Mouse Models for MEMS (Micro-Electro-Mechanical System) Based Biomarker Discovery: A Novel CTC Mouse-Trap

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INTRODUCTION

Prior to clinical patient testing, drug development programs often involve animal models as a means of determining and optimizing treatment effects on tumors. More recently, these programs have also focused on recovery of circulating tumor cells (CTCs) with the goal of providing a non-invasive means of disease detection and prognosis in response to therapy. However, the technical challenges associated with recovery and analysis of CTCs is significant. As result, new technologies for CTC recovery are in demand. The combination of micro-fluidics and the MEMS (micro-electro-mechanical system) device offers an alternative method for CTC capture and analysis. Our group has combined antibody attachment chemistry and micro-fluidics in the development of a unique MEMS-based rare cell recovery platform (CEE, Cell Enrichment and Extraction). We propose a novel approach which combines utilization of a mouse model system and CEE for investigating the role of CTCs as a biomarker.

CEE™ is a single use device that fits onto a standard microscope slide designed to either enrich cell populations or capture rare cells based on a cell-specific antibody-antigen reaction (Figure 1). The cells of interest are captured by the antibody while the remaining unwanted cell population is allowed to flow through the device for collection.

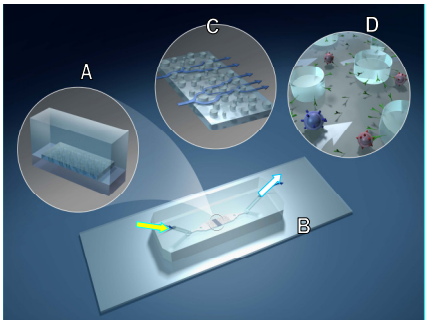

Figure 1. Enrichment using the CEE. The device is small and contained within PEG molding (A) which fits on microscope slide (B). Mathematically modeled flow rate and channel placement (C) maximize cell capture. Targeted antibodies are suspended on a surface covering the base and posts to selectively attach to target cells, creating an enriched cell sample (D).

METHODS

Female athymic nude mice were injected intraperitoneally with HeyA8 (1.25 x 10^6 cells/ml), SKOV3ip1 (5 x 10^6 cells/ml) and A2780-PAR (5 x 10^6 cells/ml) ovarian cancer cells (n=10 mice per cell line). Once tumors were palpable (approx 17-25 days after inoculation), whole blood specimens (200-500ul) were obtained from the tail vein. Each blood specimen was directly applied to an EpCAM coated CEE device. Visual confirmation of captured CTCs (human) was based on pan-cytokeratin staining and fluorescent in situ hybridization (FISH) using human-specific probes. Captured mouse cells (non-specific; stain negative) were also quantified. Observers were blinded during analysis.

RESULTS

A range of 7 to 37 tumor cells were recovered in blood of all inoculated mice tested. No human tumor cells were detected in control (tumor-free) mice. Moreover, non-specific cells in both control and inoculated blood-samples ranged only from 10 to 50 mouse cells. Representative recovery is shown in the table below.

Mouse#	Tumors Present	Results
3	NO	0 Human cells, ~ 50 mouse cells
4	NO	0 Human cells, ~ 50 mouse cells
23	NO	0 Human cells, ~ 10 mouse cells
12	YES	37 Human cells,~ 50 mouse cells
20	YES	7 Human cells, ~ 45 mouse cells
27	YES	8 Human cells, ~ 20 mouse cells
28	YES	7 Human cells, ~ 25 mouse cells
29	YES	12 Human cells, ~ 15 mouse cells

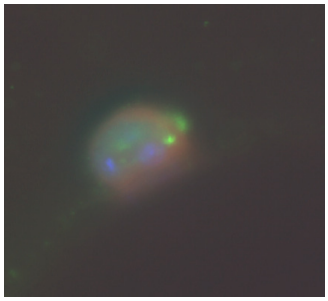

Figure 2. Simultaneous recovery and FISH detection of a human tumor cell following capture. All processes performed in the device.

CONCLUSIONS

CEE permits efficient recovery of human CTCs in mouse blood with affectively high purity. In addition, CEE is a single use MEMS device that fits onto a standard microscope slide; enabling versatility in staining options for visual interrogation of captured cells. Therefore, CEE in combination with animal models offers a new approach in defining the significance and role of CTCs as a biomarker.

The principle of the CEE™ process is the inverse of conventional magnetic beads used to capture target cells. With conventional methods, antibody-labeled beads pass through cells in suspension. In contrast, cells are passed through an immobilized matrix (channels) within the device. In addition, CEE™ design uses nanotechnology that is well known in the engineering electronics field. The design concept is a mathematically modeled series of columns that are designed to create a randomized flow pattern. The chaotic flow increases the likelihood of cell antibody interaction by maximizing the distance traveled between the inlet and exit sample ports. As demonstrated, cells captured within the device remain suitable for molecular diagnosis as either intact cells (for fluorescence in situ hybridization and immunohistochemical staining) or lysis (for DNA, RNA, proteins).

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