Gene Delivery with PCL Nanofiber in Vitro

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Abstract. Gene therapy has widely been known to be the promising treatment approach for many diseases, whether acquired or inherited through a genetic disorder. Viral and non-viral vectors are the primary research fields of gene delivery techniques which play a pivotal role in tissue engineering and cancer therapy. Even though viral vectors are well-known as higher transfection efficiency, they are also carefully applied in human because of their side effects. Non-viral vectors, such as synthetic and natural compounds and polymers which have less toxicity and low immunogenicity, can offer flexible choice of gene to deliver. However, there are some obstacles in non-viral gene delivery, including low gene transfection efficiency and sudden release. In this study, Polycaprolactone (PCL) and plasmid DNA was dissolved in acetonitrile and electrospun. Our study showed that the transfection efficiency was improved under the controlled gene delivery release in vitro condition.

1. Introduction

In the past decade, many researchers have applied physical methods in gene delivery, such as electroporation, sonoporation, magnetofection, hydroporation [1]. Ultrasound can induce cavitation of near cellular membranes to enhance transfection efficiency of nucleic acid in vitro and in vivo. In general, low-intensity ultrasound can induce beneficial and reversible cellular effects, in contrast to high-intensity ultrasound, which plays more roles in cellular death [2].
PCL, which has less toxicity and low immunogenicity, is well-known as material, extensively applied in tissue engineering [3-5]. PCL can not only overcome the barrier of safety but also be substantially synthesized in an easy way. The advantages of electrospun nanofibers gives high surface-to-volume ratio, appropriate porosity and malleability, conform to a wide variety of size and shapes. In our study, we combined electrospun PCL nanofiber with sonoporation and low pH to improve gene transfection efficiency. The structure of electrospun nanofiber is mutual layer which can protect DNA from degrading and make DNA sustained release. And ultrasound can change cellular membrane pores, followed by taking up nucleic acid. The membrane is repaired, and nucleic acid is, therefore, retained within cells [2].

2. Experiment

PCL was dissolved in acetonitrile, to which pCMVb plasmid (3.5mg/ml in TE buffer) was added. The solution was allowed to equilibrate for certain time limit with constant stirring. Polymer solution contains 700 µg plasmid DNA in 200µl TE buffer. The solution was transferred to a syringe with a plastic needle and set up for electrospinning. Process starts with controlled speed, voltage and distance. Fibroblasts were cultured by the medium pre-immersed with PCL electrospun nanofiber in a 96-well plate at 37℃ for 4 days. Then 20 µl MTT (Aldrich-Sigma Chemicals, 5mg/ml in PBS) was added to each well. After six hours, cells were cracked by 10% SDS solution, followed by reading plate at 0D 590 nm see Figure 1. The PCL electrospun nanofiber was cut into 2cm×2cm sections, and each section was incubated at 37℃ with 1 ml TE buffer in Eppendorf tubes. In order to quantify the released EGFP plasmid DNA, we collected the TE buffer of the different time points (15min, 30min, 1h, 2h, 4h, 1d, 3d, 7d) and PicoGreen(ATCC, manassas, VA, USA) was used to detect the double-stranded DNA. TE buffer were excited at 480nm, and emission was measured at 520 nm in a UV microplate reader (CytoFlour Series 4000, Perseptive Biosystems) see Figure2. Fibroblasts were seeded into petri-dish at the density of 105 cells per dish. Twenty four petri-dishes were allocated into four groups. Of the four groups, the first group was control group; the second group was treated with ultrasound; the third group was treated with reducing PH level of the medium; and the fourth group was treated with ultrasound and reducing
PH level of the medium. RT-PCR (left primer GACGTAAACGGCCACAAGTT right primer AAGTCGTGCTGCTTCATGTG product size 188 bps) was used to amplify target gene segment and 2% agarose

electrophoresis was ongoing to validate that target gene successfully see Figure 3. transducted into fibroblasts. After transfecting the fibroblasts, we directly observed the fibroblasts under laser scanning confocal microscopy (Leica Microsystems TCS Sp5-II German) see Figure 4.

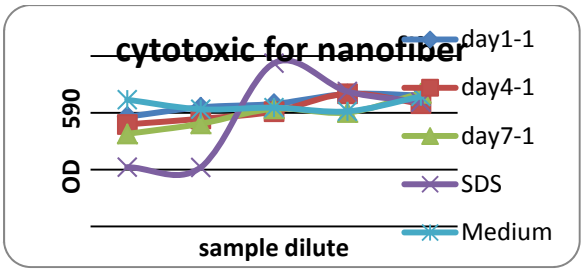

Fig.1. Cytotoxicity results for PCL nanofiber

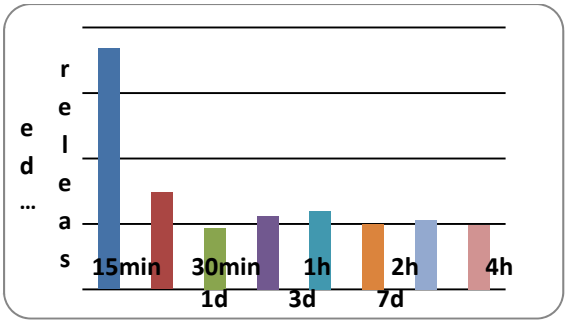

Fig.2. DNA release versus Time

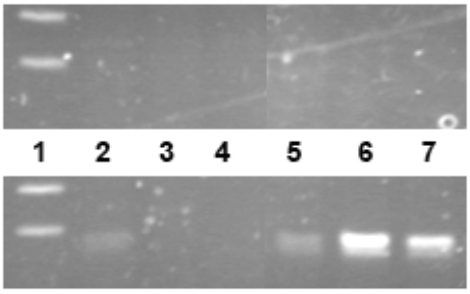

Fig.3. Lane 1 was DNA ladder. Upper lane 2, 3, 4 and lower lane 2, 3, 4 were the level of GFP gene expression in control group and in third group, respectively. Upper lane 5, 6, 7 and lower lane 5, 6, 7 were the level of GFP gene expression in second group and in fourth group, respectively.

3. Results

The data of MTT assay demonstrated that PCL electrospun nanofiber do less harm to fibroblasts. The DNA released from nanofiber could still be detected after one week. PCL electrospun nanofiber released more DNA in the first fifteen minutes than that in other time points.

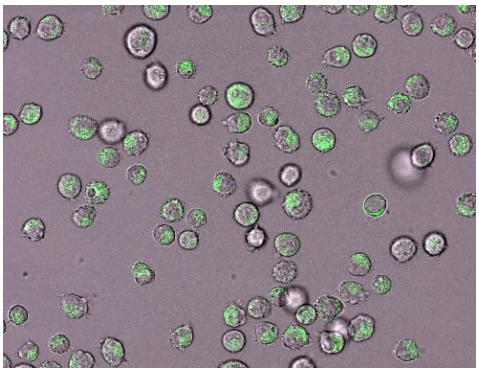

Fig.4. Treated with ultrasound and reducing PH level simultaneously, most of cells were transfected.

The target gene could be successfully transducted into fibroblasts and express functional protein. The electrophoresis image suggests that there is not gene expression in the first, second and third group. Ultrasound together with reducing PH level can dramatically improve the transfection efficiency compared with other groups.

4. Conclusions

PCL electrospun nanofiber has less cytotoxicity which can be degraded by enzymes normally existing in human body and can be a satisfying gene vector but also keep the function of target genes. As a gene vector, PCL electrospun nanofiber can continuously release plasmid DNA. Therefore, it can overcome sudden release. Particularly, with degraded by enzymes, PCL electrospun nanofiber can continuously release more target genes to express functional protein. Combining with physical methods such as ultrasound and PH level, PCL nanofiber can be a potential biomaterial applying in non-viral gene delivery. Electrospun nanofibers have high surface-to-volume ratio, appropriate porosity and malleability, conform to a wide variety of size and shapes, which make non-viral vectors, be prior to be chosen as substrates for tissue engineering as well as for delivery of cells and bioactive agents, including drugs, protein and DNA.

5. References

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