# Capture of esophageal and breast cancer cells with polymeric microfluidic devices for CTC isolation

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**Abstract.** The present study evaluated the capture efficiency of esophageal and breast cancer cells with a modified 'polymeric circulating tumor cells (CTC)-chip' microfluidic device, which was developed for the isolation of circulating tumor cells. Esophageal cancer cell lines KYSE150, KYSE220 and KYSE510, and breast cancer cell lines MCF7, SKBR3 and MDA-MB-231 were used for evaluation. The capture efficiencies of the esophageal cancer cell lines in phosphate-buffered saline (PBS) were ~0.9, irrespective of epithelial cell adhesion molecule (EpCAM) expression, which was represented as the mean fluorescent intensity from 528 to 76. In the breast cancer cell lines, efficient capture was observed for MCF7 and SKBR3 in PBS; however, a low value of ~0.1 was obtained for MDA-MB-231. Fluorescent imaging of immunolabeled cells revealed marginal EpCAM expression in MDA-MB-231. Using whole blood, no clogging occurred in the microstructure-modified CTC-chip and efficiency of capture was successfully evaluated. Capture efficiencies for KYSE220 and MCF7 in whole blood were >0.7, but were of either equal or lesser efficiency in comparison to PBS. Therefore, the modified CTC-chip appears useful for clinical application due to its cost, practicality of use, and efficient cancer cell capture.

## Introduction

Numerous studies and reviews on circulating tumor cells (CTCs) have been found to be critically useful for clinical application in the diagnosis, therapy and research of cancer (1-14). CTCs are known to exist in the peripheral blood at an extremely low concentration and are difficult to isolate from the blood. Subsequently, a number of efforts have been made to develop devices and apparatus that are able to isolate CTCs. Our previous study designed and fabricated a novel CTC isolation device, a polymeric microfluidic device termed the 'CTC-chip', which is now commercially available (15). Although most microfluidic CTC isolation devices, including the conventional CTC-chip, are made of silicon or polydimethylsiloxane (16-19), these materials are not necessarily suitable for clinical applications in terms of cost, producibility and their material properties. The current polymeric devices are comprised of light-curable resins, which were formulated for easy production and clinical usability, and our previous study demonstrated that the device overcame practical usage issues faced by conventional chips. The device captures cancer cells by targeting the epithelial cell adhesion molecule (EpCAM) expressed on the surface of cancer cells using an immobilized anti-EpCAM antibody. As EpCAM expression is considered heterogeneous among CTCs from within a single patient and varies based on cancer type, it is important to estimate the influence of EpCAM expression on the efficiency of capture by the device. The present study evaluated the efficiency of capture by use of esophageal and breast cancer cell lines. The esophageal cancer cell lines were chosen as they exhibit different EpCAM expression levels measured by flow cytometry for the evaluation. As for the breast cancer cell lines, attention was focused not only on EpCAM expression but also on subtypes of breast cancer and used cell lines representing estrogen receptor positive/progesterone receptor positive (ER⁺/PR⁺), human epidermal growth factor receptor 2 (HER2⁺) and triple-negative breast cancers.

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## Materials and methods

*Cancer cell lines and preparation of cell suspensions.* Esophageal cancer cell lines KYSE150, KYSE220 and KYSE510 were kindly provided by Dr Yutaka Shimada (one of the authors of this manuscript), and breast cancer cell lines MCF7, SKBR3 and MDA-MB-231 were obtained from the American Type Culture Collection (Manassas, VA, USA). EpCAM expression levels were measured in the esophageal cancer lines using a BD FACSCanto flow cytometer (Becton-Dickinson,
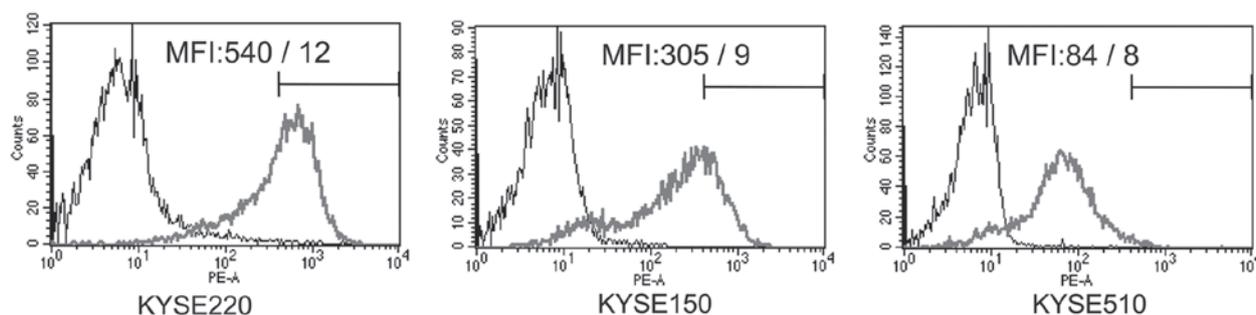
Figure 1. Epithelial cell adhesion molecule (EpCAM) expression levels of esophageal cancer cell lines measured by flow cytometry using mouse anti-EpCAM antibody and PE anti-mouse immunoglobulin G (IgG) antibody (gray lines), and control (PE anti-mouse IgG antibody only, black lines). The x-axis shows logarithmic fluorescence intensity. Mean fluorescence intensity (MFI) was calculated from the flow cytometry data.

San Jose, CA, USA), mouse anti-human EpCAM antibody (cat. no. sc-59906; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) and PE anti-mouse immunoglobulin G (IgG) antibody (cat. no. IM0551; Beckman Coulter, Brea, CA, USA).

For capture efficiency measurement, cancer cells were fluorescently labeled with CellTrace (Life Technologies, DriveRockville, MD, USA) and were either spiked into phosphate-buffered saline (PBS) containing 1% bovine serum albumin or whole blood, which had been drawn from a healthy donor (following approval by the Ethics Comittee of the University of Toyama and after obtaining written informed concent) and stored in a vacuum blood collection tube containing ethylenediaminetetraacetic acid. The cell suspensions were prepared at concentrations between 100 and 400 cells/ml.

Immunostaining of MDA-MB-231 and KYSE510 was performed using mouse anti-EpCAM and Cy3 goat anti-mouse IgG antibodies (cat. no. CLCC35010; Cedarlane, Hornby, ON, Canada). Cells cultured in 96-well microplates were fixed in 4% paraformaldehyde for 30 min and washed with PBS. Mouse anti-EpCAM antibody at a concentration of 20 $\mu$g/ml was applied to the cells for 2 h at room temperature. Cells were washed with PBS and stained with Cy3 goat anti-mouse IgG antibody at a concentration of 4 $\mu$g/ml. Following a final wash with PBS, fluorescent microscopic images were captured using a digital camera.

*Preparation of the polymeric CTC-chip.* Production of the polymeric CTC-chip and antibody coating of the chip surface were carried out as described previously (15). The microstructure of the chip consisted chiefly of an array of two different types of microposts, modified from the previous design to prevent clogging by whole blood. The gap between microposts was enlarged to 200 $\mu$m in the area around the chip inlet. Goat anti-mouse IgG antibody (cat. no. 1032-01; Southern Biotech, Birmingham, AL, USA) and mouse anti-human EpCAM antibody were used for chip coating.

*Evaluation of cell capture efficiency by the polymeric CTC-chip.* The polymeric CTC-chip was set in a holder and the efficiency of capture was evaluated using the method described previously with the cancer cell suspensions. Efficiency of capture was calculated by measuring the number of cells remaining on the chip following sample passage compared to the number of cells that passed through the chip inlet.

## Results

*EpCAM expression levels.* EpCAM expression levels in the esophageal cancer cell lines were measured by flow cytometry and represented as relative mean fluorescent intensity (MFI), which changed between 528 and 76 for the different cell lines (Fig. 1). Capture efficiencies of the esophageal cancer cell lines in PBS were ~0.9 irrespective of EpCAM expression levels (Fig. 2A). In the breast cancer cell lines, MCF7 and SKBR3 in PBS were efficiently captured, however a low value of ~0.1 was obtained for MDA-MB-231 (Fig. 2B). Fluorescent images of immunostained MDA-MB-231 and KYSE510 were captured and compared to roughly estimate EpCAM expression level of MDA-MB-231 (Fig. 3).

There was no clogging of the modified chip when using whole blood (Fig. 4) and the efficiency of cell capture was successfully evaluated. Capture efficiencies for KYSE220 and MCF7 in whole blood were >0.7, but were of either equal or lesser efficiency in comparison to PBS (Fig. 2C).

## Discussion

In the present study, the polymeric CTC-chip efficiently captured esophageal and breast cancer cells except for MDA-MB-231 in PBS. All the esophageal cancer cells used exhibited an MFI between 528 and 76, indicating EpCAM expression, with the lowest EpCAM-expressing cell line, KYSE510, still efficiently captured as shown by the bright image of the stained cells in Fig. 3B. The breast cancer lines MCF7 and SKBR3 have been previously shown to express sufficient EpCAM to allow efficient capture by other microfluidic devices (19-21), which was in accordance with the present results. Capture efficiency of SKBR3 by conventional CTC-chip was lower than the polymeric chip. Capture efficiency of MDA-MB-231 was extremely low and appeared reasonable due to marginal EpCAM expression, as shown in Fig. 3A. Previous studies have confirmed MDA-MB-231 to have extremely low EpCAM expression (22-25). However, the capture efficiency obtained for MDA-MBA-231 suggests that the extremely low level of EpCAM still had a role in capture due to obtaining a capture efficiency of only 0.02 when coating the polymeric CTC-chip with only anti-mouse IgG antibody. Low capture efficiencies of MDA-MB-231 and other cancer cell lines exhibiting down-regulation of EpCAM, often caused by epithelial-mesenchymal transition (EMT), have also been observed in PBS or whole
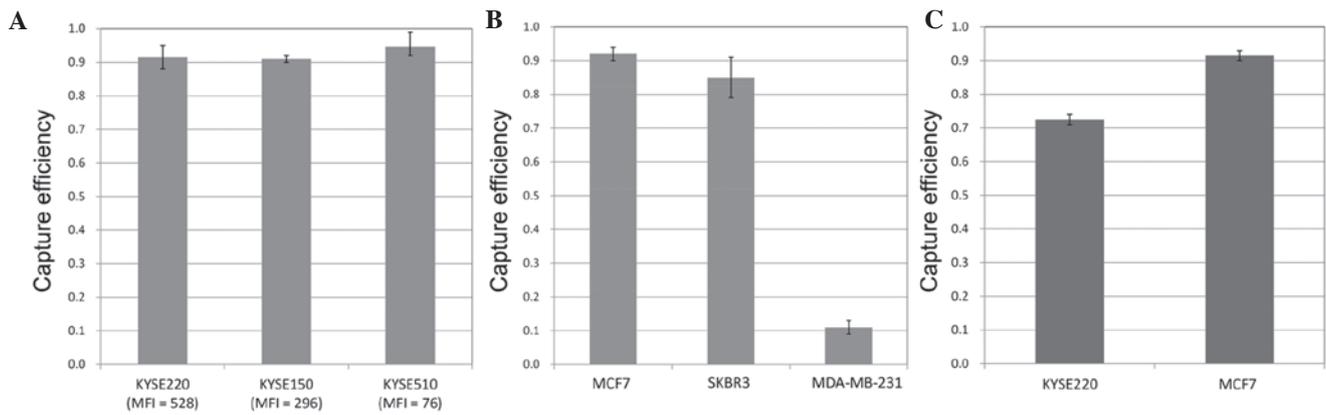
Figure 2. Capture efficiencies of the cancer cell lines by the polymeric circulating tumor cells-chip in phosphate-buffered saline (PBS) or whole blood. (A) Esophageal cancer cell lines in PBS. (B) Breast cancer cell lines in PBS. (C) KYSE220 and MCF7 in whole blood.
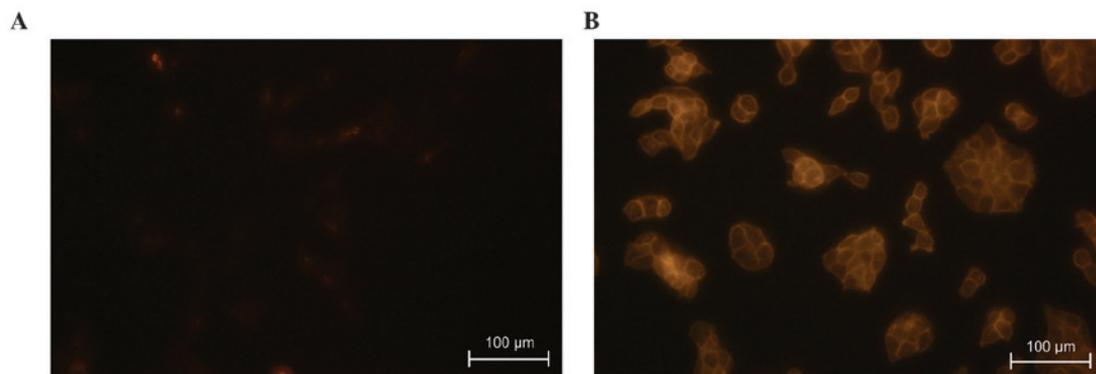


Figure 3. Fluorescent images of immunostained cells using mouse anti-epithelial cell adhesion molecule (EpCAM) antibody and Cy3 goat anti-mouse immunoglobulin G antibody. (A) MDA-MB-231. (B) KYSE510.
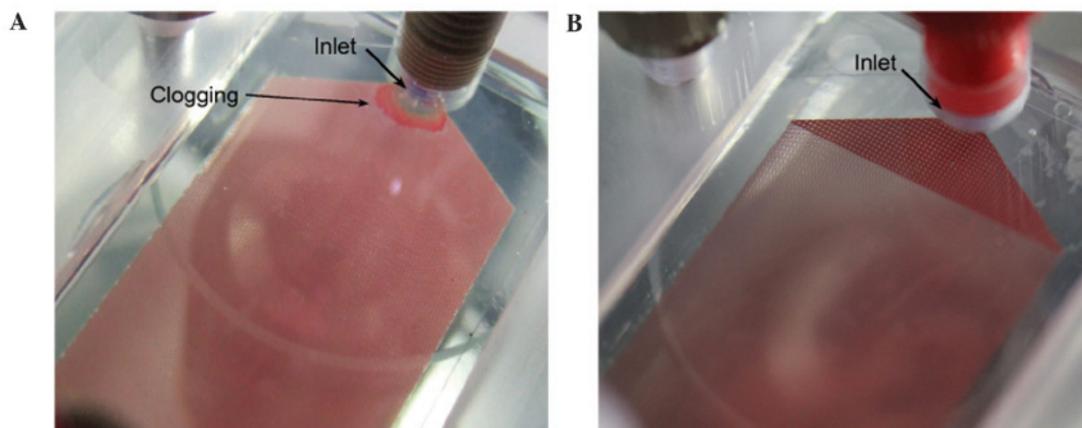


Figure 4. Images of whole blood passing through the polymeric circulating tumor cells-chip. (A) Previous chip clogged with whole blood. (B) Modified chip with a larger gap between microposts and no clogging.

blood using other microfluidic devices or methods utilizing antibody-based capture of EpCAM (20,21,23). Recently, other microfluidic devices have targeted cell surface antigens, such as prostate-specific membrane antigen (PSMA), HER2 and epidermal growth factor receptor (EGFR) by incorporating antibodies against these antigens, and have successfully isolated CTCs from the blood of cancer patients (20,26,27). MDA-MB-231 cells have been reported to be captured efficiently by a microfluidic device using anti-EGFR antibody, and this modification may be effective in the polymeric CTC-chip and easy to apply. Various antibodies are able to be simply attached to the chip surface by initial anti-IgG antibody bonding. However, these cell surface antigens are only able to target a narrow range of cancer type, such as PSMA to prostate cancer, and are known to change expression by EMT; therefore, universal targets for efficient capture of a wide range of CTCs

are still required. Much challenging work is still required to find ideal markers for the identification of cancer cells, which may subsequently be used as targets for capture (6).

Although whole blood samples caused the polymeric CTC-chip to clog in our previous study, whole blood was able to pass through the modified chip to successfully evaluate the efficiency of cell capture. This change was due to enlargement of the gap between microposts in the modified chip. Shear stress at the interface between the microposts and whole blood sample decreased by gap enlargement and local instances of blood coagulation appeared to be reduced, resulting in reduction of clogging.

As determined in the present study, capture efficiency may be generally lower in the whole blood compared to PBS. However, further studies must be carried out to clarify this issue, due to efficiency being influenced by numerous factors, such as viscosity, adsorption of cells and proteins, leading to complications.

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