Enrichment of cancer-initiating cells from colon cancer cells through porous polymeric membranes by membrane filtration method

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Materials	Abbreviation	Catalog No.	Company
Polymer			
Poly(lactide-co-glycolic acid) (lactide:glycolic=75:25)	PLGA	P1941	Sigma-Aldrich (St. Louis, MO, USA)
Silk screen	Silk	170 mesh	Yuzawaya, Tokyo, Japan
Cells			
LoVo cells	LoVo cells	60148	BCRC, Food Industry Research and Development Institute (Hsinchu, Taiwan)
Cell culture dishes			
6-well tissue culture polystyrene plate	TCPS	#353046	Corning (Corning, NY, USA)
Chemicals			
2-Hydroxyethyl agarose	Agarose	A4018	Sigma-Aldrich (St. Louis, MO, USA)
High-vacuum grease	High-vacuum grease	1658832	Corporation, Midland, MI, USA
Human CEA ELISA kit	Human CEA ELISA kit	EHCEA	Thermo Fisher Scientific Inc. (Waltham, MA, USA)
Cell culture medium and component			
DMEM	DMEM	D5648-10x1L	Sigma-Aldrich (St. Louis, MO, USA) Biological Industries,
Fetal bovine serum	FBS	04-001-1A	Kibbutz Beit-Haemek, Israel
Hoechst 33342	Hoechst	PA-3014	Lonza (Basel, Switzerland)
Surface markers			
7-AAD Viability dye	7-AAD	559925	BD Biosciences (San
FITC mouse anti-human CD44	FITC anti-CD44	555478	BD Biosciences (San Jose, CA, USA) Miltenvi Biotech
PE mouse anti-human CD133/1	PE anti-CD133	130-080-801	(Bergisch Gladbach, North Rhine-Westphalia,
FITC Mouse IgG2bк, isotype control	FITC isotype	555742	BD Biosciences (San Jose, CA, USA)
PE Mouse IgG1κ, isotype control	PE isotype	555749	BD Biosciences (San Jose, CA, USA)
Nylon mesh filter (r=11 um)	NY11	NY1104700	Merck KGaA (Darmstadt, German)
Nylon mesh filter (r=20 um)	NY20	NY2004700	Merck KGaA (Darmstadt, German)

Table S1 Materials used in this study.



Fig. S1 Flow cytometry scattergrams of the cells for analysis of CD44 expression after permeation of colon cancer LoVo cells through the NYM and PLGA/silk membranes. (a) CD44 expression on the cells in the permeate solution through the NYM-11 (i), NYM-20 (ii), P-10/silk (iii), P-5/silk (iv) and P-3/silk (v) membranes. (b) CD44 expression on the cells in recovery solution through the NYM-11 (i), NYM-20 (ii), P-10/silk (iii), P-5/silk (iv) and P-3/silk (v) membranes. (c) CD44 expression on the cells migrated through the NYM-11 (i), NYM-20 (ii), P-10/silk (iii), P-5/silk (iv) and P-3/silk (v) membranes. (d) CD44 expression of LoVo cells cultured on TPS dishes. The black solid line indicates isotype antibody expression (negative control).



Fig. S2 Flow cytometry scattergrams of cells for analysis of CD133 expression after permeation of colon cancer LoVo cells through the NYM and PLGA/silk membranes. (a) CD133 expression on the cells in the permeate solution through the NYM-11 (i), NYM-20 (ii), P-10/silk (iii), P-5/silk (iv) and P-3/silk (v) membranes. (b) CD133 expression on the cells in recovery solution through the NYM-11 (i), NYM-20 (ii), P-10/silk (iii), P-5/silk (v) membranes. (c) CD133 expression on the cells migrated through the NYM-11 (i), NYM-20 (ii), P-10/silk (iii), P-5/silk (iv) and P-3/silk (v) membranes. (c) CD133 expression on the cells migrated through the NYM-11 (i), NYM-20 (ii), P-10/silk (iii), P-5/silk (iv) and P-3/silk (v) membranes. (d) CD133 expression in LoVo cells cultured on TPS dishes. The black solid line indicates isotype antibody expression (negative control).