

奈米幾丁聚醣粒子於口腔黏膜細胞穿透之研究

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摘要

本研究首先配製濃度5 mg/mL幾丁聚醣鹽酸鹽溶液，依序加入不同量的架橋(genipin)，架橋劑濃度分別為0、0.25、0.5、0.75、及1 mg/mL，再進行噴霧乾燥法製備出幾丁聚醣鹽酸鹽奈米粒子，產物分別標示為A、B、C、D、及E對樣本作物性分析。使用場發射電子顯微鏡觀察，其各產物平均粒徑介於223至264 nm之間。隨著架橋劑濃度增加，粒徑有變小的趨勢，且粒子表面皺摺也越趨明顯。人類口腔鱗狀癌上皮細胞KOSC-3，在細胞培養中觀察到細胞型態為鱗狀上皮細胞，其生長曲線在第2~4天為對數成長期，將分別與KOSC-3進行MTT細胞毒性評估，發現經架橋劑處理的粒子在濃度100 μg/mL以下對KOSC-3細胞無明顯毒性。經過細胞切片證實KOSC-3為複層鱗狀上皮細胞，雖有細胞堆疊情形但此型態並不穩定易造成細胞死亡。經 TEER試驗得知各樣本能有效促進細胞間隙的打開，並經trypan blue染色後發現對細胞經TEER試驗後均無死亡現象，可證明此五種幾丁聚醣鹽酸鹽奈米粒子能增加細胞間隙開合的效果。

關鍵詞：幾丁聚醣鹽酸鹽、KOSC-3、細胞切片、細胞毒性、穿透試驗

目錄

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參考文獻

- 1.李素文。2003。細胞生物學實驗手冊。第92-95頁。九州圖書文物有限公司。台北，台灣。2.呂卦南。2006。幾丁質與幾丁聚醣之製備與鑑定，*康寧學報*8: 159-160。3.吳佩怡。2002。硼中子捕獲治療劑-含硼胺基酸化合物之癌細胞吸收之研究，*國立清華大學原子科學系研究所碩士論文*。第17-20頁。4.施詔銘、?耀國。2005。奈米粉末製造機之結構與改良。*中華民國新型專利第M273391號*。5.陳家全、李家維、楊瑞森。2002a。生物電子顯微鏡學。第25-55頁。行政院國科會精密儀器發展中心編印。新竹，台灣。6.陳嘉芬。2002b。細胞生物學。第303-310頁。藝軒出版社。台北，台灣。7.陳慶源。2000c。幾丁聚醣在藥物運輸系統上應用。*食品工業*32(4): 18-28。8.張喜寧，夏鎮洋。1991。穿透式及掃描式電子顯微鏡生物技術。第82-88頁。曉園出版社有限公司。台北，台灣。9.鄂征。2002。組織培養和分子細胞學技術(上冊)。第113- 120頁。九州圖書文物有限公司。台北，台灣。10.游士平。2005。輸送演要幾丁聚醣-聚丙烯酸奈米顆粒之研究。南台科技大學化學工程所碩士論文。第6頁。11.黃華民、徐淑媛。2002。組織學彩色圖譜。第27-38頁。合記圖書出版社。台北，台灣。12.蔡宏銘。2002。中藥對骨細胞活性的評估。*中國醫藥學院中國醫學研究碩士論文*。第7-27頁。13.盧永坤。2005。奈米科技概論。第195-196頁。滄海書局。台北，台灣。14.賴怡潔。2003。以CaCO-2細胞模式探討hepcidin對小腸細胞鐵蛋白含量之影響

。台灣大學農業化學研究所。台北。 15.魏育慧。2000。利用動物細胞進行抗癌藥物篩選。食品工業32(11):27-35。 16.Amir, H. S. 1998. Buccal mucosa as a route for systemic drug delivery : A review. *J Pharm Pharmaceut Sci.* 1(1):15-30. 17.Asada, M., Takahashi, H., Okamoto, H. and Danjo, K. 2004. Theophylline particle design using chitosan by the spray drying. *Int. J. Pharm.* 270:167-174. 18.Bumgardner, J. D., Yuan, Y., Chesnutt, B. M., Utturkar, G., Haggard, W. O., Yang, Y. and Ong, J. L. 2007. The effect of cross-linking of chitosan microspheres with genipin on protein release. *Carbohydrate Polymers.* 68 : 561-567. 19.Bravo, S. A., Nielsen, C. U., Amstrup, J., Frokjær J. S. and Brodin, B. 2004. In-depth evaluation of Gly-Sar transport parameters as a function of culture time in the CaCO-2 cell model. *Eur. J. Pharm. Sci.* 21 : 77-86. 20.Cerchiara, T., Luppi, B., Bigucci, F. and Zecchi, V. 2003. Chitosan salt as nasal sustained delivery systems for peptidic drugs. *Pharmacy and pharmacology.*55: 1623-1627. 21.Chiou, S. H., Wu, W. T., Huang, Y. Y. and Chung, T. W. 2001. Effects of the characteristics of chitosan on controlling drug release of chitosan coated PLLA microsphere. *Journal of Microencapsulation.* 18: 613-625. 22.Corrigan, D. O., Anne, M. H. and Corrigan, O. I. 2006. Preparation and release of salbutamol from chitosan and chitosan co-spray dried compacts and multiparticulates. *Eur. J. Pharm. Biopharm.* 62 : 295-305. 23.Desai, K. G. H. and Park, H. J. 2005. Encapsulation of vitamin C in tri-polyphosphate cross-linked chitosan microspheres by spray drying. *J. Microencapsul.* 22(2) : 179-192. 24.Eirheim, H. U., Bundgaard, C. and Nielsen, H. M. 2004. Evaluation of different toxicity assays applied to proliferating cells and to stratified epithelium in relation to permeability enhancement with glycylcholate. *Toxicol.Vitro.* 18: 649-657. 25.Ganza-Gonza^Alez, A., Anguiano-Igea, S., Otero-Espinar, F. J. and Blanco MeA^ndez, J. 1999. Chitosan and chondroitin microspheres for oral-administration controlled release of metoclopramide. *Eur. J. Pharm Biopharm.*48: 149-155. 26.Grenha, A., Seijo, B. and Remun~a'n-Lo'pez, C. 2005. Microencapsulated chitosan nanoparticles for lung protein delivery. *Eur. J. Pharm. Sci.*25: 427-437. 27.Hoogstraate, A. J., Senel, S., Cullander, C., Verhoef, J., Junginger, H. E., and Bodde, H. E. 1996. Effect of bile salts on transport porcine buccal epithelium in vitro, *J Control. Rel.* 40: 211-221. 28.He, P., Davis, S. S. and Illum, L. 1999. Chitosan microspheres prepared by spray drying. *Int. J. Pharm.* 187: 53-65. 29.Huang, Y. C., Yeh, M. K. and Chiang, C. H. 2002. Formulation factors in preparing BTM-chitosan microspheres by spray drying method. *Int. J. Pharm.* 242: 239-242. 30.Harris, D. and Robinson, J. R. 1992. Drug delivery via the mucous membranes of the oral cavity. *J. Pharm. Sci.*81:1-10. 31.Inagaki, T., Matsuware, S., Takahashi, R., Shimada, K., Fujie, K., and Maeda, S. 1994. Establishment of human oral-cancer cell line (KOSC-2 and -3) carrying p53 and C-myc abnormalities by geneticin treatment. *Int. J. Cancer.* 56: 301-308. 32.Margrethe, R. R., Jette, J., Bovan, D. and Morten, P. 1995. TR146 cells grown on filters as a model for human buccal epithelium: I. Morphology, growth, barrier properties, and permeability. *International journal of pharmacaceutics.* 125: 165-184. 33.Matinac, A., Filipovic-Grcic, J., Voinovich, D., Perissutti, B. and Franceschinis, E. 2005. Development and bioadhesive properties of chitosan-ethylcellulose microspheres for nasal delivery. *Int. J. Pharm.* 291: 69-77. 34.Mi, F. L., Sung, H. W., Shyu, S. S., Su, C. C., and Peng, C. H. 2003. Synthesis and characterization of biodegradable TPP/genipin co-crosslinked chitosan gel beads. *Polymer.* 44: 6521-6550. 35.Mazzarelli, C., Stanic, V., Gobbi, L., Tosi, G. and Mazzarelli, R. A. A. 2004. Spray-drying of solutions containing Chitosan together with polyuronans and characterization of the microspheres. *Carbohydr. Polym.* 57: 73-82. 36.Nunthanid, J., Laungana-anan, M., Sriamornsak, P., Limmatvapirat, S., Puttipipatkhachorn, S., Lim, L. Y. and Khor, E. 2004. Charakterization of Chitosan acetate as a binder for sustained release tablets. *J. Control. Release.* 99: 15-26. 37.Olivier, P., Testard, P., Marzin, D. and Abbott, D. 1995. Effect of high polyol concentrations on the natural red absorption assay and tetrazolium-MTT test of rat hepatocytes in primary culture. *Toxicol. Vitro.* 9(2): 133-138. 38.Ravi Kumar M. N. V. 2000. A review of chitin and chitosan applications. *React. Funct. Polym.* 46:1-27. 39.Rege, P. R., Garmise, R. J. and Block, L. H. 2003. Spray-dried chitosan part I: preparation and characterization. *Int. J. Pharm.* 252: 41-51. 40.Rheinwald J. G. and Beckett M. A. 1981. Tumorigenic keratinocyte lines requiring anchorage and fibroblast support cultures from human squamous cell carcinomas. *Cancer Res.* 41: 1657-1663. 41.Shih, C. M., Twu, Y. K. and Shieh, Y. T. 2006. Chitosan nanoparticles produced by a model spray drying process. *Advances in chitin science and technology:* p335, April 23-26. 42.Somashekhar, D. and Joseph, R. 1996. Chitosanases—properties and applications a review. *Bioresour. Technol.* 2:151-165. 43.Va*rum, K. M., Egeland, B. and Ellekj?r, M. R. 1995. Characterization of partially N-acetylated chitosans by near infra-red spectroscopy. *Carbohydrate Polymers.* 28: 187-193. 44.Ve'ronique, P., Anne des, R., Virginie, F., Marie G. and Yves-J, S. 2006. Nanoparticles as potential oral delivery systems of proteins and vaccines : A mechanistic approach. *Journal of controlled release.* 116: 1-27. 45.Ward, P. D., Tippin, T. K. and Thakker, D. R. 2000. Enhancing paracellular permeability by modulating epithelial tight junctions. *Pharmaceutical Sciences and Technology Today.* 3: 346-358. 46.Zhang, W. F., Chen, X. G., Li, P. W., He, Q. Z. and Zhou, H. Y. 2007. Chitosan and chitosan/-cyclodextrin microspheres as sustained-release drug carriers. *Journal of Applied Polymer Science.*103:1183-1190.