

Biocompatibility and Biodegradability of Electrolyzed Chitosan Products

陳敏貞、? ; 耀國

E-mail: 9318500@mail.dyu.edu.tw

ABSTRACT

Chitosan possesses the biochemical properties including biocompatibility, biodegradability, non-toxic and antiseptic function. Therefore, chitosan are used as biomedical materials. In this study, chitosan of various molecular weight were dissolved in acetic acid of 0.05 N to proceed electrolysis experiment. To estimate the feasibility of these chitosan products as biomedical materials, we investigated the biocompatibility and biodegradability of electrolyzed product. The test of biocompatibility consisted of blood and cell analysis. Platelet adsorption test was used for studying the compatibility of chitosan with blood. The compatibility of chitosan with cell was estimated by observing the multiplication of NIH/3T3 fibroblast on the surface of electrolyzed product. Furthermore, lysozyme extracted from egg white was used to test the biodegradability of chitosan. The results indicated that NIH/3T3 fibroblast gathered and multiplied on the films of chitosan with three different molecular weight. It presented all of them possessed good biocompatibility. Moreover, chitosan of molecular weight with 1,750 kDa possessed the best compatibility with blood. The biodegradability of chitosan increased as the chitosan molecular weight increased.

Keywords : chitosan ; electrolyzed product ; biocompatibility ; platelet ; fibroblast ; biodegradability ; lysozyme

Table of Contents

封面內頁 簽名頁 授權書.....	iii	中文摘要.....
.....iv 英文摘要.....	v 誌謝.....
.....vi 目錄.....	vii 圖目錄.....
.....xi 表目錄.....	xii 第一章 研究目的.....
.....1 第二章 文獻回顧.....	2 2.1 幾丁質與幾丁聚醣的起源.....
.....2 2.1.1 幾丁質與幾丁聚醣簡介.....	2 2.1.2 幾丁質與幾丁聚醣之結構.....
.....3 2.1.3 幾丁質之製備.....	5 2.1.4 幾丁聚醣之製備.....
.....6 2.1.5 幾丁質與幾丁聚醣之應用.....	6 2.2 電化學理論.....
.....12 2.2.1 幾丁聚醣之相關電化學文獻.....	13 2.2.2 幾丁聚醣之電解機制.....
.....14 2.3 生物相容性.....	14 2.3.1 組織相容性.....
.....15 2.3.2 細胞相容性相關研究.....	16 2.3.3 血液相容性.....
.....16 2.3.4 血液相容性相關研究.....	18 2.4 生物分解性.....
.....19 2.4.1 溶菌?特性.....	20 2.4.2 生物分解性相關研究.....
.....20 2.5 十二烷基硫酸鈉-聚丙烯醯胺膠體電泳.....	22 2.5.1 膠體的聚合反應.....
.....23 2.5.2 聚丙烯醯胺膠體電泳原理.....	25 第三章 實驗材料與設備.....
.....28 3.1 實驗材料.....	28 3.2 實驗設備.....
.....32 第四章 實驗方法.....	34 4.1 實驗流程.....
.....34 4.2 不同分子量之幾丁聚醣製備.....	35 4.2.1 原料分析.....
.....38 4.4 表面性質分析.....	40 4.4.1 接觸角 (Contact Angle, CA) 試驗.....
.....41 4.5 生物分解性研究.....	41 4.5.1 重量損失率分析.....
.....42 4.5.2 SDS-PAGE分析.....	43 4.5.3 總醣測定.....
.....46 4.6 血液相容性研究.....	47 4.6.1 血小板吸附試驗.....
.....47 4.6.2 掃描式電子顯微鏡 (SEM) 分析.....	48 4.7 細胞相容性研究.....
.....49 4.7.1 薄膜試片之準備及消毒.....	49 4.7.2 細胞復甦.....
.....50 4.7.3 細胞計數.....	50 4.7.4 細胞貼附試驗.....
.....51 第五章 結果與討論.....	52 5.1 不同分子量之幾丁聚醣製備.....
.....52 5.1.1 原料分析.....	53 5.2 幾丁聚醣電解產物製程之探討.....
.....62 5.3 表面性質分析.....	66 5.3.1 接觸角 (Contact Angle, CA) 試驗.....
.....66 5.3.2

掃描式電子顯微鏡 (SEM) 表面分析.....	68	5.4 生物分解性研究.....	70
重量損失率分析.....	70	5.4.2 SDS-PAGE分析.....	72
總醣測定.....	74	5.5 血液相容性研究.....	77
5.5.1 血小板吸附試驗.....	77	5.5.2 掃描式電子顯微鏡 (SEM) 分析.....	79
5.6 細胞相容性研究.....	82	5.6.1 細胞液配製.....	
.....82		5.6.2 細胞鏡檢.....	83
.....86		第六章 結論.....	
.....89		第七章 未來展望.....	88
.....89		參 考 文 獻.....	
.....89		圖 目 錄	
.....4		圖2.1 幾丁質、幾丁聚醣及纖維素之化學結構.....	4
.....4		圖2.2 幾丁質生物體內的排列方式.....	21
.....24		圖2.3 幾丁聚醣與溶菌酶之作用位置.....	21
.....24		圖2.4 SDS化學結構式.....	24
.....34		圖2.5 膠體聚合反應之所需成分化學結構式.....	24
.....36		圖4.1 實驗流程圖.....	34
.....39		圖4.2 奧士瓦黏度計.....	36
.....55		圖4.3 電解裝置示意圖.....	39
.....56		圖5.1 原料A之濃度對黏度關係圖.....	55
.....56		圖5.2 原料B之濃度對黏度關係圖.....	56
.....57		圖5.3 原料C之濃度對黏度關係圖.....	57
.....65		圖5.4 不同分子量之幾丁聚醣電解產物.....	65
.....69		圖5.5 SEM分析不同分子量之幾丁聚醣電解產物表面型態.....	69
.....71		圖5.6 幾丁聚醣分別於培養不同時間後之重量損失率.....	71
.....73		圖5.7 12% SDS-PAGE電泳圖.....	73
.....75		圖5.8 在波長625 nm下之葡萄糖胺標準曲線.....	75
.....76		圖5.9 幾丁聚醣於培養不同時間後之總醣含量.....	76
.....80		圖5.10 幾丁聚醣電解薄膜上之血小板吸附形態.....	80
.....82		圖5.11 以trypan blue染色之細胞形態.....	82
.....84		圖5.12 培養24 h之纖維母細胞貼附形態.....	84
.....7		表 目 錄	
.....7		表2.1 幾丁質、幾丁聚醣之生化特性.....	7
.....45		表4.1 膠體濃度配方.....	45
.....54		表5.1 不同分子量之幾丁聚醣黏度值.....	54
.....59		表5.2 幾丁聚醣原料A、B及C之分子量.....	59
.....61		表5.3 幾丁聚醣原料A、B及C之P.V.S.K消耗量與去乙酰度.....	61
.....64		表5.4 幾丁聚醣電解產物A、B及C之分子量.....	64
.....67		表5.5 不同分子量之幾丁聚醣電解產物接觸角.....	67
.....78		表5.6 不同分子量幾丁聚醣之相對血小板吸附指數.....	78

REFERENCES

- 王三郎(1999), 海洋未利用生物資源之回收再利用-幾丁質及幾丁聚醣, 生物資源 生物技術, 1 (1): 1-8.
- 王三郎(2000), 水產資源利用學, 高立圖書公司, 87-111.
- 王文憲(1994), 人體生理學(二)第六版, 合記圖書出版社, 918-921.
- 朱家瑜、潘俊良、林亮宇(1994), 組織學, 藝軒圖書文具有限公司, 81-85.
- 白其昇(1995), 植物、微生物源之溶菌酶篩選及Enterobacter cloacae M-1002所產溶菌酶抑制劑之研究, 私立大葉工學院食品工程研究所碩士論文.
- 田福助(1988), 電化學基本原理與應用, 五洲出版社, 11-20.
- 兵界中(2002), 幾丁聚醣之電化學製程與理論探討, 私立大葉大學食品工程研究所碩士論文.
- 李建武、蕭能?、余瑞元、陳麗蓉、陳雅蕙、陳來同、袁明秀(1999), 生物化學實驗原理和方法, 藝軒圖書出版社, 135-151.
- 吳襄、林坤偉(1994), 生理學大綱, 藝軒圖書出版社, 57-62.
- 金家鳳(1999), 幾丁聚醣-聚醋酸乙酯和幾丁聚醣-聚乙醇接枝共聚物之研究, 國立台灣大學材料科學與工程學研究所碩士論文.
- 林佳奴、張曉婷、吳柏昇、林睿哲(2001), 幾丁聚醣於生醫材料之應用與特性, 化工, 48 (2): 84-91.
- 林睿哲、莊文喜(2000), 血液相容性高分子生醫材料, 化工技術, 8 (10): 230-240.
- 徐世昌(2001), 生物性高分子《幾丁質與幾丁聚醣》之介紹與應用, 化工資訊, 15 (2): 36-45.
- 陳美惠、莊淑惠、吳志津(1999), 幾丁聚醣之物化特性, 食品工業月刊, 31 (10): 1-6.
- 陳家全、李家維、楊瑞森(1991), 生物電子顯微鏡學, 國科會精儀中心編印, 109-131.
- 莊仲揚(2002), 幾丁聚醣於生醫產業上的應用, 化工資訊, 16 (4): 46-50.
- 莊榮輝、吳建興、陳翰民、張世宗、林士民、劉育志(2000), 酵素化學實驗, 國立台灣大學農業化學系生物化學實驗室, 147-153.
- 程修和(2001), 以纖維素酵素與鳳梨酵素水解幾丁聚醣與羧甲基纖維素製備幾丁寡醣與纖維寡醣之比較, 國立海洋大學食品科學系碩士論文.
- 覃慧萍(2000), 水性聚胺酯/幾丁聚醣聚合物之生物適合性研究, 國立台灣大學化學工程學研究所碩士論文.
- 熊楚強、王月(1996), 電化學, 文京圖書有限公司, 1-7.
- 葉錫誼(2002), NiTi記憶金屬表面之血栓調節素固定化, 及其生物活性與血液相容性之研究, 國立成功大學化學工程學系碩士論文.
- 葉淑芬(2003), 幾丁聚醣電解產物在藥物傳輸應用之探討, 私立大葉大學食品工程研究所碩士論文.
- 謝順堂(1994), Pseudomonas aeruginosa M-1001所產溶菌酶抑制劑之研究, 私立大葉工學院食品工程研究所碩士論文.
- 闕山璋(1998), 淺談骨科生醫材料之展望, 工業材料, 136: 81-84.
- 薛家倩(2004), SDS-PAGE蛋白質電泳分離技術, 化工資訊與商情, 7: 73-79.
- 蘇遠志(2001), 幾丁質與幾丁聚醣之機能及其有效利用, 生物資源 生物技術, 3 (2): 6-19.
- Ageev, Y. P., M. A. Golub and G. A. Vikhoreva (1999), Stochastic autooscillations of electrical conductivity of chitosan film swelled in water, Materials Science and Engineering, 373-376.
- Altankov, G. and T. Groth (1994), Reorganization of substratum on hydrophilic and hydrophobic materials is related to biocompatibility, Journal of Materials Science — Materials in Medicine, 5: 732-737.
- Calvo, P., C. Remunan-Lopez, J. L. Vila-Jato, and M. J. Alonso (1997), Chitosan and Chitosan/Ethylene Oxide-Propylene Oxide Block Copolymer Nanoparticles as Novel Carriers for Protein and Vaccines, Pharmaceutical Research, 14: 1431-1436.
- Choi, U. S. (1999), Electroheological properties of chitosan suspension, Colloids and Surfaces, A: Physicochemical and Engineering Aspects, 157: 193-202.
- Haimovich, B., L. Difazio, D. Katz, L. Zhang, R. S. Greco, Y. Dror and A. Freeman (1997), A New Method for Membrane Construction on ePTFE Vascular Grafts: Effect on Surface Morphology and Platelet Adhesion, Journal of Applied Polymer Science, 63: 1393-1400.
- Hirano, S., H. Tsuchida and N.

Nagao (1989), N-acetylation in chitosan and the rate of its enzymic hydrolysis, *Biomaterials*, 10: 574-576. 33. Hsu, S. H. and W. C. Chen (2000), Improved cell adhesion by plasma - induced grafting of L-lactide onto polyurethane surface, *Biomaterials*, 21: 359-367. 34. Kenji, S., H. Saimoto and Y. Shigemasa (1999), Electric resistance of chitosan derivatives, *Carbohydrate Polymers*, 39: 145-150. 35. Kieswetter, K., Z. Schwartz, T. W. Hummert, D. L. Cochran, J. Simpson, D. D. Dean and B. D. Boyan (1996), Surface roughness modulates the local production of growth factors and cytokines by osteoblast-like MG-63 cells, *Journal of Biomedical Materials Research*, 32: 55-63. 36. Ko, T. M., J. C. Lin and S. L. Cooper (1993), Surface characterization and platelet adhesion studies of plasma-sulphonated polyethylene, *Biomaterials*, 14: 657-664. 37. Kurita, K. (2001), Controlled functionalization of the polysaccharide chitin, *Progress in polymer science*, 26: 1921-1971. 38. Lampin, M., R. Warocquier-Clerout, C. Legris, M. Degrange and M. F. Sigot-Luizard (1997), Correlation between substratum roughness and wettability, cell adhesion and cell migration, *Journal of Biomedical Materials Research*, 36: 99-108. 39. Lee, K. Y., W. S. Ha and W. H. Park (1995), Blood compatibility and biodegradability of partially N-acylated chitosan derivatives, *Biomaterials*, 16: 1211-1216. 40. Peter, M. G. (1995), Applications and environmental aspects of chitin and chitosan, *Pure Appl. Chem.*, A32 (4): 629-640. 41. Mi, F. L., Y. C. Tan, H. F. Liang and H. W. Sung (2002), In vivo biocompatibility and degradability of a novel injectable-chitosan-based implant, *Biomaterials*, 23: 181-191. 42. Ravi Kumar, M. N. V., U. Bakowsky and C. M. Lehr (2004), Preparation and characterization of cationic PLGA nanospheres as DNA carriers, *Biomaterials*, 25: 1771-1777. 43. Ravindra, R., K. R. Krovvidi, A. A. Khan (1998), Solubility parameter of chitin and chitosan, *Carbohydrate polymers*, 36: 121-127. 44. Sevastianov, V. I., R. C. Eberhart and S. W. Kim (1988), Influence of mold properties on surface structure of a polyurethane-siloxane block co-polymer, *Trans. ASAIO*, 34: 10-18. 45. Song, Y., E. E. Babiker, M. Usui, A. Saito and A. Kato (2002), Emulsifying properties and bactericidal action of chitosan- lysozyme conjugates, *Food Research International*, 35: 459-466. 46. Stokke, B. T., K. M. Varum, H. K. Holme, R. J. N. Hjerde and O. Smidsrod (1995), Sequence specificities for lysozyme depolymerization of partially N-acetylated chitosans, *Canadian Chemical processing*, 73: 1972-1981. 47. Tsaih, M. L. and R. H. Chen (1997), Effect of molecular weight and urea on the conformation of chitosan molecules in dilute solutions, *Biological Macromolecules*, 20: 233-240. 48. Twu, Y. K., C. C. Ping, I. T. Chang and C. M. Shih (2002), Theory and characterization of producing chitosan by electrochemical process, *Advances in chitin Science*, 5: 19-21. 49. Varum, K. M., H. K. Holme, M. Izume, B. T. Stokke and O. Smidsrod (1996), Determination of enzymatic hydrolysis specificity of partially N-acetylated chitosans, *Biochimica et Biophysica Acta*, 1291: 5-15. 50. Woodhouse, K. A. and J. L. Brash (1992), Adsorption of plasminogen from plasma to lysine-derivatized polyurethane surfaces, *Biomaterial*, 13: 1103-1106.