content. Journal of the European Ceramic Society 24 (2004): 2707–2715.

[10] Bigi, A.; Boanini, E.; Panzavolta, S.; Roveri, N.; and Rubini, K. Bonelike apatite growth on hydroxyapatite–gelatin sponges from simulated body fluid. Journal of Biomedical Material Research 59 (2002): 709–714.

[11] Taguchi, T.; Sawabe, Y.; Kobayashi, H.; Moriyoshi, Y.; Kataoka, K.; and Tanaka, J. Preparation and characterization of osteochondral scaffold. Materials Science and Engineering C 24 (2004): 881–885.

[12] Ijima, H.; Ohchi, T.; Ono, T.; and Kawakami, K. Hydroxyapatite for use as an animal cell culture substratum obtained by an alternate soaking process Biochemical Engineering Journal 20 (2004): 155–161.

[13] Ratner, B.D.; Hoffman, A.S.; Schoen, F.J.; and Lemons, J.E. Biomaterials. Science Academic Press (1996): 360-370.

[14] Mori, H.; and Tsukadab, M. New silk protein: modification of silk protein by gene engineering for production of biomaterials. Reviews in Molecular Biotechnology 74 (2000): 95-103.

[15] Altman, G.H.; Diaz, F.; Jakuba, C.; Calabro, T.; Horan, R.L.; Chen, J.; Lu, H.; Richmond, J.; and Kaplan, D.L. Silk-based biomaterials. Biomaterials 24 (2003): 401–416.

[16] http://www.nd.edu/~aseriann/silk.html. (June 4, 2006)

[17] http://dspace.library.drexel.edu/retrieve/4251/CHAPTER+2.pdf (March 30, 2006)

[18] Baker, R.W. Other membrane processes. Membrane Technology and Applications. 491-520. New York: John Wiley & Sons, (2004).

[19] http://www.moac.go.th/builder/mu/index.php?page=415&clicksub=415&sub=1 26 (June 11, 2007)

[20] Young, S.; Wong, M.; Tabata, Y.; and Mikos, A.G. Gelatin as a delivery vehicle for the controlled release of bioactive molecules. Journal of Controlled Release 109 (2005): 256– 274.

[21] http://en.wikipedia.org/wiki/Gelatin. (June 3, 2006)

[22] http://www.gmap-gelatin.com/about_gelatin_phys.html. (June 4, 2006)

[23] http://www.lsbu.ac.uk/water/hygel.html. (June 4, 2006)

[24] http://www.gelatin-gmia.com/html/gelatine_health.html. (June 4, 2006)

[25] http://www.csrc.com.tw/content-application.html. (June 4, 2006)

[26] Ma, P.X. Scaffolds for tissue fabrication. Materialtoday (2004)

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

[28] Rezwan, K.; Chen, Q.Z.; Blaker, J.J.; and Boccaccini, A.R. Biodegradable and bioactive porous polymer/inorganic composite scaffolds for bone tissue engineering. Biomaterials 27 (2006): 3413–3431

[29] Ozeki, M.; and Tabata, Y. *In vivo* degradability of hydrogels prepared from different gelatins by various crosslinking method. Journal of Biomaterial Science Polymer Edition (2004).

[30] 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): 765–73.

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

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

[33] http://www.vetscite.org/issue1/reviews/sultan_2_0800.htm.

[34] http://www.chemicon.com/webfiles/PDF/ECM810.pdf (August, 2007)

[35] 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.

[36] Meinel, L.; Karageorgiou, V.; Hofmann, S.; Fajardo, R.; Snyder, B.; Li, C.; Zichner, L.; Langer, R.; Novakovic, G.V.; and Kaplan, D.L. Engineering bone-like tissue *in vitro* using human bone marrow stem cells and silk scaffolds. Journal of Biomedical Material Research 71A (2004): 25–34.

[37] Kim, H.J.; Kim, U.J.; Novakovic, G.V.; Min, B.H.; and Kaplan, D.L. Influence of macroporous protein scaffolds on bone tissue engineering from bone marrow stem cells. Biomaterials 26 (2005): 4442-4452.

[38] Mosmann, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of Immunological Methods 65 (1983): 55-63.

[39] http://www.atcc.org/common/products/MttCell.cfm.

[40] Tsukada M.; Gotoh, Y.; Nagura, M.; Minoura, N.; Kasai, N.; and Freddi, G. Structural changes of silk fibroin membranes induced by immersion in methanol aqueous solution. Journal of Polymer Science 32 (1994): 961-968.

[41] Chen, X.; Knight, D.P.; Shao, Z.; and Vollrath, F. Regenerated Bombyx silk solutions studied with rheometry and FTIR. Polymer 42 (2001): 9969-9974.

[42] 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.

[43] Yamada, H.; Nakao, H.; Takasu, Y.; and Tsubouchi, K. Preparation of undegraded native molecular fibroin solution from silkworm cocoons. Materials Science and Engineering C 14 (2001): 41–46.

[44] Wang, H.; Zhang, Y.; Shao, H.; and Hu, X. A study on the flow stability of regenerated silk fibroin aqueous solution. International Journal of Biological Macromolecules 36 (2005): 66-70.

[45] Horan, R.L.; Antle, K.; Collette, A.L.; Wang, Y.; Huang, J.; Moreau, J.E.; Volloch, V.; Kaplan, D.L.; and Altman, G.H. *In vitro* degradation of silk fibroin. Biomaterials 26 (2005): 3385-3393.

[46] Minoura, N.; Aiba, S.I.; Higuchi, M.; Gotoh, Y.; Tsukada M.; and Imai Y. Attachment and growth of fibroblast cells on silk fibroin. Biochemical and Biophysical Research Communications 28 (1995): 511-516.

[47] 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.

[48] Panilaitis, B.; Altman, G.H.; Chen, J.; Jin, H.J.; Karageorgiou, V.; and Kaplan, D.L. Macrophage responses to silk. Biomaterials 24 (2003): 3079-3085.

[49] 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.

[50] Meinel, L.; Hofmann, S.; Karageorgiou, V.; Head, C.K.; McCool, J.; Gronowicz, G.; Zichner, L.; Langer, R.; Novakovic, G.V.; and Kaplan, D.L. The inflammatory responses to silk films *in vitro* and *in vivo*. Biomaterials 26 (2005): 147-155.

[51] Tamada, Y. New process to form a silk fibroin porous 3-D structure. Biomacromolecules 6 (2005): 3100-3106.

[52] Wang, Y.; Blasioli, D.J.; Kim, H.J.; Kim H.S.; and Kaplan D.L. Cartilage tissue engineering with silk scaffolds and human articular chondrocytes. Biomaterials 27 (2006): 4434-4442.

[53] Meechaisue, C.; Wutticharoenmongkol, P.; Waraput, 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.

[54] Kweon, H.Y.; Um, I.C.; and Park, Y.H. Structural and thermal characteristics of *Antheraea pernyi* silk fibroin/chitosan blend film. Polymer 42 (2001): 6651-6656.

[55] 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.

[56] Taguchi, T.; Kishida, A.; and Akashi, M. Hydroxyapatite Formation on/in poly(vinyl alcohol) hydrogel matrices using a novel alternate soaking process. Chemistry Letters (1998): 711-712.

[57] Furuzono, T.; Taguchi, T,; Kishida, A.; Akashi, M.; and Tamada, Y. Preparation and characterization of apatite deposited on silk fabric using an alternate soaking process. Journal of Biomedical Material Research 50 (2000): 344–352.

[58] 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 A 254 (2005): 159–164.

[59] Pek, Y.S.; Spector, M.; Yannas, I.V.; and Gibson, L.J. Degradation of a collagen–chondroitin-6-sulfate matrix by collagenase and by chondroitinase. Biomaterials 25 (2004): 473–482.

[60] 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.

[61] Kaplan, D.; Adams, W.W.; Farmer, B.; and Viney, C. silk polymers. Washington DC: American Chemical Society, 1993.

[62] Saitoh, H.; Ohshima, K.; Tsubouchi, K.; Yoko Takasu, Y.; and Yamada, H. X-ray structural study of noncrystalline regenerated *Bombyx mori* silk fibroin. International Journal of Biological Macromolecules 34 (2004): 259–265.

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

[64] Gil, E.S.; Frankowski, D.J.; Hudson, S.M.; and Spontak, R.J. Silk fibroin membranes from solvent-crystallized silk fibroin/gelatin blends: Effects of blend and solvent composition. Materials Science and Engineering C 27 (2007): 426–431.

[65] Motta, A.; Moschini, L.; Chamchongkaset, J.; Kanokpanont, S.; and Damrongsakkul, S. Characterization of silk proteins: A comparison of different cocoon sources. (In preparation)

[66] Wutticharoenmongkol, P.; Pavasant, P.; and Supaphol, P. Osteoblastic phenotype Expression of MC3T3-E1 cultured on electrospun polycaprolactone fiber mats filled with hydroxyapatite nanoparticles. Biomacromolecules 8 (2007): 2602-2610.

[67] 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 52 (2000): 279.

[68] Huang, Y.; Onyeri, S.; Siewe, M.; Moshfeghian, A.; and Madihally, S.V. *In vitro* characterization of chitosan–gelatin scaffolds for tissue engineering. Biomaterials 26 (2005): 7616–7627.

APPENDICES

APPENDIX A

Raw data of compressive modulus

Table A-1 Mean and SD of compressive modulus of gelatin/silk fibroin scaffolds with DHT treatment for 24 h.

Weight percentage of silk fibroin	Compressive modulus (kPa)	
	mean	SD
0	337.14	143.96
20	214.29	73.68
60	260.00	62.45
80	467.14	62.91
100	350.00	102.31

Table A-2 Mean and SD of compressive modulus of gelatin/silk fibroin scaffolds with DHT treatment for 48 h.

Weight percentage of silk fibroin	Compressive modulus (kPa)	
	mean	SD
0	302.50	34.03
20	132.50	28.72
60	220.00	24.49
80	577.50	87.32
100	442.50	66.52

Table A-3 Mean and SD of compressive modulus of hydroxyapatite/silk fibroin scaffolds.

Cycles of alternate soaking	Compressive modulus (kPa)	
	mean	SD
0	262.50	61.85
2	265.00	81.85
4	500.00	121.93
6	535.00	93.99

Table A-4 Mean and SD compressive modulus of hydroxyapatite-conjugated gelatin/silk fibroin scaffolds.

Cycles of alternate soaking	Compressive modulus (kPa)	
	mean	SD
0	506.00	151.10
2	510.00	170.88
4	550.00	234.09
6	826.00	388.63

APPENDIX B

Raw data of swelling ratios

Table B-1 Mean and SD of swelling ratios of gelatin/silk fibroin scaffolds with DHT treatment for 24 h.

Weight percentage of silk fibroin	Compressive modulus (kPa)	
	mean	SD
0	12.01	0.60
20	10.99	0.56
60	12.22	2.50
80	11.09	1.35
100	9.19	1.34

Table B-2 Mean and SD of swelling ratios of gelatin/silk fibroin scaffolds with DHT treatment for 48 h.

Weight percentage of silk fibroin	Compressive modulus (kPa)	
	mean	SD
0	13.69	2.10
20	13.75	1.85
60	13.40	1.97
80	11.77	2.28
100	10.32	2.00

Table B-3 Mean and SD of swelling ratios of hydroxyapatite/silk fibroin scaffolds.

Cycles of alternate soaking	Compressive modulus (kPa)	
	mean	SD
0	11.02	1.46
2	7.23	0.66
4	6.28	0.42
6	5.70	0.15

Table B-4 Mean and SD of swelling ratios of hydroxyapatite-conjugated gelatin/silk fibroin scaffolds.

Cycles of alternate soaking	Compressive modulus (kPa)	
	mean	SD
0	10.32	1.37
2	7.13	0.62
4	5.27	0.42
6	4.38	0.20

APPENDIX C

Standard curve of *in vitro* cell culture test

Table C-1 Absorbance at 570 nm from MTT assay for standard curve of bone-marrow derived mesenchymal stem cells (MSCs).

Replication no.	Number of cells				
	5,000	10,000	20,000	40,000	80,000
1	0.066	0.103	0.144	0.320	0.562
2	0.068	0.081	0.158	0.339	0.544
3	0.066	0.094	0.147	0.316	0.544
4	0.064	0.069	0.123	0.203	0.637
mean	0.066	0.087	0.143	0.295	0.572
SD	0.002	0.015	0.015	0.062	0.044

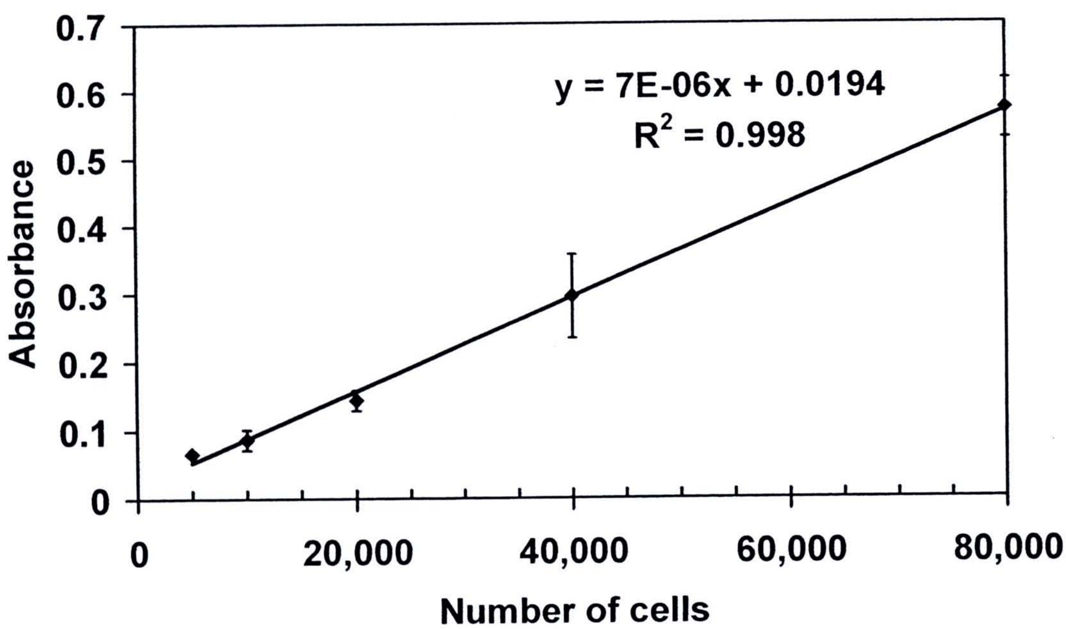

Figure C-1 Standard curve for MSCs.

Table C-2 Absorbance at 570 nm from MTT assay for mouse osteoblast-like cells (MC3T3-E1).

Replication no.	Number of cells				
	10,000	20,000	40,000	80,000	160,000
1	0.033	0.052	0.08	0.185	0.319
2	0.036	0.049	0.093	0.181	0.299
3	0.052	0.061	0.082	0.152	0.273
mean	0.040	0.054	0.085	0.173	0.297
SD	0.010	0.006	0.007	0.018	0.023

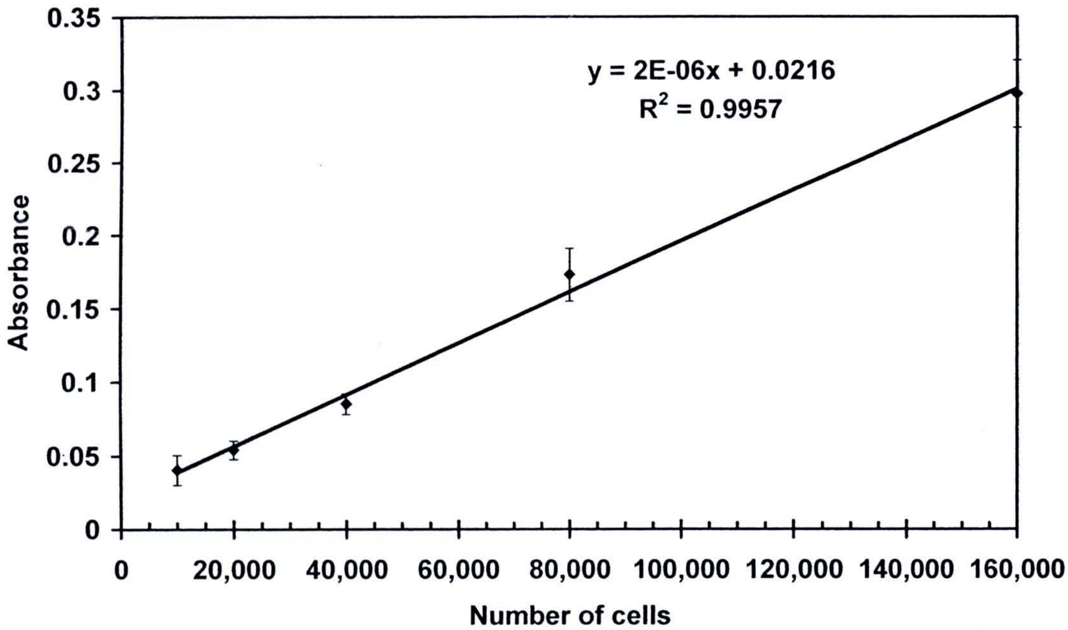

Figure C-2 Standard curve for MC3T3-E1.

VITAE

Miss Jitima Chamchongkaset was born in Bangkok, Thailand on February 7, 1983. She finished the high school education in 2000 from Bodindecha (Sing Singhaseni) school. In 2004, she received her Bachelor Degree of Engineering with a major of Chemical Engineering from Faculty of Engineering, Mahidol University. After the graduation, she pursued her graduate study to a Master of Engineering (chemical engineering), the Faculty of Engineering, Chulalongkorn University.

Some parts of this work were presented at the conferences as follows;

- Chamchongkaset, J.; Kanokpanont, S.; and Damrongsakkul, S. Morphology and biological properties of Thai silk fibroin-based scaffolds. Poster presentation in biomedical polymer at ABC2006: The Sixth Asian BioCeramics Symposium 2006, 7-10 November 2006, The Sofitel Central Plaza, Bangkok, Thailand (ABC poster presentation award)

- Chamchongkaset, J.; Kanokpanont, S.; and Damrongsakkul, S. *In vitro* study using mesenchymal stem cell on gelatin/Thai silk fibroin scaffolds. Oral presentation in biomaterials at NCBME2007: The 5th National Conference on Biomedical Engineering, 8 July 2007, The Twin Tower Hotel, Bangkok, Thailand.