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• 中文摘要

本研究爲比較經由絲瓜來源之去乙醯幾丁質對於 HeLa cell 細胞株的轉染效能，並建立結構與物性對於轉染效能的影響關係。所欲進行細胞轉染的質體 DNA 爲 p-EGFP-C1。絲瓜去乙醯幾丁質溶液(5 mM 的醋酸鈉緩衝液，pH 5.5，內含 0.02%絲瓜去乙醯幾丁質)與 DNA 的溶液(25 mM 的硫酸鈉溶液，內含 1 μg/ml 的 DNA)，進行絲瓜去乙醯幾丁質與 DNA 之奈米微球複合物(nanoparticles)之製備。取不同比例之兩種溶液，利用高速震盪器混合 15-30 秒，靜置後即可得絲瓜去乙醯幾丁質與 DNA 之奈米微球複合物，並利用電泳膠片實驗進行 DNA 被包覆之測定。與市售之幾丁質進行 DNA 包覆比較可以發現其包覆的效果並不如市售幾丁質好，可能原因爲絲瓜去乙醯幾丁質除了含有 N-acetylglucosamine 及 Glucosamine 還含有其他醣類小分子，所以帶有的正電荷不如市售幾丁質豐富，因此包覆的 DNA 較少。將 HeLa cell 培養在 6-well 的培養皿(8 x 10⁴ cells/ml)培養 24 小時後，再將絲瓜去乙醯幾丁質與 DNA 之奈米微球複合物加入培養皿中，共同培養 48 小時後，利用螢光顯微鏡和流式細胞儀對基因轉染之表現做測定及分析。由轉染實驗可以發現市售產品能將基因帶入 HeLa cell，而經由絲瓜所萃取得之絲瓜去乙醯幾丁質，雖然並沒有顯著的轉染效果，但還是具有轉染的效能。

• 英文摘要

In the present report, we compared the transfection efficiency of chitosans from various sources and the optimal condition of processing Luffa chitin into chitosan was also investigated. The plasmid p-EGFP-C1 encoding fluorescence protein was grown in Escherichia coli and extracted by a QIAGEN kit. The purity of the plasmid was checked by electrophoresis and the concentration of DNA was determined by measuring an UV absorbance at 260 nm. A 0.02% chitosan solution and a DNA solution were preheated to

55℃ separately. Both solutions in equal volume were mixed together speedily and vortexed for 15-30 s, then kept in ambient temperature for use. Complex formation was confirmed by electrophoresis on a 0.8 % agarose gel with TBE running buffer at 100 V. DNA was visualized with ethidium bromide. HeLa cell were grown in DMEM containing 10% fetal bovine serum at 37℃ under a 5% CO2 atmosphere. Cells were seeded into a 6-well plate at a density of 8×104 cell per well and grown to confluency. After 48 h incubation, the cells was removed from the culture plate and analyzed on a FACScan flow cytometer. Alternatively, cells were directly viewed under a fluorescence microscopy. Free DNA released from some chitosan/DNA complexes at a mixing ratio of 1/5, since the charge of these chitosans was insufficient to neutralize those of DNA. However, at the mixing ratio of 1/1 of Luffachitosan / DNA complex, free DNA appeared. It may reveal that, in comparison to commercial products, the Luffa chitosan bears relatively low charge, which make it carry less DNA amount than the others do. The transfection of HeLa cells with chitosan/ DNA complexes was studied by using the plasmid p-EGFP-C1 encoding fluorescence protein. The investigation of fluorescent microscopy revealed that, including Luffachitosan, those commercial chitosans we selected in this study were capable of carrying genes into HeLa cells. FASCan analysis also validated this observation except that Luffachitosan / DNA complex showed less significant but still detectable expression of transfection .