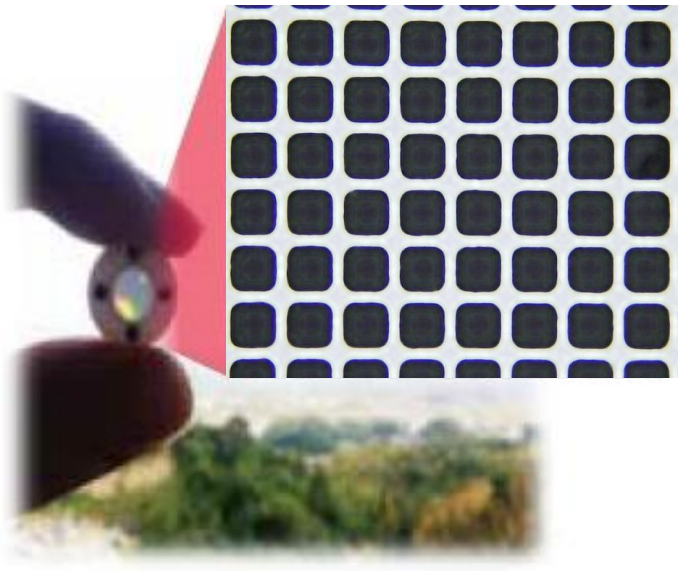
Application note Vol.2

Under Development

Specification and appearance are subject to change without notice

CELLNETTA MZM1 SERIES

Fractionation of cell clusters and single cells —Application to CTC research—



Background

There are two types of circulating tumor cells (CTCs) that circulate in the bloodstream: single cells and multicellular groupings called clusters. There has been more focus on the difference in metastatic potential of cancer between these two types. Here, we will introduce a case study of using CELLNETTA for the fractionation of simulated single-cell CTCs and cluster CTCs prepared using floating culture with fluid shear stress, conducted by Dr. Manabu Maeshiro and Associate Professor Satoru Shinriki of the Department of Molecular Laboratory Medicine at the Faculty of Life Sciences of Kumamoto University.

*The component cells of clusters fractionated using CELLNETTA have been shown to efficiently form CTC clusters in mouse blood.

(Maeshiro, M., Shinriki, S., Liu, R. et al. Colonization of distant organs by tumor cells generating circulating homotypic clusters adaptive to fluid shear stress. Sci Rep 11, 6150 (2021). doi: 10.1038/s41598-021-85743-z)

Implementation method

[CELLNETTA processing method]

(1) Culture a human oral squamous cell carcinoma cell line (SAS) of 3.0×10^6 cells in 15 mL of medium (15 mL centrifuge tube) in a rotary suspension culture.
(2) Apply hydrophilic treatment to the CELLNETTA. *1
(3) Process the cell suspension in portions not exceeding 2.0 x 10^5 cells using a 20 µm mesh CELLNETTA.
(4) Recover the cells trapped by CELLNETTA through backwashing.
(5) Repeat steps (2) to (3) five times to process the entire suspension.
(6) Recover and combine all the flow-through, and further process it using a 15 µm mesh CELLNETTA in the same way as in steps (2) to (4), dividing it into five portions.

[Method of analysis]

Photograph the mesh section of CELLNETTA under a microscope (×100) and measure the number of cell clusters and single cells trapped by the mesh.

Evaluate five visual fields for each of A, B, and C (Figure 1), and calculate the average. Analyze the flow-through in the same way by evaluating three visual fields for the 20 µm mesh and one visual field for the 15 µm mesh.

*1 For more information, please refer to the “Hydrophilic Treatment Manual” in the CELLNETTA User Guide.

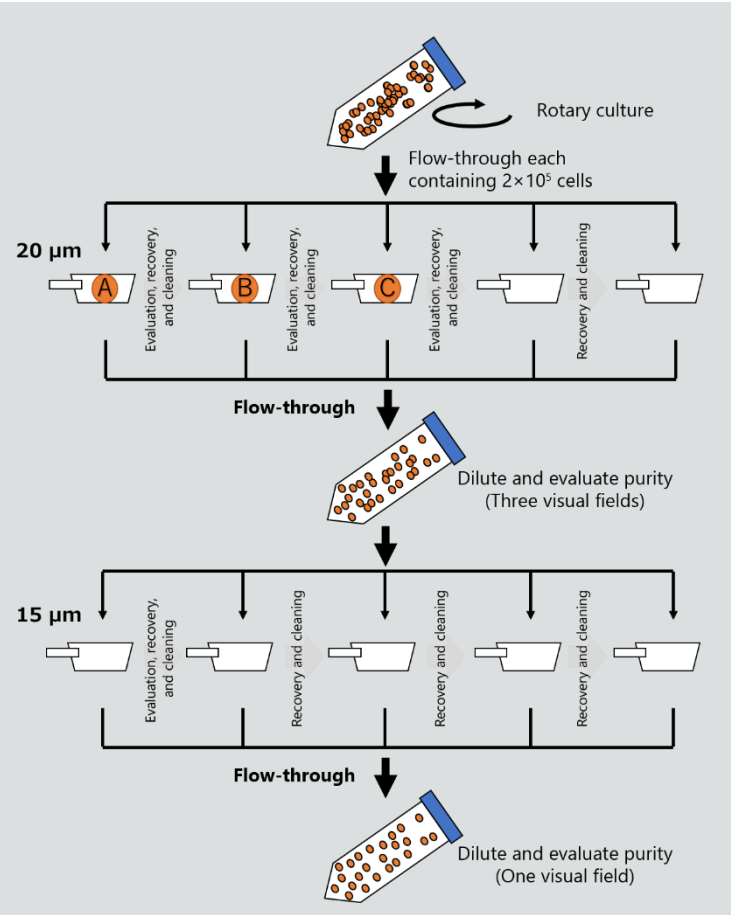


Figure 1: CELLNETTA operation procedure

Results

The abundance of cell clusters trapped using the 20 µm mesh was approximately 78% (Figure 2A). However, flow-through also contained approximately 34% clusters, the majority of which were doublets (Figure 2B). Flow-through was subsequently processed using the 15-µm mesh to ensure the purity of single cells. As a result, only approximately 4% clusters were observed in the flow-through, almost all of which were single cells (Figure 2C). When the cell clusters trapped using the 20 µm mesh were recultured, no issues in cell adhesion or cell growth were found (Figure 3).

These results suggest that cells trapped using the 20 µm mesh and the cells trapped from flow-through using the 15 µm mesh are suitable for analysis as cluster cells and single cells, respectively.

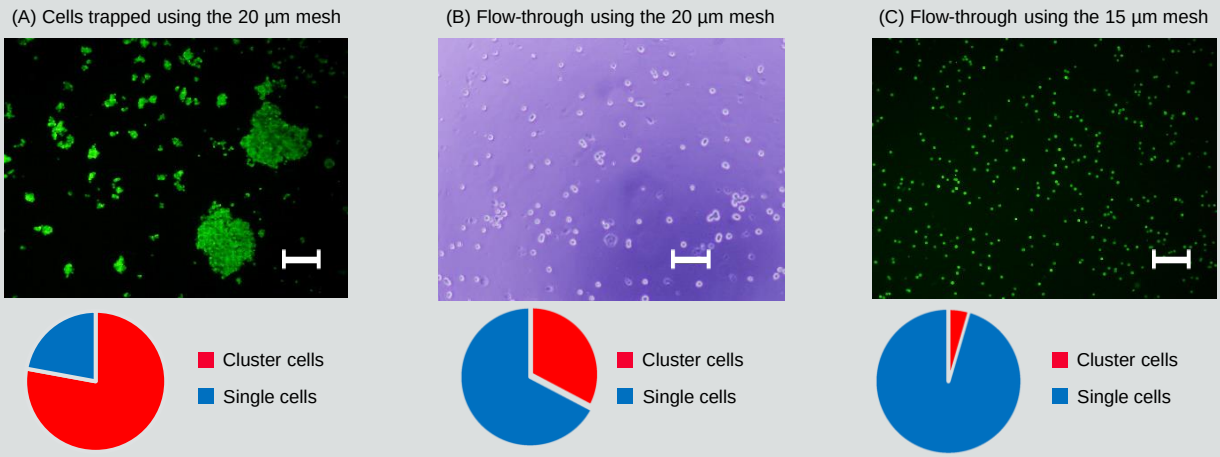


Figure 2: Micrographs of the cells obtained in each operation and the abundance of clusters. After 12 hours of rotary suspension culture, the abundance of cell clusters trapped using the 20 µm mesh was approximately 75%. The flow-through using the 15 µm mesh consisted almost entirely of single cells, with approximately 4% cluster cells. Cluster abundance (%) = 100 × (clusters)/(single cells + clusters). The scale bar is 100 µm.

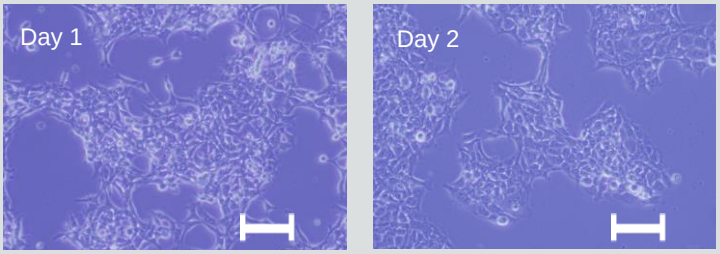


Figure 3: Recultured cluster cells that were trapped by the 20 µm mesh after three hours of rotary suspension culture. No problems with cell adhesion or cell growth were found. The scale bar is 100 µm.

Product used in this application note

Pore size	Gamma Irradiation	Product number (P/N)
15 µm	Gamma Irradiated	MZM1B015B50G
	Non-Gamma Irradiated	MZM1B015B50N
20 µm	Gamma Irradiated	MZM1B020B50G
	Non-Gamma Irradiated	MZM1B020B50N

Notes

- This product is not a medical device.
- This product is a sample for evaluation purpose.
- Please do not ship out your completed product with the sample.
- We shall not be liable for any claims on the sample in case it is shipped out to the market.

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