Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary information

Fully automated platelet isolation on a centrifugal microfluidic device for molecular diagnostics

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Supplementary Movies

Movie S1: Visualization of the platelet separation process on a disc. Fluidic motion on the disc during pure platelet separation was visualised using a built-in spinning disc imaging machine equipped with a strobe light and a camera. Images at specific disc positions were captured during the platelet isolation from whole blood. The obtained images were utilised to generate a movie.

Supplementary Figures

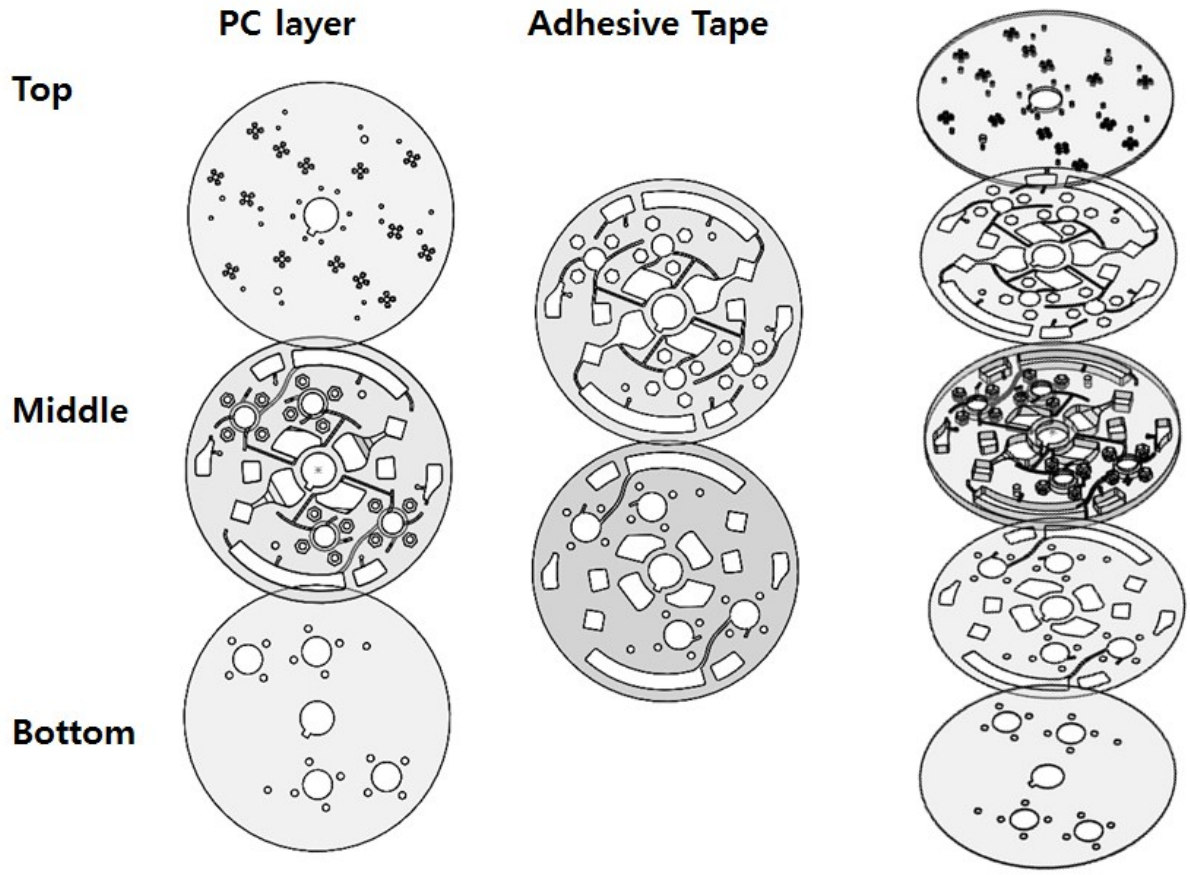

Fig. S1. Illustration of the disc assembly layers for platelet isolation. The PC layer is composed of the following: 2 mm top PC layer, 5 mm middle PC body frame, and 0.5 mm bottom PC layer. The double-sided adhesive layer is composed of 0.1 mm pressure sensitive top and bottom adhesive layers, separately in between PC layers.

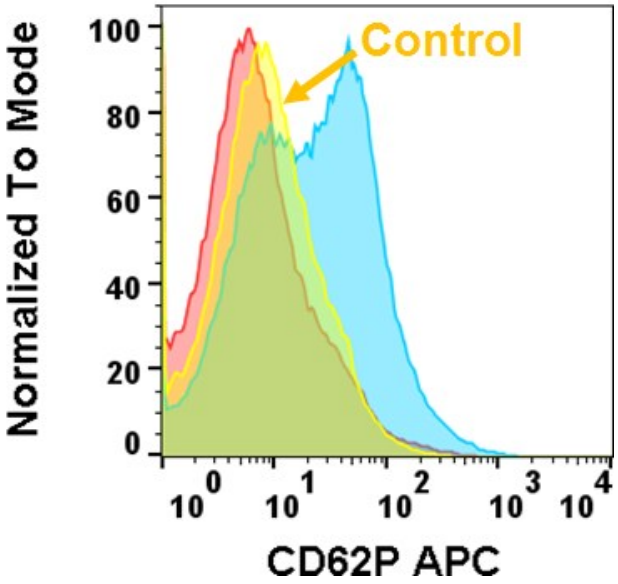

Fig. S2. flow cytometry analysis on the activation level of platelet isolated from two isolation methods. The adjunct histogram of CD62P expression for the PRP (yellow) directly immune-stained as the baseline control, the isolated platelet by disc (red), and by the centrifugation (blue). It indicates the activation of the platelet sample isolated by the disc method has no significantly different activation compared to the control, but the activation level of platelet isolated by the manual centrifugation method showed a significant shift in the CD62P peak (n=3).

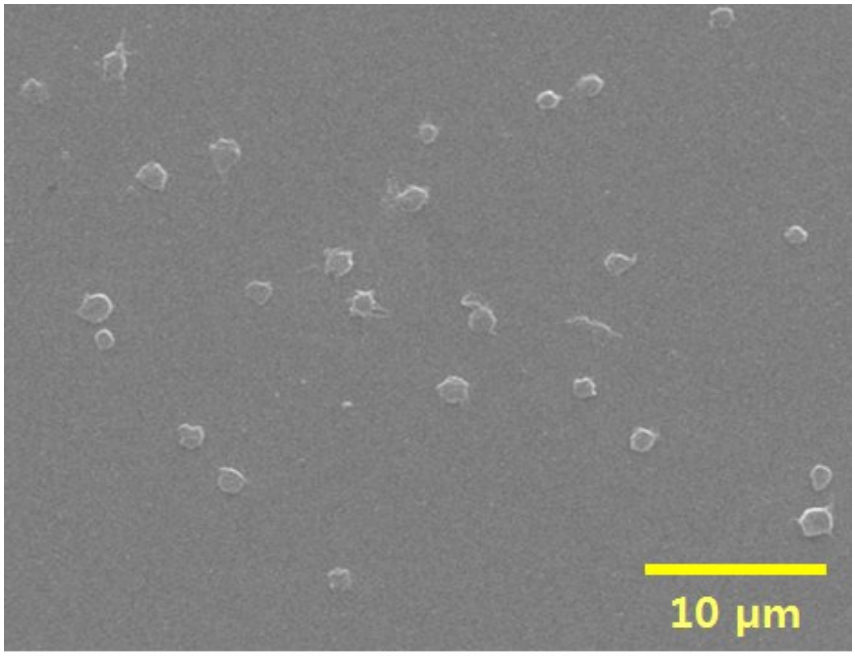

Fig. S3. SEM image of the platelets isolated by the disc.

Table S1. Operation program for the automated platelet isolation

No.	Steps	Speed (RPM)	Time (min)	Valve operation
1	Plasma (PRP) separation	3000	5	All Closed.
2	PRP transfer to filter I	1800	0.4	Valve 1 open
3	PRP transfer to filter II	2400	1	Valve 2 open
4	Washing	2400	8	Valve 1 close, Valve 3 open
5	Removal of the solution under filter II	1200	0.05	Valve 2 close, Valve 4 open
6	First elution	600	0.3	Valve 4 close, Valve 5 open
7	Elution buffer loading	1200	0.5	Valve 5 close, Valve 6 open
8	Shaking	10 Hz	0.3	Valve 6 close
9	PLT Collection	600	0.5	Valve 5 open
Total operation time			**16.1 min**	

Table S2. List of forward and reverse primer sets used for RT-qPCR of platelet RNA

Gene	5' - Forward primer - 3'	5' - Reverse primer – 3'
GAPDH	ATGGGTGTGAACCATGAGAA	GTGCTAAGCAGTTGGTGGTG
CD45	GTTTCTTAGGGACACGGCTG	TTGCTGTAGTCAATCCAGTGG
CD41	GAAGGAGAATGAGACCAGGG	TTCTTGCTCCGTATCTGCAG
CD62	TCCCAACTCCTTTGCTTCAG	CTAAGTCTGTGTAGCGATTCTGG
P2Y12*[1]	TCCATTTTGCCCGAATTCC	CAGAGTATTTTCAGCAGTGCAGTCA
PF4*[2]	TGCTGTTCCTGGGGTTGCTGC	TGCACACACGTAGGCAGCTAGTAGC

*Primer sets of P2Y12 and PF4 gene was referred to the corresponding references[1,2].

Table S3. Previous studies on platelet RNA for cancer diagnostics and/or therapy selection

Reference (year)	Blood volume (mL)	Main result
Calverley et al. (2010)[3]	45	Microarray analysis revealed the downregulation of platelet gene expression in metastatic lung cancer.
Best et al. (2015)[4]	6	mRNA sequencing of TEP RNA distinguishes cancer patients from healthy individuals with 96% accuracy.
Nilsson et al. (2016)[5]	6	RT-PCR analysis demonstrated the detection of EML4-ALK rearrangements in platelet RNA with 65% sensitivity and 100% specificity. TEP-based Crizotinib response monitoring in NSCLC patients was performed.
Best et al. (2017)[6]	4, 6, 10	RNA sequencing of TEP RNA with particle-swarm optimization algorithms resulted in accurate detection of early- and late-stage non-small-cell lung cancer (NSCLC).
Tjon-Kon-Fat et al. (2018)[7]	6	Digital-PCR revealed that the transcripts for the prostate cancer-associated biomarkers (KLK2, KLK3, FOLH1, NPY) within the platelets were uniquely present in the patient's group, but not detected in healthy controls. TEP-based abiraterone response monitoring in CRPC patients was performed.
Xing et al. (2019)[8]	2	RNA sequencing and PCR analysis of TEP RNA demonstrated that ITGA2B was significant marker for the early diagnosis of NSCLC.

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