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Development of Glass-Beads Filters for the Isolation, Culture, and Re-Collection of Cancer Cells from Blood

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Abstract

Circulating tumor cells (CTCs) are tumor cells that are considered to originate from primary cancer sites, go into the blood stream in the body, and metastasize to the other organs. Herein, we report on a simple and convenient method to trap, culture, and recollect cancer cells, the sizes of which are greater than that of normal hematologic cells by the use of glass-beads filters (GBF) having a diameter of 24 mm and a thickness of 0.4~1.2 mm, which were prepared by sintering round-shaped glass beads (diameter: 63–106 μm). A small integrated glass-beads filter (iGBF) with a diameter of ca. 9.6 mm for the use in filtering a small volume of blood was also designed and prepared. It was possible to efficiently capture mouse Lewis lung carcinoma cells expressing green fluorescent protein spiked in saline/blood by single and repeated (circulation) filtrations. In addition, we successfully captured B16 CTCs from the blood of a B16 melanoma metastasis mouse model by iGBF and grow them on/in iGBF. Filtration by GBF had negligible effect on the adherent and proliferative characteristics of cancer cells. We believe that the GBF protocols afford easy and efficient methods for early and convenient diagnosis and treatment of cancer and related diseases.

Keywords:

Glass-bead filter,

Filtration;

Circulating tumor cells;

Size based separation.

1. Introduction

Circulating tumor cells (CTCs) are tumor cells that are found in the peripheral blood of cancer patients.[1-4] It is reported that CTCs come out from the primary cancer tissues into the blood stream, travel around in the body, and then are arrested at other organ sites apart from the original cancer sites, resulting in the metastasis. It is assumed that such metastasis that causes 90% of cancer-associated human deaths could be detected, predicted, and/or managed if CTCs could be captured from the blood of cancer patients, analyzed, and used in drug testing.

Currently used methods for detection and capture of CTCs from whole blood mainly include biological and physical methods. Biological approaches include the capture of CTCs based on the expression of tumor specific antigens such as epithelial cell adhesion molecules (EpCAM), cytokeratins and so on. However, the limited number of such specific antibodies and cancer heterogeneity during epithelial-to-mesenchymal transition (EMT) hampers biological approaches as a less reliable approach.[4] On the other hand, physical methods for CTC capture are based on differences in biophysical properties such as hardness (deformity), size and shape between CTC and hematologic normal cells. It has been reported that the sizes of tumor cells are generally larger than those of hematologic cells, suggesting that the use of a size-based method for the capture of CTC using filtration systems might be a more convenient approach than biological-based methods. In this context, the use of microdevices such as microfilters, microfluidics, microsieve chips, and micropinching chips have been reported for use in the size-based enumeration and enrichment of CTCs.[5] To our knowledge, however, there is a few method that can treat a large volume (μL~L order) of non-diluted and/or diluted blood samples which isolates live cancer cells. Therefore, convenient and easy methods for isolation, growth, and re-collection of CTCs are highly required.

Herein, we report on new methods for detection of CTCs by using a particle size analyzer and PC software based on the size difference between normal blood cells and cancer cells (Figure 1).[6] For capture, growth, and re-collection of CTC, we have developed a new system using glass-bead filter (GBF) and small integrated GBF (iGBF), which possess suitable porous structures so that smaller normal hematologic cells such as red blood cells (RBC) to pass through the filters and larger cells such as cancer cells are trapped on/in the filters.[7] In this paper, these methods are reported.

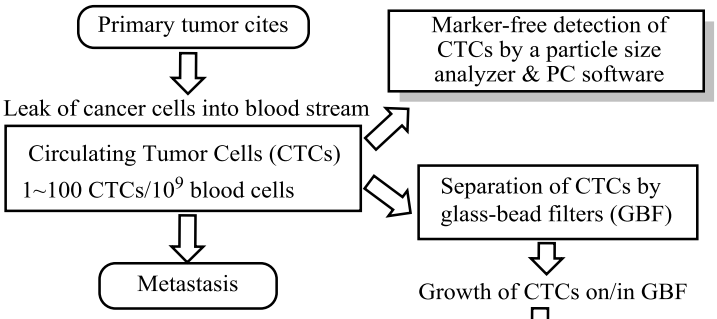
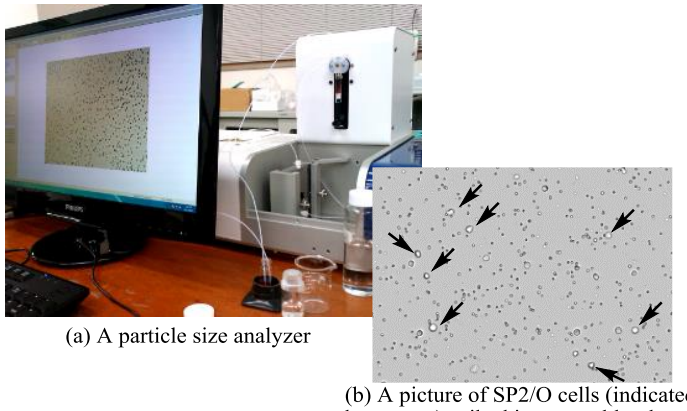


Figure 1. Convenient methods for detection, separation, growth, & re-collection of circulating tumor cells (CTC)

2. Marker-free detection of cancer cells in blood by a particle size analyzer and PC software "CCF". [6]

For the marker-free detection of cancer cells from hematologic/normal cells in blood, we have focused our effort on 1) the detection of cancer cells based on their size and shape and 2) the recognition of cancer cells based on cytomorphological deformity (surface contour). Namely, a Jasco-Occhio particle size analyzer IF nano-200 was employed for the comprehensive measurement of various size and shape parameters of cells (Figure 2). For the physical characterization of cells, a suspension of cells was introduced into a flow cell equipped with a high definition CCD camera that captures images by using stroboscopic illumination. The captured images are automatically analyzed for shape and size parameters by Particle Image Analysis Software that had been installed in particle size analyzer.[6]

In this work, SP2/O myeloma cells were chosen as the test example, because the results of our previous experiments suggested that the average inner diameter of SP2/O cells is 11.5 μm, which is greater than that of mouse red blood cells (4.4 μm on average).[4] Figure 3 displays a representative two-dimensional scatterplots of mouse blood containing SP2/O cells (the cell suspension in blood was diluted 51 X with PBS for the analysis to include 5.1 X 10^9/mL red blood cells (RBC) and ca. 2 x 10^4 SP2/O cells/mL) with respect to ISO inner diameter, which is a size parameter, on X-axis and ISO circularity, which is a shape parameter, onY-axis (each small dot in the scatterplot corresponds to a cell (particle)), suggesting that SP2/O cells and other cancer cells have greater inner diameters (≥10 μm) than those of normal cells.



(a) A particle size analyzer

(b) A picture of SP2/O cells (indicated by arrows) spiked in mouse blood taken on a particle size analyzer

Figure 2. (a) A particle size analyzer for discrimination of cancer cells from normal blood cells. (b) A picture of S/PO cells spiked in mouse blood.

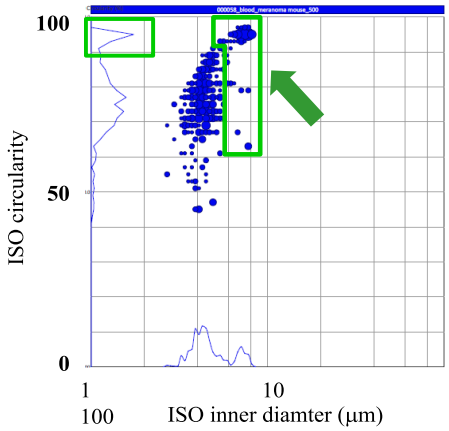


Figure 3. Two dimensional scatterplots of mouse blood containing cancer cells (SP2/O cells) with respect to ISO inner diameter (horizontal axis) & ISO circularity (vertical axis). Plots of SP2/O cells are indicated in a green box indicated by a green arrow.

It has been reported that the size of some white blood cells (WBC) is similar to that of cancer cells. In our study, we observed one WBC having an inner diameter of 10.3 μm among 777 particles (inner diameters of 4-50 μm). This information prompted us to develop a new computer software program "Cancer Cell Finder (CCF) ver. 1.0" to distinguish WBCs (inner diameter of >10.3 μm) and other normal cells from cancer cells using images obtained from the particle size analyzer. Deformity in cancer cells in contrast to normal cell is widely known and some reports have

linked the deformity in cancer cells with their metastatic propensity. In Figure 4, a WBC cell exhibits bright area in the center and darker area on the edge and smoother surface, while darkness curves of LLC are more complicated. Based on this observation, the numbers of stationary points (SP), at which the gradient of a darkness/brightness curve is zero, were counted for discriminating cancer cells from normal blood cells based on the following algorithm: 1) Prepared binarization (black and white) images of the microscopic images (or images from the particle size analyzer) of the cells with a given threshold. 2) Based on the obtained binarization images, cell areas were extracted and their center of gravity determined. 3) The defined two cross sections passing through the center of gravity of each cell (plain and dashed lines and respective luminance (darkness) functional curves of sections were obtained. 4) The total number of SP were counted on the aforementioned two luminance curves, based on which the cells were classified into two categories: i) a cell having SP number of less than or equal to 10 (≤ 10) is considered as a non-cancer cell, and ii) a cell having SP number greater than 10 (> 10) is considered as a cancer cell. The probabilities of "CCF ver. 1.0" for detecting normal cells such as WBC and lymph node cells was 83% and 100%, respectively, and for cancer cells, such as SP2/O cells and LLC cells, was 95% and 100%, respectively, as summarized in Table 1.

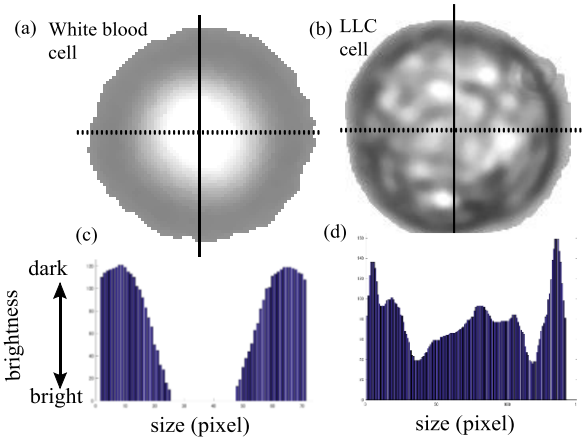


Figure 4. Particle size analyzer images of white blood cell (a) and LLC cell(b). The brightness functional curves of sections, which pass through the center of gravity of cells for white blood cell (c) and for LLC cell (d)

Table 1. Stationary poins observed for cancer and non-cancer cells as analyzed by the CLE value point method of "CCF ver. 1.0"

		Total cells	The number of cells that have >10 CLE value points	Probability of detection (%)
Normal cells	WBC	42	7	83 (as normal cells)
	Lymph node cells	42	0	100 (as normal cells)
Cancer cells	SP2/O	42	40	95
	LLC	42	42	100
	A549	41	41	100
	B16	42	41	98
	Colon-26	36	36	100
	HeLa	35	30	86
	Jurkat	42	21	50
	K562	42	41	98
	MC38	40	40	100
	MDA-MB-157	42	42	100
	MDA-MB-468	40	27	68
	Molt-4	42	10	24

3. Size-based separation of cancer cells from blood, and their growth and re-collection by means of glass-bead filters (GBF)[7]

The former GBFs were prepared by sintering (650–700°C for 1 h) commercially available round-shaped glass beads (diameter: 63–106 μm) to produce well-defined pores with an adequate porosity has a diameter of 24 mm and thickness of 1.2 and 0.4 mm, with a diameter of ca. 9 mm and thickness of ca. 2 mm (Figure 1). based on our assumption of the size threshold between cancer cells and hematologic cells. The entrapment of *in vitro* cancer cell lines and CTCs from an experimental model blood and from model mice using GBF and iGBF by a single filtration assembly and repeated (circulated) filtration system is also described. Furthermore, we also report that the cancer cells that were captured on/in GBF and iGBF could be further grown and re-collected by using the specific holder (designed especially for the culture and recollection of the trapped cancer cells). [7]

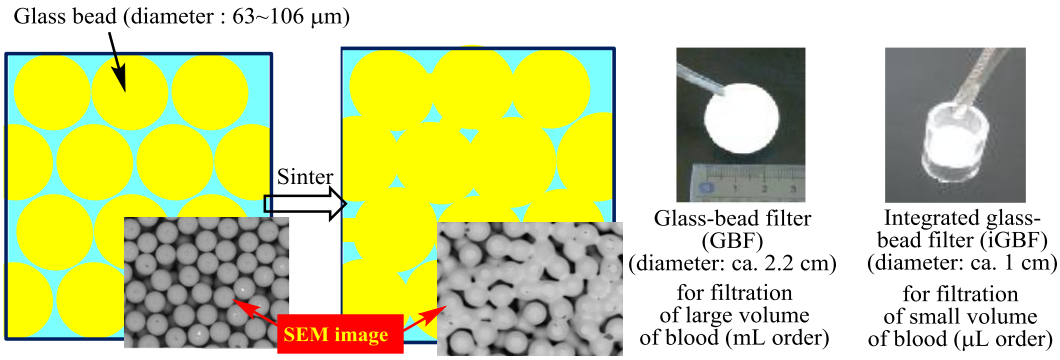


Figure 5. Preparation of glass-bead filter (GBF) by sintering glass beads

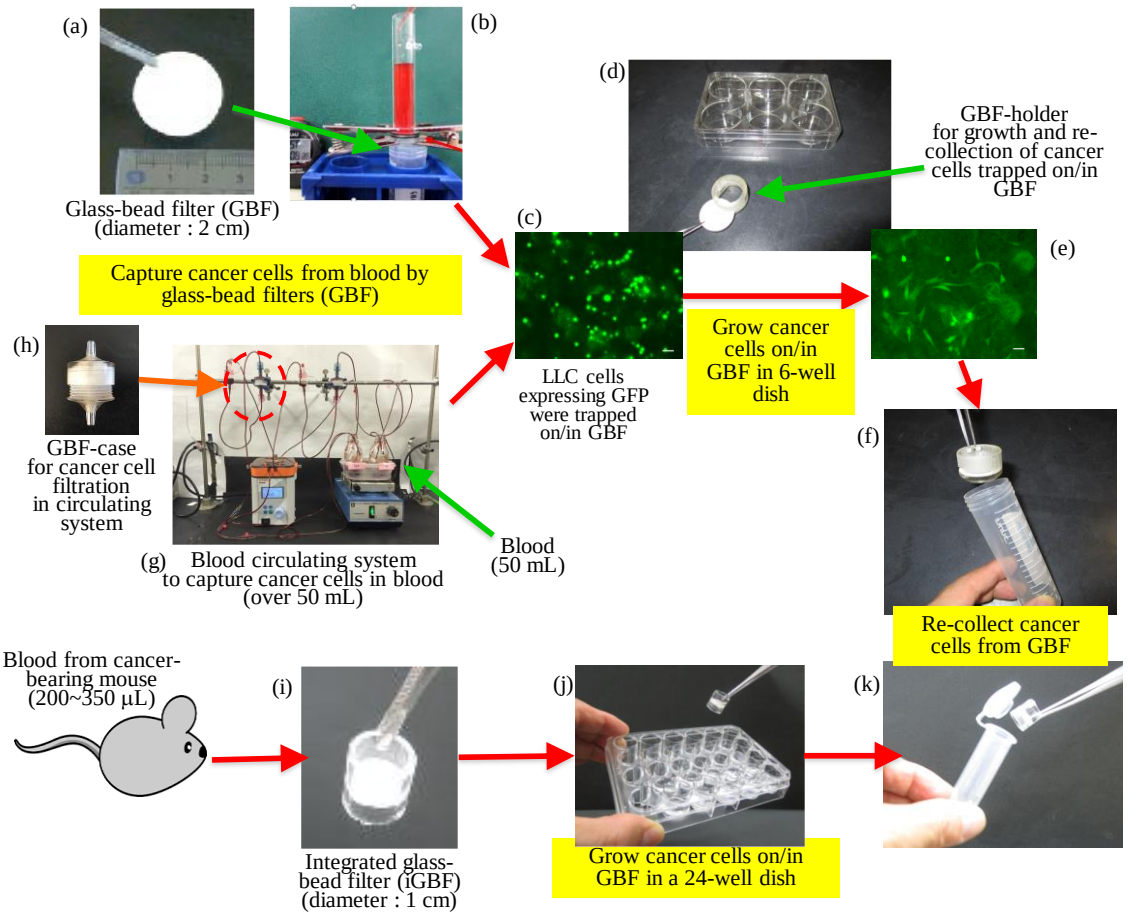


Figure 6. GBF-based methods for separatation, growth, and re-collection of CTC

4. Summary

In summary, we report on the convenient methods for detection, separation, growth, and re-collection of CTC in this paper. A convenient software program "CCF ver. 1.0" was developed to distinguish cancer cells from normal cells based on differences in the luminance (darkness) extent of curve of the cell surface. It is suitable for differentiating adherent cancer cells from WBCs and splenocytes, thus making it attractive for use in hospitals, medical facilities and laboratories, thereby overcoming the need for expensive CTC detecting devices. An analysis of blood samples (diagnosed with cancer) with a particle size analyzer or more simple and cheaper instruments assisted by a cell-recognition software such as "CCF" provides insights into possible clinical applications of our combination approach for detection CTCs and CTC clusters for evaluating the progression and theranostics of cancer and related diseases. The samples of these methods do not require any major preprocessing (e.g. cell fixation) and can be operated with any diluent, thereby permitting the

viability of the target cells to be maintained for *in vitro* establishment and for further characterization, contrary to immunological based detection which requires cells to be fixed.

5. Acknowledgements

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