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论文

脂质-鱼精蛋白-DNA复合物的构建及其对细胞的体外转染

孙逊;张志荣;

四川大学 华西药学院， 四川 成都 610041

摘要：

目的研究新型非病毒载体脂质-聚阳离子-DNA（LPD）复合物的制备方法及其对体外细胞的转染率。方法用薄膜-挤压法制备空白阳离子脂质体，与鱼精蛋白-DNA复合物在室温孵育后，得到LPD；用透射电镜观察其形态，用激光粒度仪测定其粒径和zeta电位；LPD与DNA酶I溶液在37 ℃下孵育不同时间后，用琼脂糖凝胶电泳观察其降解情况；用荧光法测定其包封率；用X-gal染色法考察了LPD对张氏（Chang）肝细胞，HepG2肝癌细胞和SMMC-7721肝癌细胞的转染率。结果LPD的形态近似于球体，平均粒径为143.5 nm，平均zeta电位为+32.6 mV；37 ℃下核酸酶作用2 h后，LPD中的DNA几乎无降解；平均包封率为93.42%；LPD对张氏（Chang）肝细胞、HepG2肝癌细胞和SMMC-7721肝癌细胞的转染率分别为(69±6)%，(43±7)%和(96.2±1.8)%。结论LPD是一种制备工艺简单、体外稳定性好、转染率高，具有应用潜力的非病毒载体系统。

关键词： 非病毒载体系统 脂质-鱼精蛋白-DNA复合物 转染率

Preparation of lipid-protamine-DNA complexes and evaluation of their transfection efficiencies *in vitro*

SUN Xun; ZHANG Zhi-rong

Abstract:

AimTo develop a novel non-viral gene delivery systems lipid-polycation-DNA complexes (LPD) and investigate their transfection efficiencies *in vitro*. MethodsLPD were prepared as follows by first mixing the plasmid DNA and protamine together, then the resulted polyplexes were incubated for 10 min at room temperature, followed by addition of preformed cationic liposomes. The morphology of LPD was observed by transmission electron microscopy. The diameter and surface charge of LPD were measured by photon correlation spectroscopy (PCS). The nuclease protection ability of LPD was evaluated by agarose gel electrophoresis. Estimation of transfection efficiency was performed by galactosidase assay in Chang, HepG2 and SMMC-7721 cells. ResultsThe average diameter and the zeta potential of LPD were 143.5 nm and 32.6 mV, respectively. LPD could protect the plasmid DNA from nuclease degradation after 2 hours incubation at 37 ℃ while the naked DNA degraded rapidly. The average transfection efficiencies were (69±6)%, (43±7)% and (96.2±1.8)% in Chang cells, HepG2 cells and SMMC-7721 cells respectively. ConclusionLPD could be prepared easily with small particle sizes and high transfection activities. LPD may be good non-viral vectors for applications in gene delivery.

Keywords: lipid-protamine-DNA complexes transfection efficiency non-viral gene delivery systems

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通讯作者: 张志荣

作者简介:

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