

HIGH THROUGHPUT PURIFICATION DEVICE FOR GENE DELIVERY MULTIFUNCTIONAL ENVELOPE-TYPE NANODEVICE

Daisuke Shigenaka^{1,2}, Masami Ukawa³, Noritada Kaji^{1,2}, Yukihiro Okamoto², Manabu Tokeshi^{2,4}, Hidetaka Akita³, Hideyoshi Harashima³ and Yoshinobu Baba^{1,2,5}

¹Department of Applied Chemistry, Nagoya University, JAPAN,

²FIRST Research Center for Innovative Nanobiodevices, Nagoya University, JAPAN,

³Faculty of Pharmaceutical Sciences, Hokkaido University, JAPAN,

⁴Division of Biotechnology and Macromolecular Chemistry, Hokkaido University, JAPAN and

⁵Health Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Japan

ABSTRACT

Multifunctional envelope-type nanodevices (MEND) developed by Harashima *et al.* is one of the novel non-viral DNA vectors expected as a safe gene delivery system. Our group has already attained fast and easy preparation of MEND by using the microfluidic device. However, purification of MEND should be performed in a high-throughput manner because prepared MEND contains impurities such as plasmid-cationic polymer complex. In this paper, we report a novel purification method for MEND by free flow electrophoresis based on microfluidic device, which purification principle is based on the zeta potential difference between MEND and impurities.

KEYWORDS

MEND, Drug delivery system, Free-flow electrophoresis.

INTRODUCTION

MEND is a novel non-viral DNA vector as a safe gene delivery system (Figure 1) and constructed via three steps: (i) core formation by DNA condensation with a polycation, (ii) lipid film hydration for the electrostatic binding with the condensed DNA, and (iii) packaging the condensed DNA with lipids by sonication [1]. Since the conventional method requires troublesome procedures and has prevented the clinical application of MEND, new method to construct MEND is highly required to overcome these problems. Although our group has already developed on-chip MEND construction method [2,3], which is very simple, rapid, convenient, and cost-effective compared

with conventional methods, to improve the step of purification of MEND has still remained as a major problem because present method needs longer time to purify MEND from impurities such as DNA core.

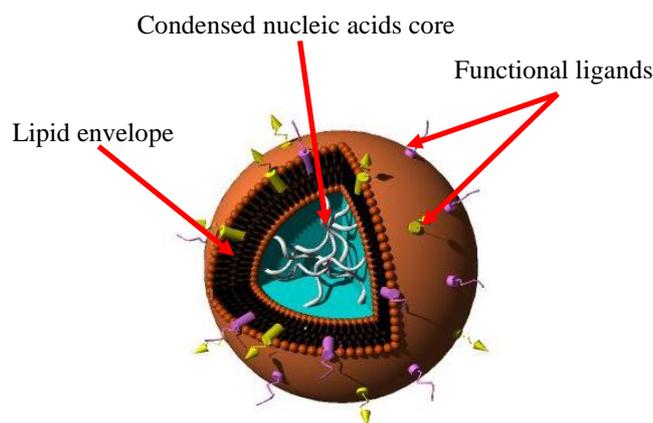


Figure 1. Schematic representation of the MEND

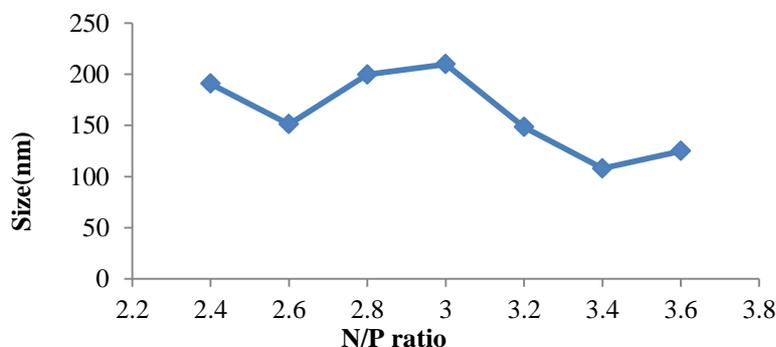


Figure 2. Relation between nitrogen/phosphate (N/P) ratio and core size.

Free-flow electrophoresis (FFE) is a continuous separation method, providing bands along the separation channel and a continuous supply of separated components [4]. In FFE, charged particles are injected into a thin carrier flow

with an electrical field applied perpendicular to the flow. The charged particles are deflected from the general flow direction at an angle determined by the flow velocity, electrophoretic mobility and electrical field strength. So we designed microchip-based free-flow electrophoresis method. This method is expected high throughput purification of the MEND.

EXPERIMENTAL

We prepared double-staining MEND for fluorescent observation and fluorescence detection. Double-staining MEND was prepared with fluorescein labeled DNA core and rhodamine labeled DOPE according to following procedures: fluorescein labeled DNA core was prepared by mixing fluorescein labeled plasmid DNA and poly(ethylene imine) (PEI), and its size's dependency on the mixing ratio between DNA and PEI, i.e., nitrogen/phosphate (N/P) ratio, was investigated. Figure 2 indicates that we can successfully fabricate 100 nm size cores at a N/P ratio of 3.4. At this ratio, we attempted to fabricate MEND with rhodamine labeled DOPE and fluorescein labeled DNA core by lipid hydration method. Prepared MEND's size distribution and zeta potential are shown in Figure 3 and indicate that we can successfully prepared MEND suitable for gene delivery. Furthermore, fluorescence spectrum of prepared MEND was measured.

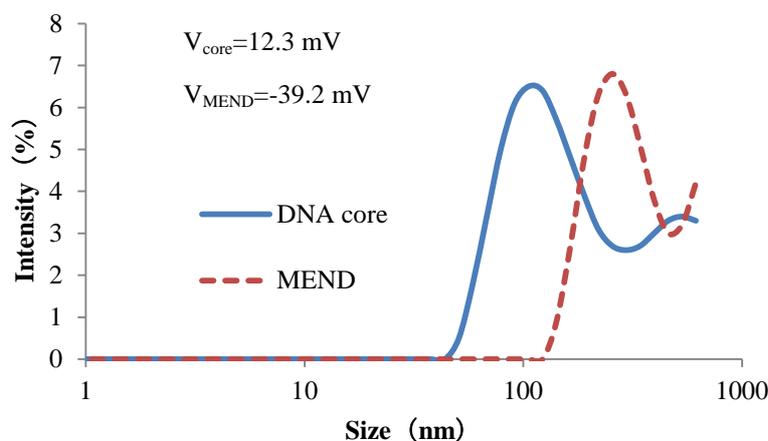


Figure 3. DNA core and MEND size distribution. The peak size of DNA core is 105.7 nm and the peak size of MEND is 255 nm.

Subsequently, the microfluidic device for purification of MEND was constructed, which separation principle was based on the difference of zeta potential between MEND and others. This microfluidic device consists of electrodes prepared by Au vapor deposition on a glass and a PDMS channel as shown in Figure 4. With this microfluidic device, we introduce the fluorescein solution in HEPES and applied a voltage in the separation channel.

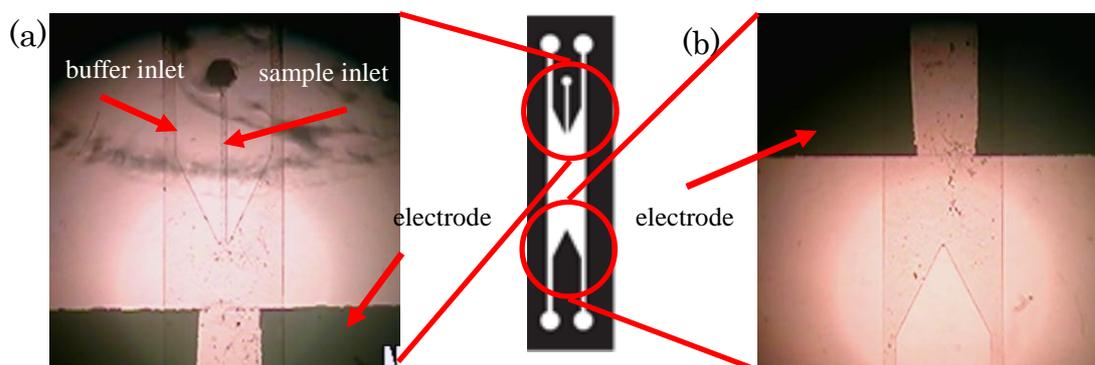


Figure 4. Overall view of the microfluidic device. The width of the separation area is 2.0 mm and the length of the separation area is 7.5 mm. The channel depth is 50 μm . (a) Inlet side image. (b) Outlet side image.

RESULTS AND DISCUSSIONS

When light with a wavelength of 492 nm was applied for the sample solution of MEND, 518 nm fluorescence was observed, and when the light of the wavelength which is 557 nm was applied, 571 nm fluorescence was observed. Figure 5 shows that prepared MEND was doubly stained with fluorescein and rhodamine.

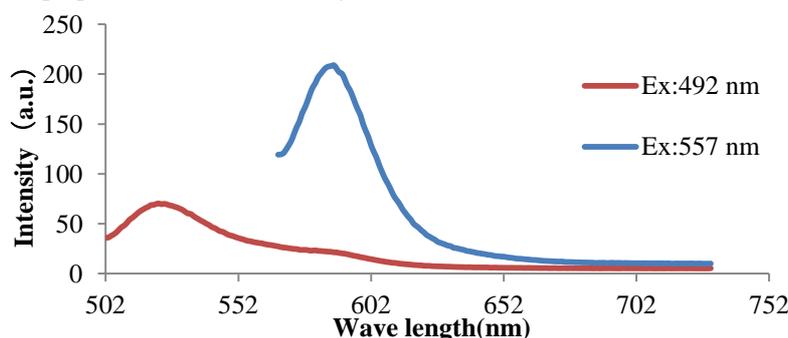


Figure 5. Fluorescence spectrum of MEND. This result indicates accomplishment of double labeling.

With this device, we could form the sheath flow by the control of the flow velocity as shown in Figure 6(a). In addition, fluorescein molecules in HEPES buffer migrated to the anode when we applied voltage of 40 V as shown in Figure 6(b). Fluorescein molecules is negatively charged under the condition of pH=7.4, so the sample stream is deflected into anode side.

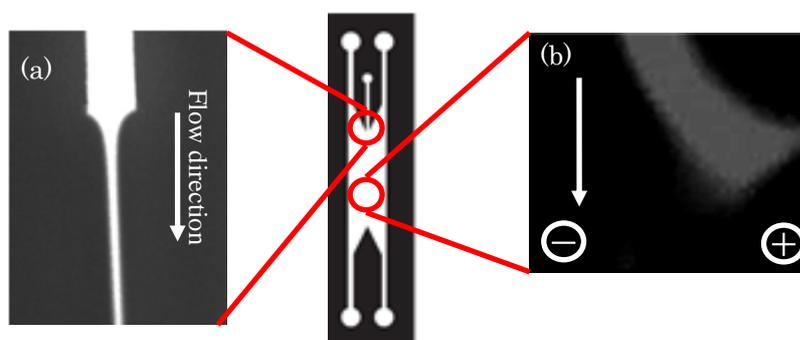


Figure 6. (a) Picture of sheath flow. (b) FFE of fluorescein in 10 mM HEPES (pH=7.4)

CONCLUSIONS

In this study, we developed the high-throughput purification device for MEND. Since the device is designed to integrate with the microfluidic device for on-chip MEND construction we developed [1], the integrated microfluidic devices will be applicable to the clinical trials for MEND.

ACKNOWLEDGEMENTS

This research is partially supported by the Japan Society for the Promotion of Science (JSPS) through its “Funding Program for World-Leading Innovative R&D on Science and Technology (FIRST Program)”, and the Grant-in-Aid for Scientific Research (S).

REFERENCES

- [1] K. Kogure, R. Moriguchi, K. Sasaki, M. Ueno, S. Futaki, H. Harashima, “Development of a non-viral multifunctional envelope-type nano device by a novel lipid film hydration method” *J. Control Release*, **98**, pp. 317-323, (2004)
- [2] K. Kitazoe, J. Wang, N. Kaji, Y. Okamoto, M. Tokeshi, K. Kogure, H. Harashima, Y. Baba, “A touch-and-go lipid wrapping technique in microfluidic channels for rapid fabrication of multifunctional envelope-type gene delivery nanodevice” *Lab Chip*, **11**, pp. 3256-3262, (2011)
- [3] K. Kitazoe, Y.-S. Park, N. Kaji, Y. Okamoto, M. Tokeshi, K. Kogure, H. Harashima, Y. Baba. “Fabrication of functionalized double-lamellar multifunctional envelope-type nanodevices using a microfluidic chip with a chaotic mixer array” *PLoS One*, **7**, e39057, (2012)
- [4] D. Kohlheyer, G. A. J. Besselink, S. Schlautmann, R. B. M. Schasfoort, "Free-flow zone electrophoresis and Isoelectric focusing using a microfabricated glass device with ion permeable membranes" *Lab Chip*, **6**, pp.374-380, (2006)

CONTACT

*Daisuke Shigenaka, shigenaka.daisuke@g.mbox.nagoya-u.ac.jp