ON-CHIP IMMUNOELECTROPHORESIS FOR EVALUATING SURFACE PROTEINS OF EXOSOMES AT SINGLE-PARTICLE LEVEL FOR DIAGNOSTIC APPLICATION

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ABSTRACT

Surface proteins of individual exosomes was evaluated by an on-chip immunoelectrophoresis system by laser dark-field microscopy. Using such a system, sensitive tracking of exosomes was performed successfully. A positive shift of the zeta potential of exosomes secreted from human breast cancer cells in vivo and in vitro was observed after the immunoreaction. These results suggested that on-chip immunoelectrophoresis is a promising methodology for single-exosome analysis toward the future application of exosomes as disease biomarkers for low-invasivity diagnostics.

KEYWORDS: Cancer Detection, Capillary Electrophoresis, Exosome, Immunoelectrophoresis, Zeta potential

INTRODUCTION

Recently, cell-secreted nanovesicles (extracellular vesicles, ECVs) such as exosomes have attracted considerable attention. The fact that exosomes deliver genetic material including proteins, mRNAs, and miRNAs suggests that they play a pivotal role in intercellular communication. In addition, exosomes are also expected to be potential disease biomarkers [1]. To characterize exosomes, they were evaluated by flow cytometry after binding exosomes to exosomal-marker-protein-coated beads and to fluorescent-dye-labeled antibodies of objective proteins and by western blot analysis [2]. However, these methods are not suitable for obtaining the precise profile of exosomes when the samples are mixtures of heterogeneous particles since they cannot perform particle-by-particle measurement. In this study, we demonstrated the electrokinetic evaluation of surface proteins of individual exosomes by on-chip immunoelectrophoresis.

EXPERIMENTAL

Figure 1 shows a schematic image of the immunoelectrophoresis of exosomes. The electrophoretic mobility (EPM) of an exosome changes when the exosome binds with specific antibodies. The immunoreactivity of an exosome can be evaluated by the positive shift of the zeta potential of the exosome, which can be caused by the addition of the positive charge of antibodies to exosomes.

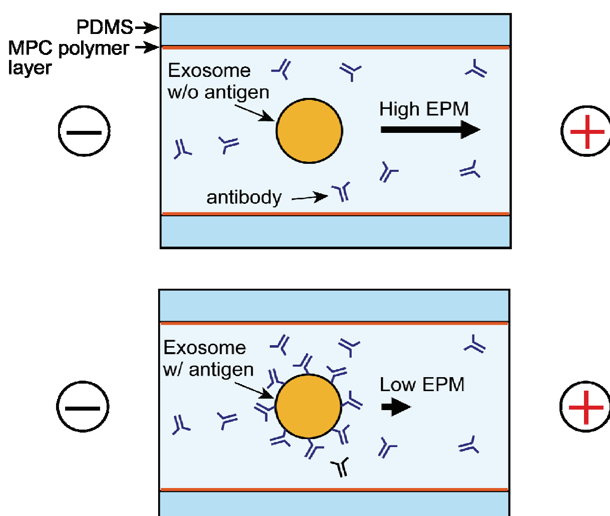

Figure 1 Schematic image of immunoelectrophoresis in a μCE chip. The immunoreactivity of exosomes can be estimated on the basis of the change in zeta potential, calculated from EPM by the Smoluchowski formula, at the single-particle level. To suppress nonspecific adsorption and electroosmotic flow, the inner surface of the microchannel was coated with 2-methacryloyloxyethylphosphorylcholine (MPC) polymer.

Figure 2 shows a schematic diagram of an on-chip microcapillary electrophoresis (μCE) system equipped with a laser dark-field microscope. A μCE chip was fabricated by soft lithography. The inner surface of the microchannel was coated with 2-methacryloyloxyethylphosphorylcholine (MPC) polymer to suppress nonspecific adsorption and electroosmotic flow. With this system, the sensitive tracking of individual exosomes was successfully performed and the system was found to be suitable for the particle-by-particle measurement of the zeta potential of exosomes.

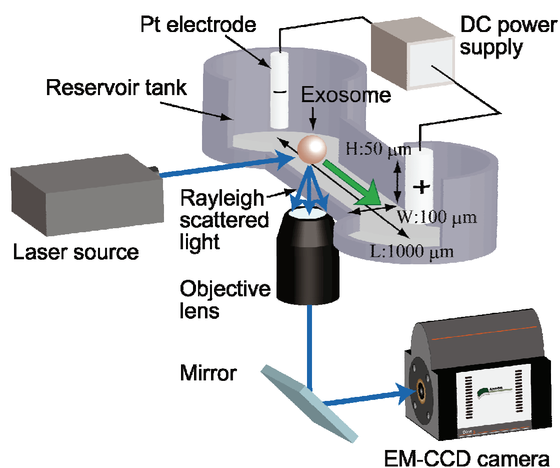

Figure 2 Schematic diagram of the on-chip microcapillary electrophoresis (μCE) system with a laser dark-field microscope. This system has enabled the sensitive imaging of individual exosomes in a dark field by detecting laser light scattered from exosomes using an EM-CCD camera.

A human breast cancer cell line (MDA-MB-231) was cultured to confluence in an RPMI medium followed by 24 h culture with a serum-free RPMI medium. Exosomes were extracted from the medium by differential ultracentrifugation. The samples were introduced into the flow channel of a μCE chip. To perform the electrophoresis experiments, migrating exosomes were recorded under an electric field of 50 V/cm using a laser dark-field microscope. The zeta potential of individual exosomes was calculated from EPM using the Smoluchowski equation.

RESULTS AND DISCUSSION

To explore the feasibility of on-chip immunoelectrophoresis for analyzing exosomes, the zeta potentials of exosomes extracted from a culture medium of human breast cancer MDA-MB-231 cells by differential ultracentrifugation were evaluated after incubation with anti-human CD63 (hCD63) mouse antibodies or mouse IgG (isotype control) for 30 min at 4°C. Figure 3 shows the zeta-potential distributions of exosomes derived from MDA-MB-231 cells by immunoelectrophoresis. The averages ± standard deviations were −7.4 ± 2.2 mV (isotype control) and −2.0 ± 1.3 mV (anti-hCD63 antibody). Positive shifts of the zeta potential of exosomes were observed after the immunoreaction.

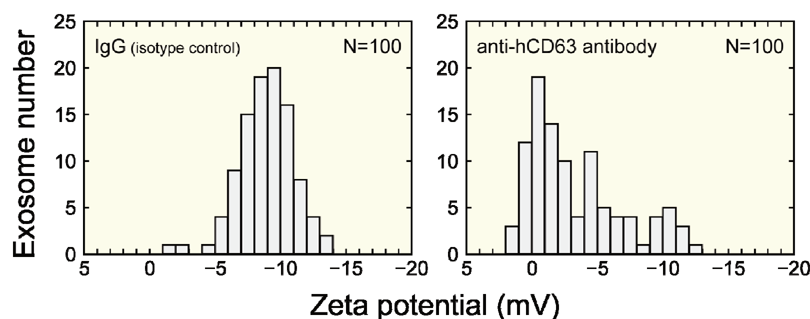

Figure 3 Effect of immunoreaction with anti-hCD63 antibody on zeta-potential distribution of exosomes. The zeta potential of exosomes extracted from a culture medium of human breast cancer (MDA-MB-231) cells was evaluated after treatment with anti-hCD63 antibody. CD63 is an exosomal marker protein. Normal IgG was used as an isotype control antibody to estimate the nonspecific binding of anti-hCD63 antibody.

Furthermore, to investigate the applicability of on-chip immunoelectrophoresis to in vivo analysis, the zeta-potential of exosomes extracted from the blood of nude mice implanted with human breast cancer (MDA-MB-231) cells was evaluated after the immunoreaction. As shown in Fig. 4, the distribution of the zeta potential was found to have a peak at approximately 0 mV after the immunoreaction of exosomes with anti-hCD63 (exosomal marker) and anti-hCD44 (breast cancer marker) antibodies, whereas the distribution has no peak at approximately 0 mV after treatment with mouse IgG (isotype control). Therefore, the possibility of on-chip immunoelectrophoresis for the in vivo analysis of exosomes was revealed.

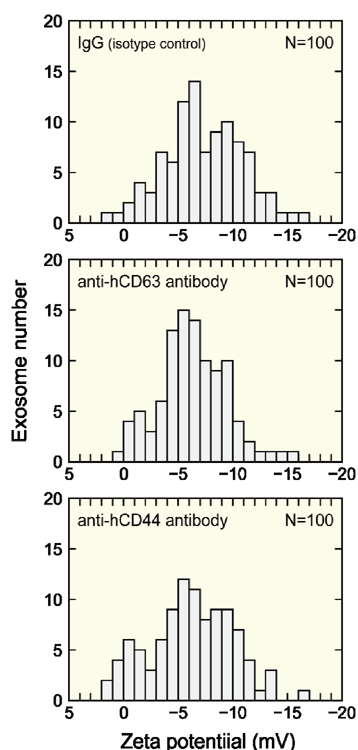

Figure 4 Zeta-potential distribution of exosomes extracted from the blood of nude mice implanted with MDA-MB-231 cells after treatment with antibodies. hCD63 and hCD44 are exosomal and breast cancer marker proteins, respectively. Normal IgG was used as an isotype control antibody to estimate the nonspecific binding of anti-hCD63 and anti-hCD44 antibodies.

CONCLUSION

The accurate electrokinetic evaluation of individual exosomes and on-chip immunoelectrophoresis were successfully demonstrated for evaluating surface proteins of individual exosomes. The present results suggested that on-chip immunoelectrophoresis is a promising methodology for single exosome analysis toward the future application of exosomes as disease biomarkers for low-invasive diagnostics.

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