Analysis & Sensing

Supporting Information

Isolation of Circulating Biomarkers for Liquid Biopsy using Immunoaffinity-Based Stimuli-Responsive Hybrid Hydrogel Beads

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Figures & Table

S1. An Overview of the Overall Process

The fabrication and verification of the present hydrogel beads was proceeded as follows.

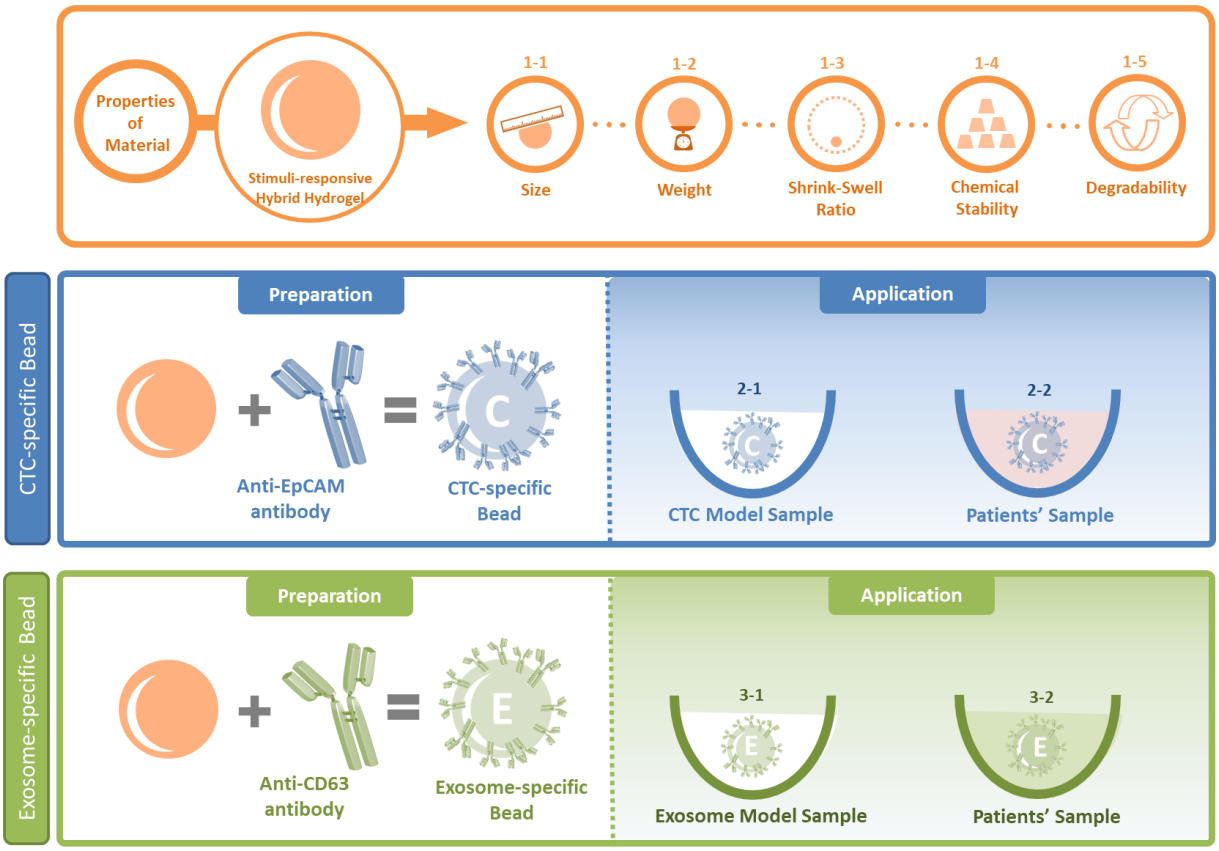

Fig. S1 An overview of the overall experiment using the present hydrogels

The process of the present study consists of three parts: (a) the property of materials such as size, weight, shrink-swell ratio, chemical stability, and degradability; (b) the preparation and sample application of CTC-specific beads; (c) the preparation and sample application of exosome-specific beads. In order to verify the clinical utility, samples from 5 patients suffered from lung cancer was applied to CTC-specific beads and exosome-specific beads, simultaneously. Lastly, the isolated and collected CTCs and exosomes were verified using in-vivo imaging system and nanoparticle tracking analysis (NTA) system, respectively.

S2. Long-Term Stability of the Stimuli-Responsive Hydrogel Beads

In order to prove the long-term stability of the present hydorgel, we conducted two types of experiment: (a) the weight of the beads was repetitively measured in the course of shriking and swelling cycles for 10 times (n=5); (b) the weight of the beads, which had been incubated in PBS solution, was measured at intervals of one month (n=5). The prepared beads were comparatively uniform and balanced despite measurement error. At first, the weight of the beads was slightly dispersed, but the value was converged after a few cycles. Considering rapid dehydration inside convection oven, we have concluded that the beads were not considerably damaged during the process. Meanwhile, the other groups were prepared for long-term incubating in PBS solution: group a (control, just-prepared), group b (1-month incubation), group c (2-month incubation), group d (3-month incubation), group e (4-month incubation), group, f (5-month incubation), group g (6-month incubation), group h (7-month incubation), group i (8-month incubation), group j (9-month incubation), group k (10-month incubation). **Fig. S2** shows the changes variation over time regarding the average weight and shrink-swell ratio. The average weight varies between 4.42 and 4.52, and the shrink-swell ratio distributed between 19.7 and 20.9, within the margin of error (**Table S1**). Therefore, we have concluded that the inherent characteristics of the beads was thoroughly preserved for at least 10 months.

Table S1. Average weight per bead and shrink-swell ratio of the present hydrogel beads during long-term incubation.

Group	a	b	c	d	e	f	g	h	i	j	k
Duration (Months)	just prepared	1	2	3	4	5	6	7	8	9	10
Avg. weight (mg)	4.51 ± 0.19	4.46 ± 0.16	4.52 ± 0.21	4.45 ± 0.16	4.46 ± 0.26	4.42 ± 0.14	4.43 ± 0.09	4.45 ± 0.10	4.50 ± 0.10	4.47 ± 0.19	4.45 ± 0.12
Shrink-swell Ratio	20.9 ± 1.5	20.1 ± 1.3	20.3 ± 1.1	19.7 ± 1.7	20.4 ± 1.3	20.4 ± 1.5	20.0 ± 0.9	20.3 ± 0.8	19.4 ± 1.2	20.9 ± 1.0	20.3 ± 1.3

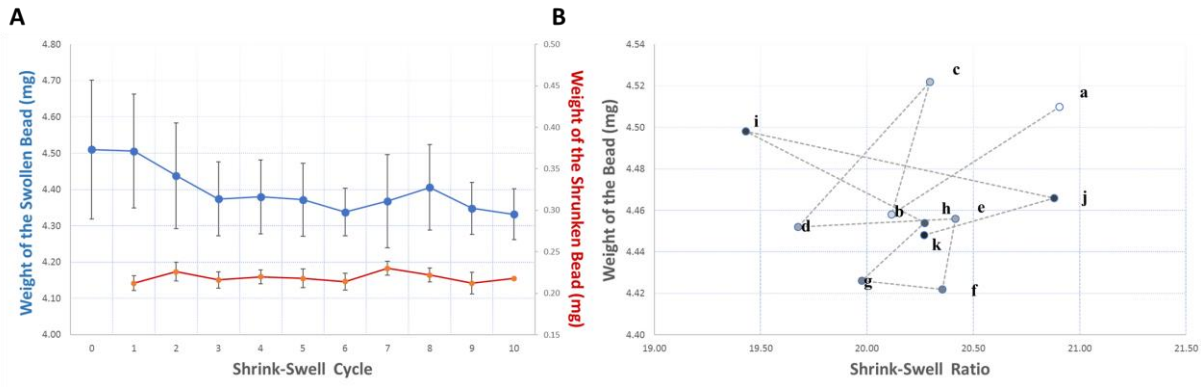

Fig. S2. Long-term stability of the present hydrogel beads. **A**) Weight of the beads at shrunken and swollen state, during 10 shrink-swell cycle; **B**) The change of shrink-swell ratio during 10 month incubation

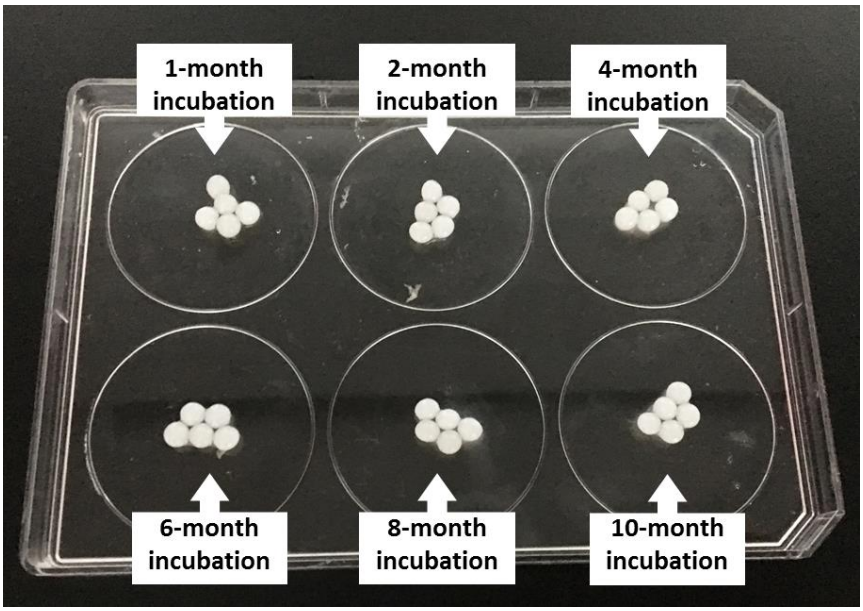

Fig. S3. Photographic image of the present beads after long-term incubation. The beads were incubated at the same condition (PBS solution, 4℃)

Fig. S4. Size of ^{LB}Beads for experiments with clinical samples

S3. FE-SEM images of the Specified Beads

Fig. S5 show the present hydrogel beads and the isolated circulating markers on their their specific beads. The size of the isolated cancer cells ranged between 15 to 20 μm, and they showed dendritic-like surface morphology (**Fig. S5B**). However, the size of the isolated WBCs was smaller than or equal to a certain size of cancer cells (between 10 to 15 μm), and showed aciniform (grape-like) morphology (**Fig. S5C**). In order to help the readers understand, we also present reengineered and colored images. The program used for this work was Adobe Photoshop CS6 (Adobe, USA). Briefly, we roughly selected the Lasso Tool in order to distinguish cell-captured region. The selected area smoothed by the function of Feather, and then made as a new layer. We utilized each color as a sort of symbol in our schematic diagram: red, blue, and green represents circulating tumor cells, white blood cells, and circlating exosomes, respectively. Therefore we filled the same color into the newly created layer, and the edges parts of the cells were carefully refined with the Erase. Lastly, we merged the modified layer with the background, and degree, magnitude, and saturation were properly adjusted. **Fig. S5E** and **Fig. S5F** show the isolated circulating tumor cell (red-colored) and white blood cell (blue-colored), respectively. They are clearly distingshed by size and shape.

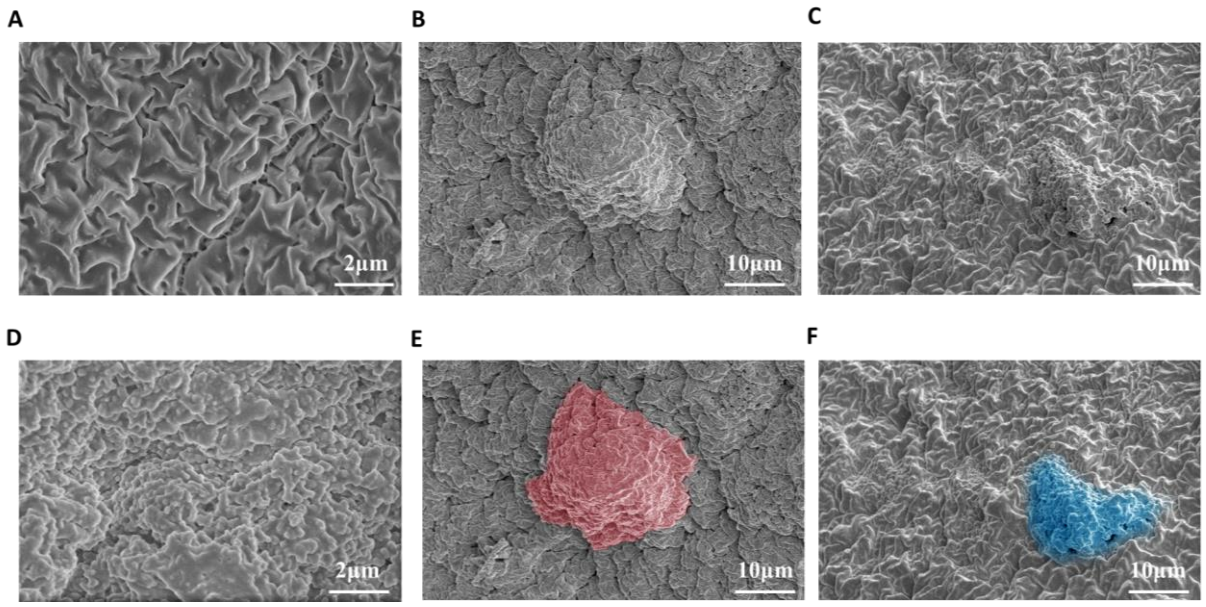

Fig. S5 FE-SEM image of the isolated cells onto the present hydrogels (^{LB}Beads). **A**) The surface of the control bead; **B**) The isolated CTCs using CTC-specific bead; **C**) The eliminated WBCs using WBC-specific bead; **D**) The isolated circulating exosomes using exosome-specific bead; **E**) Reengineered color image of (**B**); **F**) Reengineered color image for (**C**).

S4. CTC recovery performance of CTC Beads using model samples

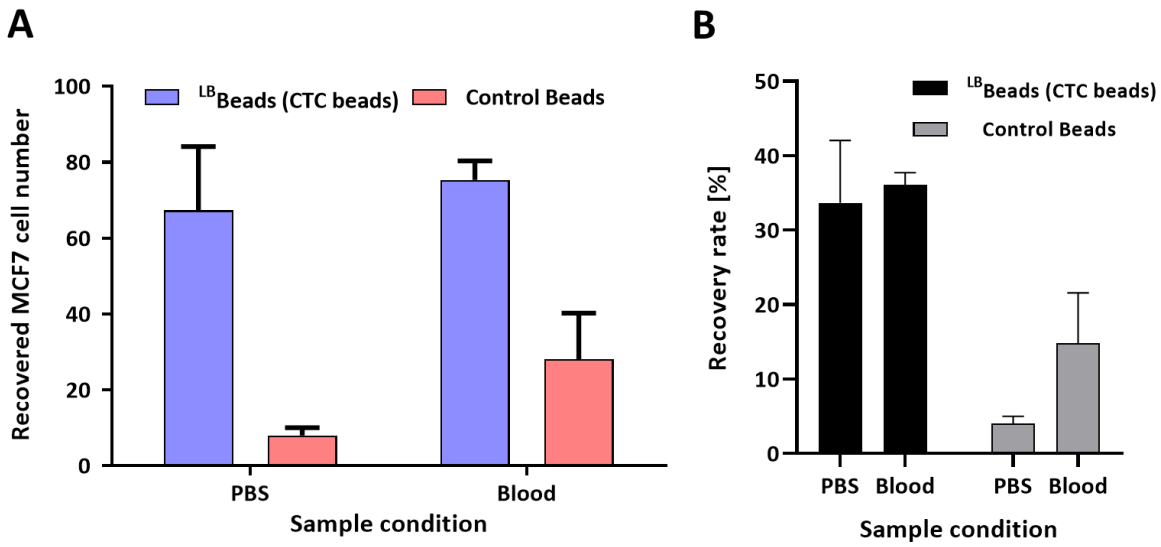

Fig. S6. CTC recovery performance of ^{LB}Beads using MCF7 cancer cells spiked in PBS and Blood

S5. Clinical sample CTC characterization using label-free CTC isolation

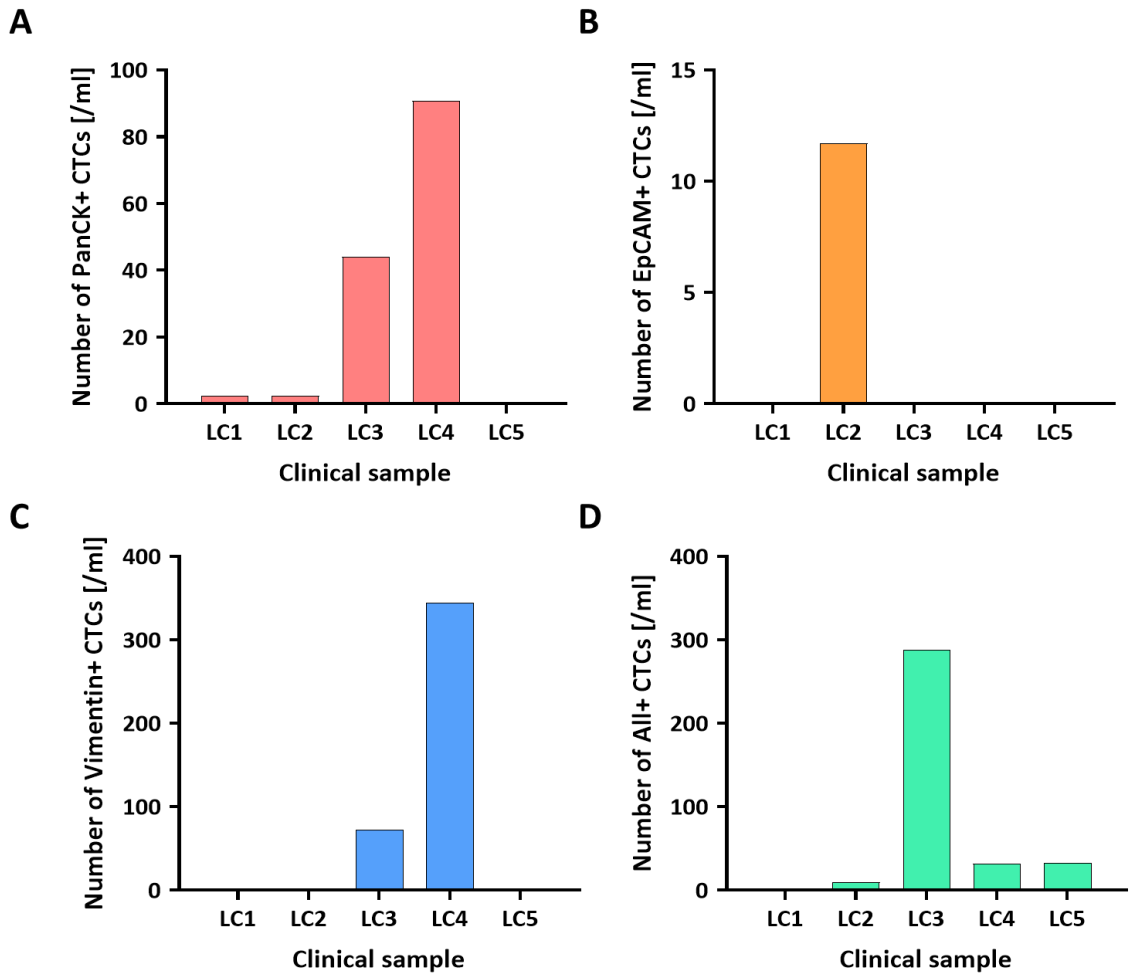

Fig. S7. Profiling of CTCs isolated using label-free CTC isolation microfluidic device, Labyrinth, stained with three different cancer-associated protein markers. **A)** pan-cytokeratin positive only; **B**) EpCAM positive only; **C**) vimentin positive only; **D**) all three marker positive.

S6. Clinical sample information

Table S2. Clinical information of patient samples

ID	Sex	Age	Location	Stage	Node status	Tumor	Adenocarcinoma subtype	Metastasis
LP1	F	61	Lung	IV	N3	T2	EGFR	-
LP2	M	58	Lung	IV	Nx	T3	Alk	Brain
LP3	F	82	Lung	IV	NA	NA	EGFR	-
LP4	M	67	Lung	IIIB	NA	NA	EGFR	-
LP5	M	66	Lung	IV	N0	T1a	EGFR	Brain

S7. Circulating marker isolation performance comparison

Table S3. The comparison table between previous circulating marker isolations and the present study

Type	Aim of study	Sample type	Processing time	Resources		Cost	Sample release	Ref
				Pump	Magnet			
Microfluidics	CTC isolation	Whole blood	~1-3h	O	O/X	Moderate (12-35USD)	X	[S1]
	Exosome isolation	Serum/ Plasma		O	X		O	[S2]
Magnetic beads	CTC isolation	Whole blood	~1.5h	X	O	Expensive (15-40USD)	X	[S3]
	Exosome isolation	Plasma		X	O		X	[S4]
Present study (LBBeads)	Dual-profiling (CTCs+Exo.)	Whole blood	~1h	X	X	cheap (~10USD)	O	-

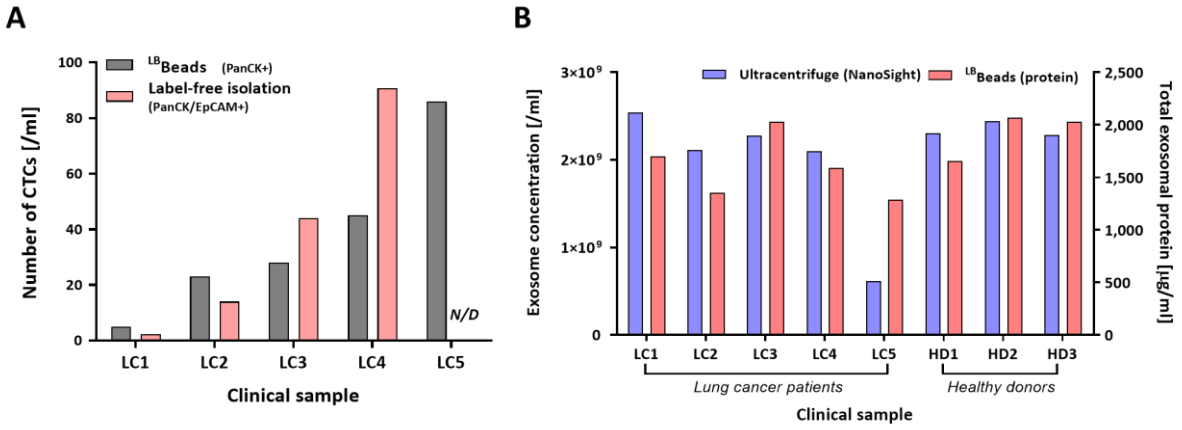

Fig. S8. Circulating markers isolation performance comparison between LBBeads and conventional methods

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