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Complex Micelles Applied Ultrasound-Mediated Gene Transfection

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1. Introduction

One of the vital issues in gene therapy is lack of proper transfection method. While viral vectors attracting much attention at the beginning, nonviral transfection methods are gathering more and more interest due to higher site specificity and much lower toxicity. Ultrasound, especially with the appearance of contrast agents, is recently developed as a noninvasive and nonviral targeting delivery method [1]. Ultrasonic waves can increase cell membrane permeability temporally by inducing transient holes, termed as sonoporation, in the phospholipids bilayer and thus allow the transfer of large DNA molecular into the cell [2]. However, an obvious deficiency of ultrasound mediated delivery method is the low transduction efficiency [3].

To enhance the stability, DNA plasmids encoding for green fluorescent protein (GFP) were combined to block polymers and then deliverd into cells in present study using Mega-Hertz ultrasound with the presence of microbubbles. The transfection efficiency is confirmed.

2. Materials and methods

A system sketch of ultrasound exposure system is shown in **Fig.1**. The system is comprised of a function arbitrary waveform generator (model WF1944A, NF Corporation, Yokohama, Kanagawa, Japan), a 50 dB gain radio frequency amplifier (2100L, E&I, Rochester, NY, USA), an oscilloscope, and a custom designed single piezoceramic element plane transducer (13.5 mm diameter, Imasonic, Besancon, France). The central frequency of the transducer is 2 MHz, and a 40 cycle burst pulse with pulse repetition frequency at 5 kHz and the overall exposure time at 60 seconds. The transducer was inserted into wells of a 24-well plate, which was laid on sound absorbing material in a 37°C water bath. Mouse embryonic fibroblast cell lines (NIH3T3) were used as in vitro biological model for gene transfection. Transfection efficiency was then measured with flow cytometry and cell viability was measured by utilizing highly water-soluble tetrazolium salt.

The polyplex micelle was formed by combining plasmids and block copolymer (**Fig.2a**). Block copolymer consists of a polyethyleneglycol-group and poly-lysine. In solution, lysine is positively charged, while plasmid DNA is negatively charged. Thus, poly-lysine and DNA interact electrostatically, causing the block copolymer and DNA to form a polymer micelle (**Fig.2b**) [4]. Upon formation of a polymer micelle, DNA becomes compacted [5]. A toroid-shaped polymer micelle was applied, which corresponds to an negative-positive ratio of 1.5. The size of this polymer micelle is approximately 300 nm.

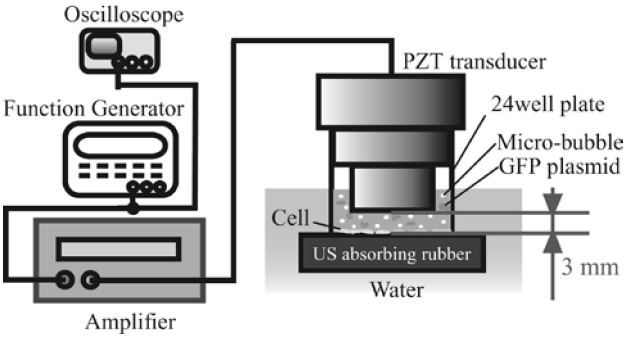
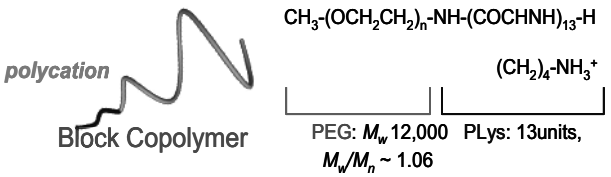
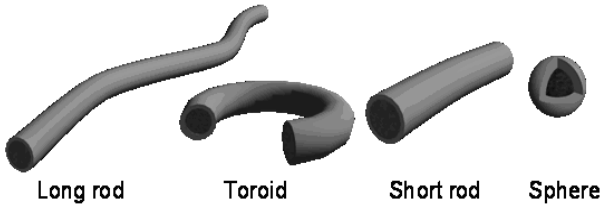


Fig.1 System setup



a) Block copolymer



b) Formation of micelle

Fig.2 Complex micelles

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3. Results and Discussions

The formation of complex micelle was confirmed using atomic force microscope, indicated by **Fig.3**.

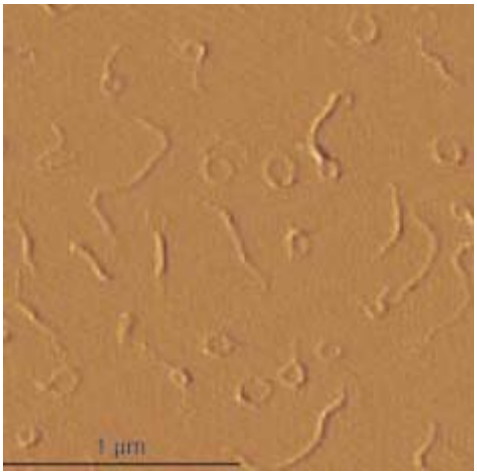

Fig.3 AFM image of complex micelle

Naked DNA are susceptible to attack and degradation by nucleolytic enzymes in serum-containing media [6] and, therefore, cannot be expressed. However, the activity of these enzymes against DNA can be blocked by forming polymer micelles, which can be confirmed by electrocataphoresis.

Plasmid DNA in solution has a supercoiled (SC) structure but will collapse and the plasmid DNA becomes an open circular (OC) structure if DNA is attacked by nucleolytic enzymes and suffers a loss of at least one base. These two types of DNA, SC and OC, can be separated by electrophoresis. Both naked DNA and polymer micelle were added to the culture medium used in the ultrasound exposure experiment for different periods of time. As shown in **Fig.4**, most of the naked DNA plasmids are degraded within 15 minutes. On the other hand, some polymer micellized DNA plasmids are immune to attacks for 60 minutes, suggesting enhanced stability.

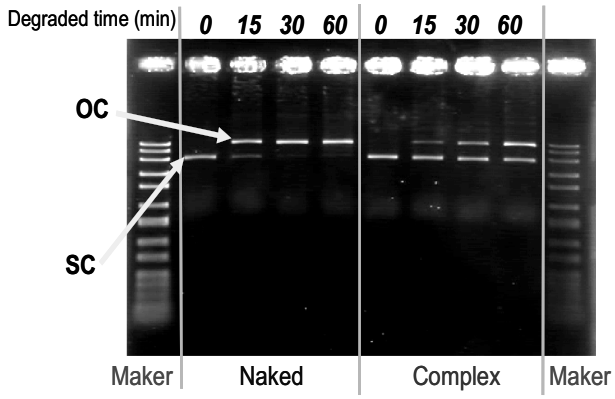

Fig.4 Electrophoresis results

The transfection ratio was thus enhanced, seen in the **Fig.5**. For all the cases irradiated, the DNA plasmids concentration is 15μg/ml; the microbubble density is around 10^5count/mm^3; the ultrasound intensity is 7.72 W/cm^2; the irradiation time is 60 seconds. The results were averaged from three independent groups and the sample volume is 12. The results were given by averaged value and standard deviation. Ultrasound is effective in inducting genes has been long proved and here it is obvious that applying complex micellized plasmids are also effective in enhancing transfection efficiency.

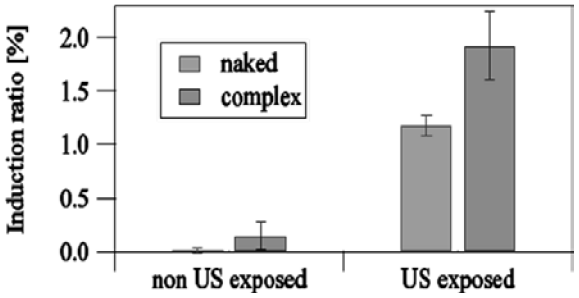

Fig.5 Transfection efficiency

4. Conclusion

The stability of plasmid DNA in culture containing serum can be enhanced when combined to polymer and the complex micelle formed can hold its existence longer. The gene transfection efficiency can thus be improved by applying this complex micelle method.

Acknowledgment

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