

## REFERENCES

1. Hutmacher, D. W. Scaffold design and fabrication technologies for engineering tissue-state of the art and future perspectives. *J. Biomat. Sci-Polym. Ed.* **2001**, *12*, 107-124.
2. Perry, T. E., Kaushal, S., Sutherland, F. W. H., Guleserian, K. J., Bischoff, J., Sacks, M., and Mayer, J. E. Bone marrow as a cell source for tissue engineering heart valves. *Annal. Thorac. Sur.* **2003**, *75*, 761-767.
3. Yang, F., Murugan, R., Ramakrishna, S., Wang, X., Ma, Y. X., and Wang, S. Fabrication of nano-structured porous PLLA scaffold intended for nerve tissue engineering. *Biomaterials* **2004**, *25*, 1891-1900.
4. Sato, T., Chen, G., Ushida, T., Ishii, T., Ochiai, N., Tateishi, T., and Tanaka, J. Evaluation of PLLA-collagen hybrid sponge as a scaffold for cartilage tissue engineering *Mater. Sci. Eng. C* **2004**, *24*, 365-372.
5. Newman, K. D., and MacBurney, M. W. M. W. Poly(D, L lactic-*co*-glycolic acid) microspheres as biodegradable microcarriers for pluripotent stem cells. *Biomaterials* **2004**, *25*, 5763-5771.
6. Koegler, W. S., and Griffith, L. G. Osteoblast response to PLGA tissue engineering scaffolds with PEO modified surface chemistries and demonstration of patterned cell response. *Biomaterials* **2004**, *25*, 2819-2830.
7. Hafemann, B., Ensslen, S., Erdmann, C., Niedballa, R., Zühlke, A., Ghofrani, K., and Kirkpatrick, C. J. Use of a collagen/elastin-membrane for the tissue engineering of dermis. *Burns* **1999**, *25*, 373-384.
8. Lu, Q., Ganesan, K., Simionescu, D. T., and Vyawahare, N. R. Novel porous aortic elastin and collagen scaffolds for tissue engineering. *Biomaterials* **2004**, *25*, 5227-5237.

9. Kang, H. W., Tabata, Y., and Ikada, Y. Fabrication of porous gelatin scaffolds for tissue engineering. *Biomaterials* **1999**, *20*, 1339-1344.
10. Chang, C. H., Liu, H. C., Lin, C. C., Chou, C. H., and Lin, F. H. Gelatin-chondroitin-hyaluronan tri-copolymer scaffold for cartilage tissue engineering. *Biomaterials* **2003**, *24*, 4853-4858.
11. Ma, J., Wang, H., He, B., and Chen, J., A preliminary *in vitro* study on the fabrication and tissue engineering applications of a novel chitosan bilayer material as a scaffold of human neofetal dermal fibroblasts. *Biomaterials* **2001**, *22*, 331-336.
12. Hoemann, C. D., Sun, J., Légaré, A., McKee, M. D., and Buschmann, M. D. Tissue engineering of cartilage using an injectable and adhesive chitosan-based cell-delivery vehicle. *Osteoarthr. Cartil.* **2005**, *13*, 318-329.
13. Hirano, S. Chitin and chitosan as novel biotechnological materials. *Polym. Int.* **1999**, *48*, 732-734.
14. Biagini, G., Bertani, A., Mazzarelli, R., Damadei, A., Dibenedetto, G., Belligolli, A., and Ricotti, G. Wound management with *N*-carboxybutyl chitosan. *Biomaterials* **1991**, *12*, 281-286.
15. Nettles, D. L., Elder, S. H., and Gilbert, J. A. Potential use of chitosan as a cell scaffold material for cartilage tissue engineering. *Tissue Eng.* **2002**, *8*, 1009-1016.
16. Xia, W., Liu, W., Cui, L., Liu, Y., Zhang, W., Liu, D., Wu, J., Chua, K., and Cao, Y. Tissue engineering of cartilage with the use of chitosan-gelatin complex scaffolds. *J. Biomed. Mater. Res.* **2004**, *71B*, 373-380.
17. Zhang, Y., Ni, M., Zhang, M., and Ratner, B. Calcium phosphate chitosan composite scaffolds for bone tissue engineering. *Tissue Eng.* **2003**, *9*, 337-345.

18. Risbud, M. V., Karamuk, E., Schlosser, V., and Mayer, J. Hydrogel-coated textile scaffolds as candidate in liver tissue engineering: II. Evaluation of spheroid formation and viability of hepatocytes. *J. Biomater. Sci. Polym. Ed.* **2003**, *14*, 719-731.
19. Gingras, M., Paradis, I., and Berthod, F. Nerve regeneration in a collagen-chitosan tissue-engineered skin transplanted on nude mice. *Biomaterials* **2003**, *24*, 1653-1661.
20. Nehrer, S., Breinan, H. A., Ramappa, A., Young, G., Shortkroff, S., Louie, L. K., Siedge, C. B., Yannas, I. V., and Spector, M. Matrix collagen type and pore size influence behavior of seeded canine chondrocytes. *Biomaterials* **1997**, *18*, 769-776.
21. Weadock, K. S., Miller, E. J., Bellincampi, L. D., Zawadsky, J. P., and Dunn, M. G. Physical crosslinking of collagen fibers: comparison of ultraviolet radiation and dehydrothermal treatment. *J. Biomed. Mater. Res.* **1995**, *29*, 1373-1379.
22. Li, Z., Ramay, H. R., Hauch, K. D., Xiao, D., and Zhang, M. Chitosan-alginate hybrid scaffolds for bone tissue engineering. *Biomaterials* **2005**, *26*, 3919-3928.
23. Jameela, S. R., and Jayakrishnan, A. Glutaraldehyde crosslinked chitosan as a long acting biodegradable drug delivery vehicle: studies on microspheres in rat muscle. *Biomaterials* **1995**, *16*, 769-775.
24. Jayakrishnan, A., and Jameela, S. R. Glutaraldehyde as a fixative in bioprostheses and drug delivery matrices. *Biomaterials* **1996**, *17*, 471-484.
25. Hennink, W. E., and Van Nostrum, C. F. Novel crosslinking methods to design hydrogels. *Adv. Drug. Deliv. Rev.* **2002**, *54*, 13-36.

26. McIntire, L. V., Greisler, H. P., Griffith, L., Johnson, P. C., Mooney, D. J., Mrksich, M., Parenteau, N. L., and Smith, D. Tissue engineering research. *WTEC panel final report; International technology research institute*. Baltimore: Maryland, **2002**; pp1-5.
27. Langer, R., and Vacanti, J. P. Tissue engineering. *Science* **1993**, *260*, 920-926.
28. Griffith, L. G., and Naughton, G. Tissue engineering-current challenges and expanding opportunities. *Science* **2002**, *295*, 1009-1014.
29. Sachols, E., and Czernuszka, J. T. Making tissue engineering scaffolds work. Review on the application of solid freeform fabrication technology to the production of tissue engineering scaffolds. *Eur. Cells. Mat.* **2003**, *5*, 29-40.
30. Richter, E., Fuhr, G., Müller, T., Shirley, S., Rogaschewski, S., Reimer, K., and Dell, C. Growth of anchorage-dependent mammalian cells on microstructures and microperforated silicon membranes. *J. Mater. Sci.: Mater. Med.* **1996**, *7*, 85-97.
31. Yoshimoto, H., Shin, Y. M., Terai, H., and Vacanti, J. P. A biodegradable nanofiber scaffolds by electrospinning and its potential for bone tissue engineering. *Biomaterials* **2003**, *24*, 2077-2082.
32. Li, J., Pan, J., Zhang, L., and Yu, Y. Culture of hepatocytes on fructose-modified chitosan scaffolds. *Biomaterials* **2003**, *24*, 2317-2322.
33. Nam, Y. S., and Park, T. G. Porous biodegradable polymeric scaffolds prepared by thermally induced phase separation. *J. Biomed. Mater. Res.* **1999**, *47*, 8-17.
34. Madihally, S. V., and Matthew, H. W. T. Porous chitosan scaffolds for tissue engineering. *Biomaterials* **1999**, *20*, 1133-1142.

35. Schoof, H., Apel, J., Heschel, I., and Rau, G. Control of pore structure and size in freeze-dried collagen sponges. *J. Biomed. Mater. Res.* **2001**, *58*, 352-357.
36. Mikos, A. G., Thorsen, A. J., Czerwonka, L. A., Bao, Y., and Langer, R. Preparation and characterization of poly (L-lactic acid) foams. *Polymer* **1994**, *35*, 1068-1077.
37. Chow, K. S., and Knor, E. Novel fabrication of open-pore chitin matrixes. *Biomacromolecules* **2000**, *1*, 61-67.
38. Mooney, D. J., Baldwin, D. F., Suh, N. P., Vacanti, J. P., and Langer, R. Novel approach to fabricate porous sponges of poly (D, L-lactic-*co*-glyclic acid) without the use of organic solvents. *Biomaterials* **1996**, *17*, 1417-1422.
39. Mikos, A. G., Bao, Y., Cima, L. G., Ingber, D. E., Vacanti, J. P., and Langer, R. Preparation of poly (glycolic acid) bonded fibres structures for cell attachment and transplantation. *J. Biomed. Mater. Res.* **1993**, *27*, 183-189.
40. Kurita, K. Chitin and chitosan derivatives. *American Chemical Society* Washington DC, **1997**. pp. 239-259.
41. Singh, D. K., and Ray, A. R. Biomedical applications of chitin, chitosan, and their derivatives. *Rev. Macromol. Chem. Phys.* **2000**, *C40(1)*, 69-83.
42. Lu, J. X., Psudhommeaux, F., Meunier, a., Sedal, L., and Guillemin, G. Effects of chitosan on rat knee cartilages. *Biomaterials* **1999**, *20*, 1937-1944.
43. Haipeng, G., Yinghui, Z., Jianchun, L., Yandao, G., Nanming, Z., and Xiufang, Z. Studies on nerve cell affinity of chitosan-derived materials. *J. Biomed. Mat. Res.* **2000**, *52*, 285-295.

44. Prasitsilp, M., Jenwithisuk, R., Kongsuwan, K., Damrongchai, N., and Watts, P. Cellular responses to chitosan *in vitro*: the importance of deacetylation. *J. Mater. Sci.: Mater. Med.* **2000**, *11*, 773-778.
45. Shanmugasundaram, N., Ravichandran, P., Reddy, P. N., Ramamurty, N., Pal, S., and Rao, K. P. Collagen-chitosan polymeric scaffolds for the *in vitro* culture of human epidermoid carcinoma cells. *Biomaterials* **2001**, *22*, 1943-1951.
46. Mi, F. L., Wu, Y. B., Shyu, S. S., Chao, A. C., Lai, J. Y., and Su, C. C. Asymmetric chitosan membrane prepared by dry/wet phase separation: a new type of wound dressing for controlled antibacterial release. *J. Membr. Sci.* **2003**, *212*, 237-254.
47. Jorge-Herrero, E., Fernandez, P., Turnay, J., Olmo, N., Calero, P., Garcia, R., Freile, I., and Castillo-Olivares, J. L. Influence of different chemical cross-linking treatments on the properties of bovine pericardium and collagen. *Biomaterials* **1999**, *20*, 539-545.
48. Moore, M. A., Bohachevsky, I. K., and Cheung, D. T. Stabilization of pericardial tissue by dye-mediated photooxidation. *J. Biomed. Mater. Res.* **1994**, *28*, 611-618.
49. Moore, M. A., Chen, W. M., Phillips, R. E., Bohachevsky, I. K., and Mallroy, B. K. Shrinkage temperature versus protein extraction as measure of stabilization of photooxidized tissue. *J. Biomed. Mater. Res.* **1996**, *32*, 209-214.
50. Weadock, K. S., Miller, E. J., Keuffel, E. I., and Dunn, M. G. Effect of physical crosslinking methods on collagen fiber durability in proteolytic solutions. *J. Biomed. Mater. Res.* **1996**, *32*, 221-226.

51. Patite, H., Rault, I., Huc, A., Menasche, P., and Herbage, D. Use of the acyl azide method for cross-linking collagen-rich tissues such as pericardium. *J. Biomed. Mat. Res.* **1990**, *24*, 179-187.
52. Ono, K., Saito, Y., Yura, H., Ishikawa, K., Kurita, A., Akaike, T., and Ishihara, M. Photocrosslinkable chitosan as a biological adhesive. *J. Biomed. Mater. Res.* **2000**, *49*, 289.
53. Zhu, A., Zhang, M., Wu, J., and Shen, J. Covalent immobilization of chitosan/heparin complex with a photosensitive hetero-bifunctional crosslinking reagent on PLA surface. *Biomaterials* **2002**, *23*, 4657-4665.
54. Obara, K., Ishihara, M., Ishizuka, T., Fujita, M., Ozeki, Y., Maehara, T., Saito, Y., Yura, H., Matsui, T., and Hattori, H. Photocrosslinkable chitosan hydrogel containing fibroblast growth factor-2 stimulates wound healing in healing-impaired *db/db* mice. *Biomaterials* **2003**, *24*, 3437-3444.
55. Mao, C., Zhu, A.P., Qiu, Y. Z., Shen, J., and Lin, S. C. Introduction of *O*-butyrylchitosan with a photosensitive hetero-bifunctional crosslinking reagent to silicone rubber film by radiation grafting and its blood compatibility. *Colloids Surf B Biointerfaces* **2003**, *30*, 299-306.
56. Dusseault, J., Leblond, F. A., Robitaille, R., Jourdan, G., Tessier, J., Ménard, M., Henley, N., and Hallé, J. Microencapsulation of living cells in semi-permeable membranes with covalently cross-linked layers. *Biomaterials* **2005**, *26*, 1515-1522.
57. Masuoka, K., Ishihara, M., Asazuma, T., Hattori, H., Matsui, T., Takase, B., Kanatani, Y., Fujita, M., Saito, Y., Yura, H., Fujikawa, K., and Nemoto, K. The interaction of chitosan with fibroblast growth factor-2 and its protection from inactivation. *Biomaterials* **2005**, *26*, 3277-3284.

58. Zhu, A., Zhang, M., Wu, J., and Shen, J. Covalent immobilization of chitosan/heparin with a photosensitive hetero-bifunctional crosslinking reagent on PLA surface. *Biomaterials* **2002**, *23*, 4657-4665.
59. Smith, M. B., March, J. *Advanced organic chemistry: reactions, mechanisms, and structure*. New York: Wiley press, **1992**, pp.253-254.
60. Carey, F. A., Sundberg, R. I. Nitrenes and related intermediates. *Advance organic chemistry: Part B: Reactions and synthesis*. New York: Plenum press, **1993**, pp.535-536.
61. Fringuelli, F., Piermatti, O., Pizzo, F., and Vaccaro, L. Ring opening of epoxides with sodium azide in water. A regioselective pH-controlled reaction. *J. Org. Chem.* **1999**, *64*, 6094-6096.
62. Lutje Spelberg, J. H., Tang, L., Kellogg, R. M., and Janssen, D. B. Enzymatic dynamic kinetic resolution of epichlorohydrins. *Tetrahedron Asymmetry* **2004**, *15*, 1095-1102.
63. Kiepura, R. T., and Sanders, B. R. *ASM Handbook* Vol 9. The United States of America: ASM international pressed, **1992**, pp.123-134.
64. Chung, T. W., Yang, J., Akaike, T., Cho, K. Y., Nah, J. W., Kim, C. S., Su, C. S., and Cho, C. S. Preparation of alginate/galactosylated chitosan scaffold for hepatocyte attachment. *Biomaterials* **2002**, *23*, 2827-2834.
65. Kang, H. W., Tabata, Y., Ikada, Y. Fabrication of porous gelatin scaffolds for tissue engineering. *Biomaterials* **1999**, *20*, 1339-1344.
66. Wang, H., Li, W., Lu, Y., and Wang, Z. Studies on chitosan and poly (acrylic acid) interpolymer complex. I. Preparation, structure, pH-sensitivity, and salt sensitivity of complex-forming poly (acrylic acid): chitosan semi-interpenetrating polymer network. *J. Appl. Polym. Sci.* **1997**, *65*, 1445-1450.

67. Lee, J. W., Kim, S. Y., Kim, S. S., Lee, Y. M., Lee, K. H., and Kim, S. J. Synthesis and characteristics of interpenetrating polymer network hydrogel composed of chitosan and poly (acrylic acid). *J. Appl. Polym. Sci.* **1999**, *73*, 113-120.
68. Sionkowska, A., Wisniewski, M., Skopinska, J., Kennedy, C. J., and Wess, T. J. The photochemical stability of collagen-chitosan blends. *J. Photochem. Photobiol. A: Chem.* **2004**, *162*, 545-554.
69. Billmeyer, F. W. *Textbook of polymer science*. Chapter 12. Canada: Wiley press, **1984**, pp.339-349.



ศูนย์วิทยทรัพยากร  
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## **APPENDICES**

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## APPENDIX A

**Table A1:** Pore size of 1%wt shrimp chitosan scaffolds freezing at temperature –10°C.

Pore size (μm)	Sample				
	1	2	3	4	5
L <sub>1</sub>	43.9	52.7	33.0	37.7	43.9
L <sub>2</sub>	51.5	59.5	53.8	39.5	34.8
L <sub>3</sub>	62.3	62.3	62.3	39.7	43.6
L <sub>4</sub>	52.6	61.0	53.2	41.2	52.9
L <sub>5</sub>	49.7	58.0	58.0	38.6	49.7
L <sub>6</sub>	52.3	72.7	60.2	45.7	61.7
L <sub>7</sub>	36.4	72.7	48.5	48.5	48.5
L <sub>8</sub>	48.9	58.6	43.5	43.8	53.0
Average			50.8		
Standard deviation			7.4		

**Table A2:** Pore size of 1%wt squid chitosan scaffolds freezing at temperature –10°C.

Pore size (μm)	Sample				
	1	2	3	4	5
L <sub>1</sub>	37.7	37.7	33.0	33.0	52.7
L <sub>2</sub>	43.5	43.5	38.9	43.8	49.2
L <sub>3</sub>	43.6	54.5	54.5	62.3	62.3
L <sub>4</sub>	61.0	52.6	52.3	61.0	61.7
L <sub>5</sub>	58.0	69.5	49.7	69.5	58.0
L <sub>6</sub>	46.0	73.6	52.6	52.6	61.0
L <sub>7</sub>	48.5	54.5	54.5	54.5	54.5
L <sub>8</sub>	38.4	43.2	42.9	41.9	59.5
Average			51.5		
Standard deviation			4.4		

**Table A3:** Pore size of 2%wt shrimp chitosan scaffolds freezing at temperature  $-10^{\circ}\text{C}$ .

Pore size ( $\mu\text{m}$ )	Sample				
	1	2	3	4	5
$L_1$	43.9	37.7	29.3	33.0	26.4
$L_2$	30.7	37.8	30.2	33.6	30.2
$L_3$	36.4	39.7	33.6	31.2	31.2
$L_4$	33.3	40.9	28.3	33.5	26.6
$L_5$	31.6	31.6	29.0	31.6	29.0
$L_6$	33.7	28.5	30.9	33.7	27.3
$L_7$	43.6	33.6	31.2	36.4	33.6
$L_8$	30.2	33.6	27.5	37.8	33.3
Average			32.9		
Standard deviation			2.8		

**Table A4:** Pore size of 2%wt squid chitosan scaffolds freezing at temperature  $-10^{\circ}\text{C}$ .

Pore size ( $\mu\text{m}$ )	Sample				
	1	2	3	4	5
$L_1$	37.7	43.9	43.9	43.9	52.7
$L_2$	50.8	52.3	43.5	60.9	75.6
$L_3$	54.5	62.3	48.5	62.3	72.7
$L_4$	61.7	73.6	52.9	52.9	52.6
$L_5$	43.5	69.5	58.0	43.5	69.5
$L_6$	51.9	61.0	50.0	60.2	61.4
$L_7$	54.5	62.3	62.3	62.3	62.3
$L_8$	43.5	59.5	43.8	50.0	60.9
Average			55.7		
Standard deviation			6.1		

**Table A5:** Pore size of 3%wt shrimp chitosan scaffolds freezing at temperature –10°C.

Pore size ( $\mu\text{m}$ )	Sample				
	1	2	3	4	5
L <sub>1</sub>	33.0	26.4	33.0	26.4	26.4
L <sub>2</sub>	38.1	30.5	34.6	25.8	30.7
L <sub>3</sub>	39.7	36.4	39.7	31.2	31.2
L <sub>4</sub>	37.5	37.5	37.7	33.3	28.5
L <sub>5</sub>	34.8	31.6	38.6	34.8	26.7
L <sub>6</sub>	33.5	33.1	36.6	30.7	30.1
L <sub>7</sub>	33.6	36.4	31.2	33.6	33.6
L <sub>8</sub>	27.1	37.5	30.5	28.7	32.8
Average			32.8		
Standard deviation			2.4		

**Table A6:** Pore size of 3%wt squid chitosan scaffolds freezing at temperature –10°C.

Pore size ( $\mu\text{m}$ )	Sample				
	1	2	3	4	5
L <sub>1</sub>	33.0	29.3	29.3	37.7	33.0
L <sub>2</sub>	38.4	34.1	34.6	34.6	34.1
L <sub>3</sub>	39.7	39.7	39.7	33.6	39.7
L <sub>4</sub>	37.5	41.7	41.4	33.5	37.3
L <sub>5</sub>	34.8	34.8	34.8	38.6	38.6
L <sub>6</sub>	30.3	40.4	40.9	40.2	36.4
L <sub>7</sub>	33.6	33.6	36.4	36.4	36.4
L <sub>8</sub>	30.7	38.4	31.1	38.6	30.5
Average			36.0		
Standard deviation			0.8		

**Table A7:** Pore size of the mole ratio of 2%wt shrimp chitosan to DAZ (1:0.05) at UV irradiation time 40 min freezing at temperature –10°C.

Pore size ( $\mu\text{m}$ )	Sample				
	1	2	3	4	5
L <sub>1</sub>	49.0	36.8	27.8	33.2	33.7
L <sub>2</sub>	37.0	37.0	37.0	37.0	37.0
L <sub>3</sub>	32.0	34.0	43.7	42.4	38.5
L <sub>4</sub>	33.1	53.8	47.8	47.8	43.1
L <sub>5</sub>	45.6	45.6	33.2	36.9	36.5
L <sub>6</sub>	34.4	38.2	38.2	49.1	38.2
L <sub>7</sub>	36.5	40.3	32.9	51.8	33.2
L <sub>8</sub>	33.1	43.1	35.9	43.1	39.1
Average			39.2		
Standard deviation			2.5		

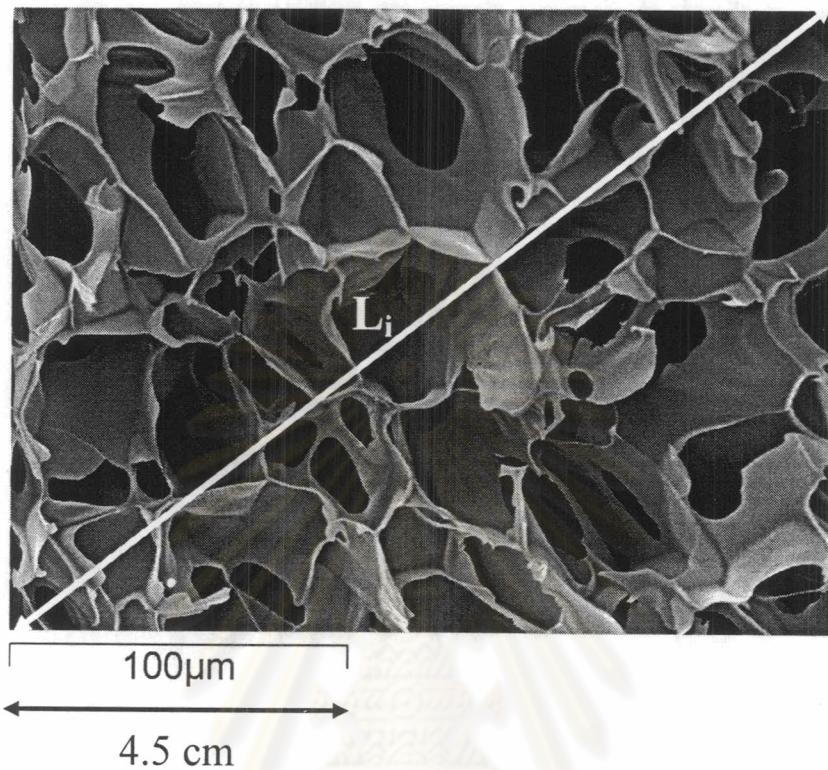
**Table A8:** Pore size of the mole ratio of 2%wt shrimp chitosan to DAZ (1:0.1) at UV irradiation time 40 min freezing at temperature –10°C.

Pore size ( $\mu\text{m}$ )	Sample				
	1	2	3	4	5
L <sub>1</sub>	42.0	47.9	49.0	47.8	35.6
L <sub>2</sub>	43.1	51.8	51.8	51.8	43.1
L <sub>3</sub>	47.8	50.6	46.7	32.7	41.7
L <sub>4</sub>	47.9	53.8	47.8	47.8	39.1
L <sub>5</sub>	52.8	44.7	51.4	45.6	45.6
L <sub>6</sub>	47.9	47.9	49.1	49.1	34.4
L <sub>7</sub>	40.8	52.8	52.1	44.1	44.1
L <sub>8</sub>	47.8	47.8	47.8	39.2	47.8
Average			46.6		
Standard deviation			3.5		

**Table A9:** Pore size of the mole ratio of 2%wt shrimp chitosan to DAZ (1:0.5) at UV irradiation time 40 min freezing at temperature –10°C.

Pore size ( $\mu\text{m}$ )	Sample				
	1	2	3	4	5
L <sub>1</sub>	32.4	30.6	45.4	30.4	43.0
L <sub>2</sub>	37.0	37.0	43.1	32.4	37.0
L <sub>3</sub>	44.4	37.4	37.6	38.2	46.4
L <sub>4</sub>	39.1	35.9	35.9	33.1	39.1
L <sub>5</sub>	60.4	36.5	52.1	36.2	40.0
L <sub>6</sub>	49.1	42.9	49.1	38.2	49.1
L <sub>7</sub>	52.4	40.8	53.4	41.0	45.9
L <sub>8</sub>	43.1	39.1	43.1	43.1	53.8
Average			42.0		
Standard deviation			3.8		

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### Calculation of pore size

$$\text{Pore size} = \frac{\sum_{i=1}^n (L_i/a) \times 100 \mu\text{m}}{N \times 4.5 \text{ cm}}$$

Where  $L_i$  = test line length (cm)

$a$  = number of pores within total length of random test lines

$N$  = number of samples

## APPENDIX B

**Table B1:** Compressive modulus of 2%wt chitosan scaffolds freezed at temperature -10, -80 and -196°C.

Compressive modulus (MPa)	Freezing temperature (°C)					
	Shrimp chitosan			Squid chitosan		
	-10	-80	-196	-10	-80	-196
1	0.68	1.70	2.39	0.97	1.11	1.26
2	0.74	1.33	2.73	1.19	1.23	1.39
3	0.84	1.65	2.02	1.34	1.53	1.11
4	1.03	1.02	1.05	1.19	1.72	1.31
5	1.22	0.47	1.05	1.05	1.69	1.11
6	0.97	1.24	2.11	0.54	1.66	1.80
7	1.18	1.12	1.75	1.00	1.01	0.81
8	1.60	0.70	1.95	0.97	1.57	0.93
9	0.95	1.69	1.91	0.80	0.76	1.20
10	1.13	1.61	1.44	0.82	1.40	1.35
Average	1.03	1.25	1.84	0.99	1.37	1.23
Standard deviation	0.27	0.43	0.54	0.23	0.33	0.27

**Table B2:** Compressive modulus of 3%wt chitosan scaffolds freezed at temperature -10, -80 and -196°C.

Compressive modulus (MPa)	Freezing temperature (°C)					
	Shrimp chitosan			Squid chitosan		
	-10	-80	-196	-10	-80	-196
1	1.31	1.41	5.34	1.53	3.63	2.74
2	1.05	2.47	5.48	1.77	1.84	4.50
3	0.92	1.94	3.50	0.95	5.01	2.29
4	0.69	3.10	4.54	1.34	1.62	3.70
5	1.46	2.89	3.41	0.73	2.55	2.37
6	1.31	2.64	4.91	0.83	2.30	1.57
7	1.38	1.98	4.66	1.67	2.15	3.59
8	1.59	1.51	4.67	1.29	2.35	2.96
9	1.39	2.26	4.22	0.91	3.31	2.19
10	1.11	1.09	2.37	1.54	2.00	3.61
Average	1.22	2.13	4.31	1.26	2.68	2.95
Standard deviation	0.27	0.66	0.96	0.37	1.03	0.89

**Table B3:** Compressive modulus of mole ratio of 2% shrimp chitosan to DAZ (1:0.05) at various irradiation times freezing at  $-10^{\circ}\text{C}$ .

Compressive modulus (MPa)	UV irradiation time (min)		
	0	40	60
1	0.16	1.09	0.88
2	0.82	1.13	0.70
3	1.38	0.94	0.87
4	0.92	1.25	0.66
5	1.35	1.16	0.61
6	0.89	0.94	0.66
7	0.83	1.03	0.59
8	1.12	0.74	0.59
9	0.67	0.54	0.61
10	0.72	0.73	0.54
Average	0.97	0.95	0.67
Standard deviation	0.26	0.22	0.12

**Table B4:** Compressive modulus of mole ratio of 2% shrimp chitosan to DAZ (1:0.1) at various irradiation times freezing at  $-10^{\circ}\text{C}$ .

Compressive modulus (MPa)	UV irradiation time (min)		
	0	40	60
1	0.96	1.07	0.73
2	0.95	1.26	0.68
3	0.74	0.99	0.49
4	0.98	1.13	0.64
5	1.05	1.27	0.72
6	0.51	0.97	0.69
7	0.57	0.92	0.45
8	0.49	1.06	0.86
9	0.49	0.89	0.65
10	0.61	0.80	0.76
Average	0.73	1.04	0.67
Standard deviation	0.23	0.15	0.12

**Table B5:** Compressive modulus of mole ratio of 2% shrimp chitosan to DAZ (1:0.5) at various irradiation times freezing at  $-10^{\circ}\text{C}$ .

Compressive modulus (MPa)	UV irradiation time (min)		
	0	40	60
1	0.65	0.19	0.20
2	0.47	0.16	0.21
3	0.41	0.20	0.15
4	0.42	0.14	0.20
5	0.27	0.12	0.22
6	0.70	0.11	0.18
7	0.29	0.18	0.15
8	0.37	0.25	0.16
9	0.49	0.23	0.18
10	0.33	0.18	0.23
Average	0.44	0.18	0.19
Standard deviation	0.14	0.04	0.03

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## APPENDIX C

**Table C1:** Density of 1%wt chitosan scaffolds freezed at temperature -10, -80 and -196°C.

Density (g/ml)	Freezing temperature (°C)					
	Shrimp chitosan			Squid chitosan		
	-10	-80	-196	-10	-80	-196
1	0.022	0.020	0.021	0.025	0.023	0.022
2	0.024	0.025	0.027	0.026	0.028	0.021
3	0.021	0.023	0.024	0.020	0.029	0.028
Average	0.022	0.023	0.024	0.024	0.027	0.024

**Table C2:** Density of 2%wt chitosan scaffolds freezed at temperature -10, -80 and -196°C.

Density (g/ml)	Freezing temperature (°C)					
	Shrimp chitosan			Squid chitosan		
	-10	-80	-196	-10	-80	-196
1	0.032	0.032	0.033	0.034	0.036	0.055
2	0.031	0.031	0.037	0.034	0.037	0.046
3	0.030	0.033	0.034	0.035	0.039	0.043
Average	0.031	0.032	0.035	0.034	0.037	0.048

**Table C3:** Density of 3%wt chitosan scaffolds freezed at temperature -10, -80 and -196°C.

Density (g/ml)	Freezing temperature (°C)					
	Shrimp chitosan			Squid chitosan		
	-10	-80	-196	-10	-80	-196
1	0.051	0.048	0.048	0.058	0.055	0.061
2	0.050	0.050	0.048	0.059	0.056	0.061
3	0.050	0.050	0.050	0.058	0.055	0.060
Average	0.050	0.049	0.049	0.058	0.055	0.061

**Table C4:** Density of 1, 2 and 3%wt chitosan films.

Density (g/ml)	Concentration of chitosan films (%wt)					
	Shrimp chitosan			Squid chitosan		
	1	2	3	1	2	3
1	1.30	1.24	1.33	1.31	1.32	1.38
2	1.30	1.29	1.31	1.35	1.24	1.38
3	1.25	1.27	1.34	1.28	1.30	1.37
Average	1.28	1.27	1.33	1.31	1.29	1.38

**Table C5:** %Porosity of 1, 2 and 3%wt chitosan scaffolds freezed at temperature -10, -80 and -196°C.

%Porosity	Concentration of chitosan scaffolds (%wt)					
	Shrimp chitosan			Squid chitosan		
	1	2	3	1	2	3
-10	98.3	97.6	96.2	98.2	97.3	95.8
-80	98.2	97.5	96.3	97.9	97.1	96.0
-196	98.2	97.3	96.3	98.3	96.3	95.6


  
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## APPENDIX D

**Table D1:** Degree of crosslinking of mole ratio of 2% shrimp chitosan to DAZ (1:0.1) at various irradiation times.

UV irradiation time (min)	Degree of crosslinking (%)		Average	Standard deviation
	X1	X2		
40	12.1	5.7	8.9	4.5
100	43.4	35.4	39.4	5.7
150	69.6	73.4	71.5	2.7
200	69.3	72.4	70.9	2.2

**Table D2:** Degree of crosslinking of mole ratio of 2% shrimp chitosan to DAZ (1:0.5) at various irradiation times.

UV irradiation time (min)	Degree of crosslinking (%)		Average	Standard deviation
	X1	X2		
40	57.6	62.8	60.20	3.68
100	67.5	65.6	66.55	1.34
150	68.9	70.4	69.65	1.06
200	84.1	82.6	83.35	1.06

**Table D3:** Degree of crosslinking of mole ratio of 2% shrimp chitosan to DAZ (1:1) at various irradiation times.

UV irradiation time (min)	Degree of crosslinking (%)		Average	Standard deviation
	X1	X2		
40	56.9	57.9	57.4	0.71
60	73.5	78.2	75.9	3.32
150	77	77.6	77.3	0.42
200	79.7	80.4	80.1	0.49

## APPENDIX E

**Table E1:** Dimension increasing of mole ratio of 2% shrimp chitosan to DAZ (1:0.1) at various irradiation times (pH5)

UV irradiation time (min)	Dimension increasing (%)			Average	Standard deviation
	X1	X2	X3		
20	397.3	327.1	424.9	383.1	50.5
40	198.5	213.5	201.3	204.4	8.0
100	119.5	118.4	110.3	116.1	5.0
150	83.9	120.8	116.5	107.1	20.2

**Table E2:** Dimension increasing of mole ratio of 2% shrimp chitosan to DAZ (1:0.5) at various irradiation times (pH5)

UV irradiation time (min)	Dimension increasing (%)			Average	Standard deviation
	X1	X2	X3		
20	75.7	87.4	79.6	80.9	5.9
40	45.6	52.5	50.7	49.6	3.6
100	32.0	36.8	35.6	34.8	2.5
150	30.5	26.2	30.0	28.9	2.4

**Table E3:** Dimension increasing of mole ratio of 2% shrimp chitosan to DAZ (1:1) at various irradiation times (pH5)

UV irradiation time (min)	Dimension increasing (%)			Average	Standard deviation
	X1	X2	X3		
20	45.5	36.4	46.2	42.69	5.46
40	42.3	48.6	37.9	42.94	5.38
100	18.8	25.6	26.8	23.71	4.33
150	22.4	15.9	25.7	21.33	5.00

**Table E4:** Dimension increasing of mole ratio of 2% shrimp chitosan to DAZ (1:0.1) at various irradiation times (pH7)

UV irradiation time (min)	Dimension increasing (%)			Average	Standard deviation
	X1	X2	X3		
20	13.6	16.9	15.8	15.4	1.7
40	11.0	13.7	14.0	12.9	1.7
100	19.6	21.0	11.5	17.4	5.2
150	13.4	14.2	11.2	12.9	1.5

**Table E5:** Dimension increasing of mole ratio of 2% shrimp chitosan to DAZ (1:1) at various irradiation times (pH7)

UV irradiation time (min)	Dimension increasing (%)			Average	Standard deviation
	X1	X2	X3		
20	10.1	12.7	9.5	10.7	1.7
40	8.4	10.1	12.0	10.2	1.8
100	15.5	13.7	18.5	15.9	2.5
150	14.1	13.5	14.3	13.9	0.4

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## VITAE

Miss. Temsiri Wangtaveesab was born in Bangkok, Thailand, on May 28<sup>th</sup>, 1977. She received Bachelor Degree of Science in 1998 from Department of Chemistry, Faculty of Science, King Mongkut's Institute of Technology Ladkrabang. She started as a Master Degree student in Petrochemistry and Polymer Science, Chulalongkorn University in 2002 and completed the program in 2005.

