

Studies of magnetic chitosan/Fe₃O₄ micro/Nanoparticles for nattokinase delivery

劉佳瑜、

E-mail: 321900@mail.dyu.edu.tw

ABSTRACT

In this study, chitosan/iron (II, III) oxide/ nattokinase micro/nano particles were produced by low-temperature spray-drying. The magnetic micro/nano particles were further study the transportation behavior between cells using an in vitro model of human oral squamous cell carcinoma (KOSC-3). In order to prepare micro/nano particles, three suspensions of 0.5、1、1.5% (w/v) chitosan with iron oxide were first mixed with nattokinase and then spray-dried in a low temperature condition. The samples were analyzed by field emission scanning electron microscopy (FESEM). It was found that the size of all the particles were in the range of 767 nm to 2.1 μm. The cytotoxicity of micro/nano particles was determined by MTT assay. No observable toxicity was noted on KOSC-3 cells by incubation with chitosan/iron (II, III) oxide/ nattokinase micro/nano particles. In vitro studies performed on KOSC-3 cell showed a pronounced opening of the cell junctions obtained by transepithelial electrical resistance (TEER) assay. In the meantime, the medium in the outside of insert was taken and a QuantiProTM BCA assay kit was used to investigate the release and transportation of nattokinase. The results indicated that the samples from multi-stage electromagnet collector had significantly improved transportation properties by the attraction of a magnet. However, the largest and smallest particles behave differently and that is worthy of further study.

Keywords : chitosan、Fe₃O₄、nattokinase、KOSC-3、drug release

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 x 表目錄 xii 1. 緒論 1 2. 文獻回顧 2 2.1 藥物傳輸
簡介 2 2.1.1 藥物傳輸技術 2 2.1.2 藥物傳輸之途徑 3 2.1.3 奈米顆粒之藥物傳輸 3 2.1.4 藥物控制釋放 6 2.2 幾丁質與幾丁聚
醣簡介 7 2.2.1 幾丁質及其衍生物結構 7 2.2.2 幾丁聚醣藥物載體的特性 10 2.3 磁性材料 10 2.4 納豆激? 略 12 2.5 低溫噴霧
乾燥 15 3. 材料與方法 20 3.1 實驗架構 20 3.2 實驗材料 21 3.2.1 藥品 21 3.2.2 耗材 22 3.2.3 儀器設備 23 3.2.4 細胞株 24 3.2.5
培養基與試劑配製 25 3.3 幾丁聚醣/四氧化三鐵懸浮液之製備 29 3.4 低溫噴霧乾燥法製備磁性微奈米複合顆粒 29 3.5 產物
分析 32 3.5.1 場發射電子顯微鏡 (FESEM) 32 3.5.2 原子吸收光譜儀 (AAS) 32 3.5.3 傅立葉紅外線光譜儀 (FTIR) 32 3.5.4 酶素
活性測定 33 3.6 細胞分析 33 3.6.1 細胞活化 33 3.6.2 繼代培養 34 3.6.3 細胞毒性 (MTT) 試驗 34 3.6.4 細胞穿透電阻 (TEER)
試驗 35 3.6.5 釋放試驗 36 3.6.6 細胞存活率 (Trypan blue) 試驗 37 4. 結果與討論 38 4.1 磁性幾丁聚醣/四氧化三鐵/納豆激?
L奈米粒子製備 38 4.2 場發射電子顯微鏡 (FESEM) 觀察 40 4.3 原子吸收光譜儀 (AAS) 分析 48 4.4 傅立葉紅外線光譜儀
(FTIR) 分析 50 4.5 酶素活性測定 53 4.6 細胞培養 56 4.7 細胞毒性 (MTT) 試驗 58 4.8 細胞穿透電阻 (TEER) 試驗 61 4.9 釋放
試驗 64 4.10 細胞存活率 (Trypan blue) 試驗 67 5. 結論 70 參考文獻 72

REFERENCES

1. 林芳新、董瑞安。2006。奈米核殼複合粒子的製備及生醫應用。科儀新知 28 (1):7-16。新竹，台灣。
2. 范瓊藝。2006。利用餉料發酵生產納豆激?，*碩士論文*。大同大學生物工程研究所碩士論文。台北，台灣。
3. 施詔銘、*奈米粉末製造機之結構改良*。中華民國專利局。新型第 M273391 號。台北，台灣。
4. 徐世昌。2001。生物高分子-幾丁質與幾丁聚醣之介紹與應用。化工資訊 15 (2):36-45。新竹，台灣。
5. 黃新義、*奈米粉末製造及收集設備之結構改良*。中華民國專利局。新型第 M353785 號。台北，台灣。
6. 梁晃千。2000。以天然交聯劑 genipin 交聯明膠的藥物制放微粒載體：體外與體內性質評估:11-15。國立中央大學化學工程研究所碩士論文。桃園，台灣。
7. 彭一凡。2007。探討照光強度對納豆菌生長與納豆激?：*芝山尬v響:13-17*。國立中央大學化學工程與材料工程研究所碩士論文。桃園，台灣。
8. 葉晨聖。2004。磁性奈米粒子的製備與其在生醫領域之應用。奈米專輯:1 (7):64-72。新竹，台灣。
9. 張安華。2005。實用奈米技術。第 233-235 頁。新文京開發出版股份有限公司。台北，台灣。
10. 傅昭銘、王昱峰。2003。奈米磁顆粒之放射性標化及應用簡介。物理雙月刊 25 (3):1-4。台北，台灣。
11. 趙蘭英。2003。奈米科技應用於藥物傳輸的技術發展趨勢。第二章第 1-3 頁。工研院 IEK 生醫與生活組。新竹，台灣。
12. 劉伊郎、陳恭。2000。氧化鐵 (Fe₃O₄) 薄膜與超晶格。物理雙月刊 22 (6):592-605。
13. 鄭宇書。2008。低溫奈米噴霧乾燥設備開發及應用:27-33。大葉大學生物產業科技系碩士論文。彰化，台灣。
14. Amidi, M., Romeijn, S. G., Borchard, G., Junginger, H. E., Hennink, W. E. and Jiskoot, W. 2006. Preparation and characterization of protein-loaded N-trimethyl chitosan nanoparticles nasal delivery system. Journal of Controlled Release. 111:107-116.
15. Berry, C. C. and Curtis, A. S. G. 2003. Functionalisation of magnetic nanoparticles for application in biomedicine. Journal of Physics D: Applied Physics. 36:198-206.
- 16.

Carpenter, E. E. 2001. Iron nanoparticles as potential magnetic carriers. *Journal of Magnetism and Magnetic Materials*. 225 : 17-20. 17.

Chatterjee, J., Haik, Y. and Chen, C. J. 2003. Size dependent magnetic properties of iron oxide nanoparticles. *Journal of Magnetism and Magnetic Materials*. 257 : 113-118. 18. 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. 19. Denkbas, E. B., Kilicay, E., Birlikseven, C. and ?頃t?卣k, E. 2002. Magnetic chitosan microspheres:preparation and characterization. *Reactive & Functional Polymers*. 50 : 225-232. 20. Desai, K. G. H., and Park, H. J. 2005. Preparation and characterization of drug-loaded chitosan-tripolyphosphate microspheres by spray drying. *Drug Development Research*. 64:114-128. 21. Donadel, K., Felisberto, M, D. V., Favere ,V. T., Rigoni, M., Batistela, N. J., and Laranjeira, M. C. M. 2008. Synthesis and characterization of the iron oxid magnetic particle coated with chitosan biopolymer. *Material Science and Engineering C*. 28 : 509-514. 22. Furlani, E. F. and Fulani, P. 2007. A model for predicting magnetic targeting of multifunctional particles in the microvasculature. *Journal of Magnetism and Magnetic Materials*. 312 : 187-193. 23. Grenha, A., Seijo, B. and Remu??鴨-L?櫟ez, C. 2005. Microencapsulated chitosan nanoparticles for lung protein delivery. *European Journal of Pharmaceutical Sciences* 25:427-437. 24. Guo, Y. H., Li, F. R., Bao, S. Y., Han, T., Cao, J. J. and Zhou, H. X. 2007. Preparation and characteristic of carboplatin-Fe@C-loaded chitosan nanoparticle with dual physical drug – loaded mechanisms. *Current Applied Physics*. 7S1 : e97-e102. 25. Gupta, K. A., and Gupta, M. 2005. Synthesis and surface engineering of iron oxide nanoparticle for biomedical application. *Biomaterials*. 26 : 3995-4021. 26. Hong, J., Xu, D., Gong, P., Sun, H., Dong, L. and Yao, S. 2007. Covalent binding of - chymotrypsin on the magnetic nanogels covered by amino groups. *Journal of Molecular Catalysis B:Enzymatic*.45:84-90. 27. Huang, S. Y., Shieh, Y. T., Shih, C. M. and Twu, Y. K. 2010. Magnetic chitosan/iron (II, III) oxide nanoparticles prepared by spray-drying. *Carbohydrate Polymers*, Vol. 81:906-910. 28. Huber, D. L. 2005. Synthesis properties and applications of iron nanoparticle. *Small*. 5:482-501. 29. Ito, A., Shinkai, M., Honda, H. and Kobayashi, T. 2005. Medical Application of functionalized magnetic nanoparticles. *Journal of Bioscience and Bioengineering*. 1:1-11. 30. Jeong, J. R., Chul, S., Lee, S. J., and Kim, J. D. 2005. Magnetic properties of superparamagnetic - Fe₂O₃ nanoparticles prepared by coprecipitation technique. *Journal of Magnetism and Magnetic Materials*. 286 : 5-9. 31. Jia, Z., Yujun, W., Yangcheng, L., Jingyu, M. and Guangsheng, L. 2006. In situ preparation of magnetic chitosan/Fe₃O₄ composite nanoparticles in tiny pools of water-in-oil microemulsion. *Reactive & Functional Polymer*. 66:1552-1558. 32. Jotania, R. B., Khomane, R. B., Chauhan, C. C., Menon, S. K. and Kulkarni, B. D. 2008. Synthesis and magnetic properties of barium-calcium hexaferrite particle prepared by sol-gel and microemulsion techniques. *Journal of Magnetism and Magnetic Materials*. 320 : 1095-1101. 33. Kim, E. H., Ahn, Y. and Lee, H. S. 2007. Biomedical applications of superparamagnetic iron oxide nanoparticles encapsulated within chitosan. *Journal of Alloys and Compounds*. 434/435:633-636. 34. Li, G. Y., Jiang, Y. R., Huang, K. L., Ding, P. and Chen, J. 2008. Preraration and properties of magnetic Fe₃O₄-chitosan nanoparticles. *Journal of Alloys and Compounds*. 466:451-456. 35. Liu, T. Y., Hu, S. H., Liu, K. H., Liu, D. M. and Chen, S. Y. 2006. Preparation and characterization of smart magnetic hydrogels and its use for drug release. *Journal of Magnetism and Magnetic Materials* . 304 : 397-399. 36. Maruyama, M., and Sumi, H. 1998. Effect of natto diet on blood pressure. *Basic and Clinical Aspects of Japanese Traditional Food Natto II*. 1-3. 37. Nguyen, T. T. B., Hein, S., Ng, C. H. and Stevens, W. F. 2007. Molecular stability of chitosan in acid solutions stored at various conditions. *Journal of Applied Polymer Science*, Vol.107, 2588-2593. 38. Rana, S., Gallo, A., Srivastava R.S. and Misra R.D. 2007. On the suitability of nanocrystalline ferrites as a magnetic carrier for drug delivery:functionalization conjugation and drug release kinetics. *Acta Biomaterialia*. 3:233-42. 39. Sumi, H., Hamada, H., Nakanishi, K. and Hiratani, H. 1990. Enhancement of the fibrinolytic activity in plasma by oral administration of Nattokinase I. *Acta Haematol* 84:139-43. 40. Sumi, H., Hamada, H., Tushima, H., Mihara, H. and Muraki, H. 1987. A novel fibrinolytic enzyme (Nattokinase) in the vegetable cheese Natto; a typical and popular soybean food in the Japanese diet. *Experientia* 43:1110-1. 41. Sun, K. Y., Ma, M., Zhang, Y. and Gu, N. 2004. Synthesis of nanometer- size maghemite particles from magnetite. *Colloids and Surfaces A:Physicochemistry and Engineering Aspects*. 245:15-19. 42. Takahashi, H., Chen, R., Okamoto, H., and Danjo, K. 2005. Acetaminophen particle design using chitosan and a spray-drying technique. *Chemical and Pharmaceutical Bulletin*. 53:37-41. 43. V?熳um, K. M., Egelalndal, B. And Ellekj?禱, M. R.1995. Characterization of partially N-acetylated chitosans by near infra-red spectroscopy. *Carbohydrate Polymers*. 28:187-193. 44. Vaughan, D. E., 2001. Angiotensin, fibrinolysis, and vascular homeostasis. *American Journal of Cardiology*. 87:18-24. 45. V?臆onique, P., Anne des, R., Virginie, F., Marie G. and Yves-J, S. 2006. Nanopaticles as potential oral drlicery systems of proteins and vaccines : A mechanistic approach. *Journal of Controlled Release*. 116: 1-27. 46. Wang, J., Zhang, K., Peng, Z. and Cheng, Q. 2004. Magnetic properties improvement in Fe₃O₄ nanoparticles grow under magnetic fields. *Journal of Crystal Grow*. 266 : 500-504. 47. Xu, C., and Sun, S. 2007. Monodisperse magnetic nanoparticle for biomedical application. *Polymer International*. 56 : 821-826. 48. Zhang, W., Shen, H., Xie, M. Q., Zhuang, L., Deng, Y. Y., Hu, S. L. and Lin, Y. Y. 2008. Synthesis of carboxymethyl-chitosan-bound magnetic nanoparticles by the spraying co-precipitation method. *Scripta Material*. 59 : 211-214. 49. Yu, J. H., Lee, D. W., Kim, B. K. and Jang, T. 2006. Synthesis and properties of magnetic fluid based on iron nanoparticles prepared by a vapor-phase condensation process. *Journal of Magnetism and Magnetic Materials*. 304 :16-18.