Electronic Supplementary Material (ESI) for RSC Advances.

[Supplementary Information]

Enhancement of isolation sensitivity for the viable heterogeneous circulating tumor cells swelled by hypo-osmotic pressure

Jiyoon Bu,[a] Young-Ho Cho, [a*] and Sae-Won Han [b]

[a] Cell Bench Research Center,

Korea Advanced Institute of Science and Technology (KAIST)

291 Daehak-ro, Yuseong-gu, Daejeon 34141, Republic of Korea

[b] Colorectal Cancer Center, Seoul National University Cancer Hospital,

101 Daehak-ro, Jongno-gu, Seoul 03080, Republic of Korea

[*]Corresponding Author

Tel.: +82-42-350-8691 / Fax +82-42-350-8690 / E-mail nanosys@kaist.ac.kr

Contents

S1. Epithelial-to-Mesenchymal Transition Induced by TGF-β1

Epithelial-to-mesenchymal transition (EMT) induced by TGF-β1 treatment was confirmed by quantitative real-time polymerase chain reaction (qRT-PCR). We measured the mRNA expressions of Vimentin and E-Cadherin, which are well-known EMT-related markers for validating mesenchymal and epithelial characteristics, respectively [S1]. We also analyzed the expression level of Snail and Twist, which are key effectors for inducing EMT [S2]. As shown in Figure S1, expressions of Vimentin, Snail, and Twist have been highly enhanced, while E-Cadherin has decreased after 48 h of TGF-β1 treatment (10 ng/mL). Therefore, TGF-β1 treated cancer cells were regarded as mesenchymal-like CTC model in our study.

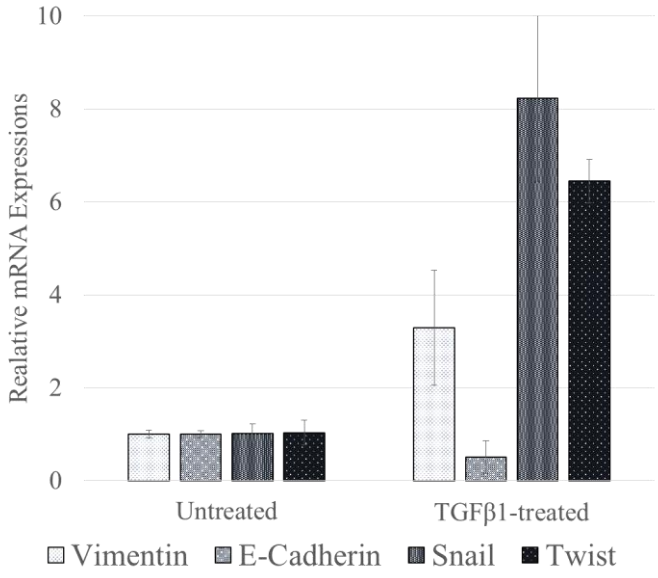


Figure S1. Relative mRNA expression of Vimentin, E-Cadherin, Snail, and Twist after 48 h treatment of TGF-β1 (10 ng/mL).

S2. Circulating Tumor Cell Isolation Using Fabric Filters

We used our mass-producible fabric filters [S3] as a representative size-selective CTC isolation devices. Figure S2 shows the scanning electron microscope (SEM) image of slots formed in between the neighboring warps and wefts on the fabric filter.

The slot sizes and its uniformity is well described in our previous work [S3]. In brief, the fabric filters are made of 20 denier polyester monofilaments (d = 57.1 ± 3.6 μm), having density of 592 epi (ends per inch) and 200 ppi (picks per inch). The warps and wefts are twilled in 2 by 2. As a result, the slot size was 7.6 ± 1.8 μm (warp to warp) × 48.2 ± 2.9 μm (weft to weft).

The entire manufacturing process of our fabric filter was conducted by Se Hong Tradings (Daegu, South Korea). Fabric sheets were prepared in ten A4-sized sheets, which was cut from the identical fabric. We cut A4-sized fabric sheets into circle (D = 20 mm) and inserted between the jigs. Top and bottom jigs were attached to the sample container and commercial 10 mL syringe, respectively. Flow was given by negatively pressure, via syringe pump. Details for the structure of our fabric filter and isolation processes are given in the paper entitled "*Lab on a fabric: Mass producible and low-cost fabric filters for the high-throughput viable isolation of circulating tumor cells*."

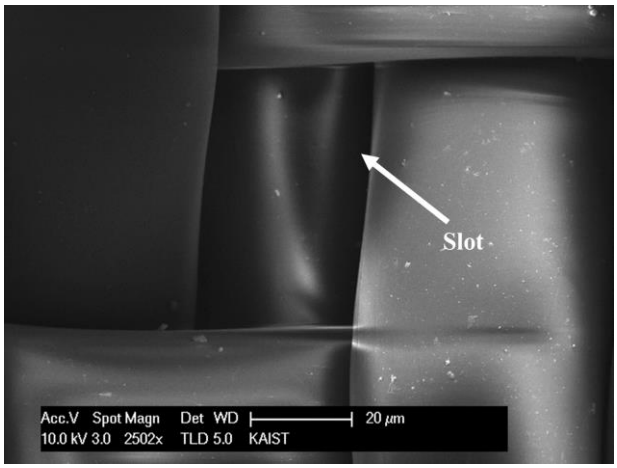


Figure S2. Scanning electron microscope image of the slot formed between the warps and the wefts of polyester fabric filters.

S3. Differentiation Index for the Cell Volume Changes

Differentiation index (DI) is widely used to evaluate whether two different groups are distinguishable or not [S4]. In most of the cases, DI value is calculated based on the real value. In our case, DI was calculated by comparing the volume increment rate of cancer cells and leukocytes after inducing hypo-osmotic swelling (normalized value), to intuitively show how the cancer cells and leukocytes swell differently under hypotonic condition. DI was defined as followed [S5]:

$$DI = \frac{U_m \cdot U_A}{L_M \cdot L_A} \quad (1)$$

where U_m, U_A, L_M, and L_A indicate the minimum value of upper level (cell type which shows higher relative size increment at the given time), average value of the upper level, maximum value of the lower level (cell type which shows lower relative size increment at the given time), and average value of the lower level, respectively. Therefore, DI higher than 1 indicates that the relative cell size increment of two groups are highly distinguishable at the given time period. DI for ECTCs versus leukocytes and MCTCs versus leukocytes were all higher than 1 in between 0 to 15 min (Fig. S3), indicating that the cancer cells swell larger and more rapidly compared to the leukocytes.

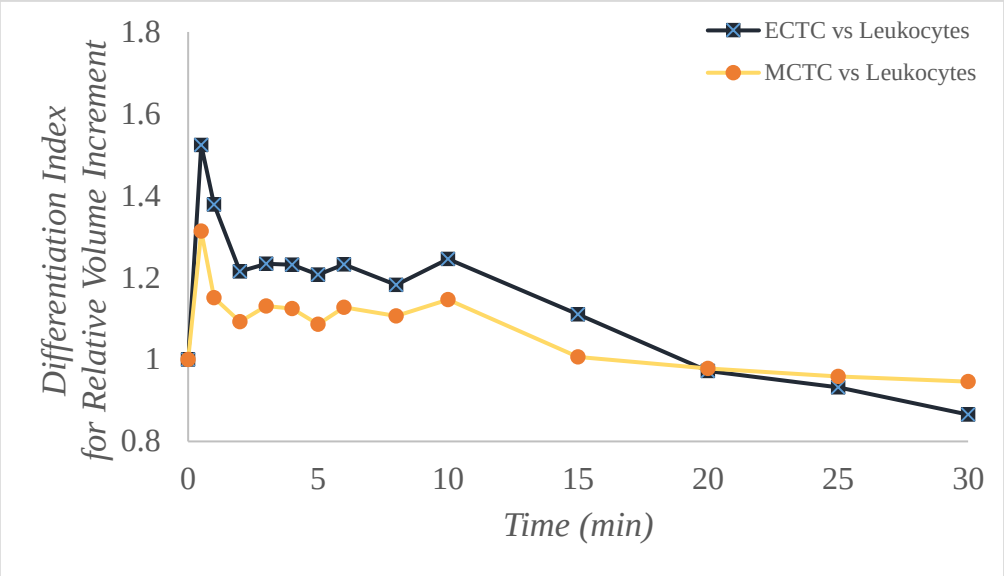
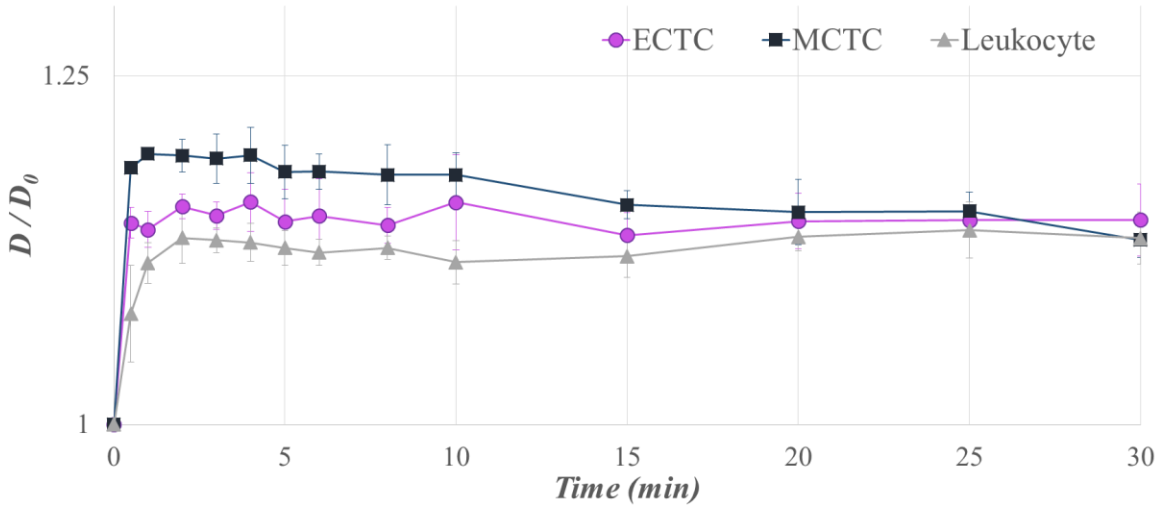


Figure S3. Differentiation indices of the cell volume changes induced by hypo-osmotic swelling (190 mOsm/kg). Note that the initial cell diameter of ECTCs, MCTCs, and leukocytes were 12.7 ± 2.2 μm, 13.7 ± 0.2 μm, and 6.7 ± 0.1 μm, respectively.

S4. Cell Size Increment in Terms of Diameter

We have modified the cell volume into diameter, since the filters are generally designed to capture cells or particles having diameters larger than the slot size (Fig. S4). As described in the main manuscript, cancer cells showed larger size increment compared to the leukocytes, with the maximum difference of 6.0 % and 9.7 % for ECTCs and MCTCs, respectively.

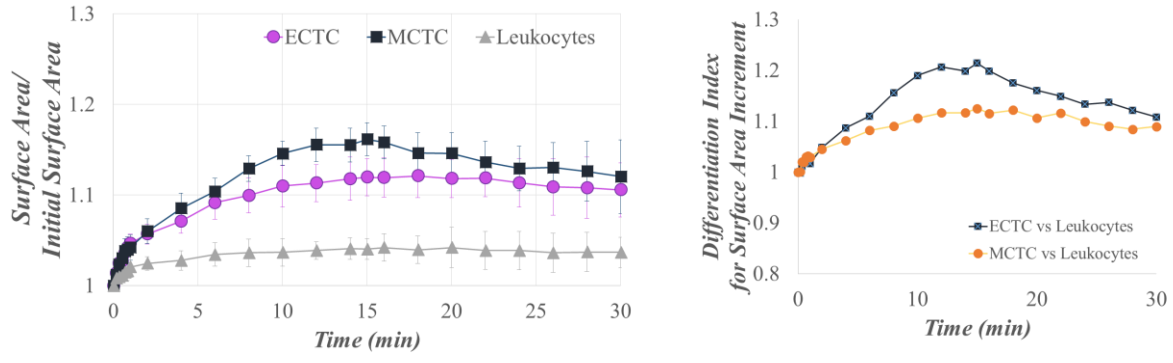


***D_0: Initial cell diameter**

Figure S4. Cell size increment due to the hypo-osmotic swelling at the osmolality of 190 mOsm/kg, expressed in terms of diameter.

S5. Cell Surface Area Increased by Hypo-Osmotic Pressure

We further analyzed the changes of cell size in terms of surface area, by acquiring and analyzing the images for 30 min after inducing hypotonic pressure. Similar to the changes in volume, the size of cancer cells increased more sharply and swelled larger compared to the leukocytes (Fig. S5A). As a result, DIs between cancer cells and leukocytes were higher than 1 between 20s to 30 min (Fig. S5B). However, there was a remarkable discrepancy with the method based on volume measurement. All three cell types show much slower increase in their surface area, compared to the volume changes obtained from cell counter. The reason for the discrepancies are mainly derived from the increase in membrane tension. Hypotonia not only increases the cell size but also causes the tension in cell membrane. Increase in the membrane tension allows cells to have more spherical geometry and causes them to detach from the bottom plate [S6. S7]. Furthermore, when deionized water is supplied to the chamber, difference in the height of the fluid results in out focusing. Therefore, surface area measurement based on image processing might not be reliable as the volume measurement. In spite of these errors, changes in surface area of cancer cells and leukocytes are still distinguishable and support our hypothesis that the cancer cells would swell larger and more rapidly.



(a) (b)

Figure S5. Changes in surface area after inducing hypotonic pressure: (a) changes in surface area for each cell type; (b) differentiation indices for cancer cells and leukocytes.

S6. Verification of CTC Isolation Enhanced by Hypo-Osmotic Swelling Using Colorectal Cancer Cells

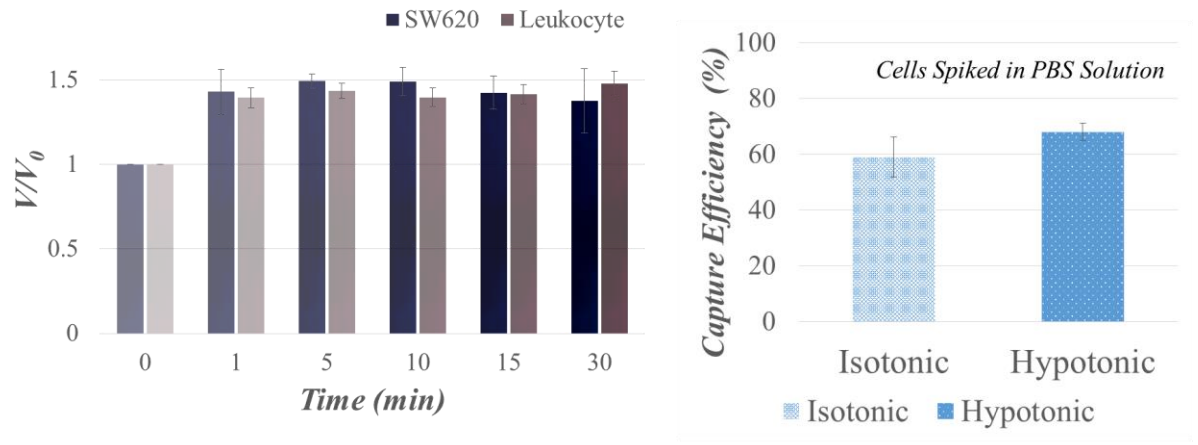
We used colorectal cancer cell line SW620, to verify that our method is not only confined to the breast cancer cell lines.

Cell Culture: Human colorectal cancer cell line SW620 was maintained in RPMI 1640 culture medium (Invitrogen), supplemented by 10% (v/v) fetal bovine serum (Gibco) and 1 % (v/v) penicillin-streptomycin (Invitrogen), and grown in humidified atmosphere (5 % CO_2) at 37 °C. Cells were passaged at 70% confluence until passage 3.

Verification of Cell Size Increment in Hypotonic Solution (190 mOsm/kg): The volume increment of SW620 cells after inducing hypotonic pressure is supported in Fig. S6A. Similar to the breast cancer cell lines, SW620 cells swelled rapidly for initial 5 min, increased their volume gradually for 10 min, and started to shrink in between 10 to 15 min. Differentiation index, compared to the relative volume increment of leukocytes, was also higher than 1 in between 5 to 10 min. Therefore, we conclude that the volume increment of SW620 was not remarkable as the breast cancer cell lines, but still swell more rapidly and larger compared to the leukocytes.

Verification of Sensitivity Enhancement: We spiked SW620 cell lines in PBS solution having osmolality of 280 mOsm/kg (isotonic) and 190 mOsm/kg (hypotonic), respectively. Capture sensitivity for SW620 cells was enhanced 1.15-fold after inducing hypotonic pressure (68.1 ± 3.2 % vs. 59.0 ± 3.2 %; $p = 0.091$). Statistical accuracy has been decreased compared to the

breast cancer cell line tests, but we can still observe a weak tendency showing differences in capture sensitivity depending on the osmolality (Fig. S4B).



(a) (b)

Figure S6. Verification of CTC isolation enhanced by hypo-osmotic swelling using colorectal cancer cell line SW620: (a) the volume increment of SW620 cells compared to the leukocytes at the osmolality of 190 mOsm/kg; (b) osmolality-dependent capture efficiency of SW602 cells spiked into PBS solution.

S7. Clinical Information of Cancer Patients

Clinical information of colorectal cancer patients involved in our study is supplied in table S1.

Table S1. Clinical information of colorectal cancer patients.

ID	Sex	Age	Location	Stage	State	Metastasis	KRAS
P1	M	59	Rectal	III	Recurred	Lung	G12D
P2	F	63	Sigmoid	I	Recurred	Liver	WT
P3	M	85	Ascending	IV	Initial State	Liver	G12D
P4	F	55	Transverse	III	Recurred	Peritoneal seeding	G12C
P5	M	57	Ascending	IV	Initial State	Lung, Bone, Lymph node	L19F
P6	M	63	Descending	IV	Initial State	Lung, Liver, Peritoneal seeding	A164T

S8. Leukocyte Contamination

The number of leukocytes for each blood sample was measured in order to quantify the effect of hypotonic pressure on purity of retrieved samples (Table S2). The number of isolated leukocytes are presented in table S2. Despite of slight increase in the number of leukocytes under hypotonic condition, the purity has been enhanced since the number of CTCs increased more sharply (Table S3).

Table S2. The number of leukocytes remained in retrieved sample after CTC isolation from 1 mL of human blood samples.

	Sample ID	**The Number of Leukocytes in retrieved sample (cells mL^{-1})**	
		Hypotonic	**Isotonic**
Patients	***P1***	348	339
	P2	316	307
	P3	237	235
	P4	220	240
	P5	287	273
	P6	331	315
Healthy Volunteers	***H1***	422	412
	H2	376	337
	H3	217	211
	H4	305	286

Table S3. The purity of retrieved samples. The ratio of retrieved CTCs compared to the total number of cells in retrieved samples were calculated for both hypotonic and isotonic condition. CTC purity has enhanced in hypotonic condition ($p = 0.011$).

	Sample ID	Purity (%)	
		Hypotonic	Isotonic
Patients	***P1***	3.33	2.59
	P2	5.11	4.06
	P3	2.47	2.49
	P4	1.35	0.83
	P5	1.37	0
	P6	2.36	1.25
	Average	2.66 ± 1.29	1.87 ± 1.33

Reference

[S1] C. S. Scanlon, E. A. Van Tubergen, R. C. Inglehart and N. J. D'Silva, *J Dent Res*, 2013, 92, 114-121.

[S2] A. G. de Herreros, S. Peiro, M. Nassour and P. Savagner, *J Mammary Gland Biol*, 2010, 15, 135-147.

[S3] J. Bu, Y. T. Kang, Y. S. Lee, J. Kim, Y. H. Cho and B. I. Moon, *Biosens Bioelectron*, 2017, 91, 747-755.

[S4] Y. Park, H. W. Kim, J. Yun, S. Seo, C. J. Park, J. Z. Lee and J. H. Lee, *Biomed Res Int*, 2016, DOI: Artn 8748023

[S5] G. Kang, Y. J. Kim, H. S. Moon, J. W. Lee, T. K. Yoo, K. Park and J. H. Lee, *Biomicrofluidics*, 2013, 7.

[S6] N. Groulx, F. Boudreault, S. N. Orlov and R. Grygorczyk, *J Membrane Biol*, 2006, 214, 43-56.

[S7] C. E. Morris and U. Homann, *J Membrane Biol*, 2001, 179, 79-102.