Electronic Supplementary Information

I. Generation of pulsatile pressure by oscillator

The oscillator unit in Figure S1A has two valves (V_1 and V_2) and two elastomeric membranes (M_1 and M_2), which change an elastic energy variation to a pressure change and vice versa.

The numbers shown in Figure S1A refer to the port number. For the oscillator unit, input and output wells were connected to ports 1 and 2, respectively. Port 3 was connected to a reference well. The pressures at ports 1, 2, and 3 (P_1, P_2, P_3) are constants. Owing to the height difference among the wells, the pressure condition is $P_1 > P_3 > P_2$. The open and closed states of the valves and the deflection of the membranes in the oscillator unit are self-controlled by the interaction of the microfluidic components. This change in the states in the valves generates pulsatile pressure (P_O) in point A. As shown in Figure S1B, the system has three layers. The top and bottom layers are marked in gray and orange, respectively. The middle layer is an elastomeric membrane located between the top and bottom layers. The fluid flows across the top and bottom layers through holes (green circles in Figure S1B).

The opening of V_i (i = 1, 2) suddenly increases the pressure on the bottom side of M_i, whereas the closing of V_i rapidly decreases that of M_i, owing to the high input pressure and low output pressure. When V_1 opens, V_2 closes (State 1 in Figure S1B). This is because the opening of V_1 suddenly increases the pressure on the bottom side of M_1, and this pressure closes V_2 through the motion of control liquid (arrow with green lines). Then, the high pressure at the bottom side of V_2 is gradually decreased through the channel connected to port 3. Afterwards, V_2 opens and V_1 closes (State 2 in Figure S1B). In this manner, the opened and closed states of V_1 and V_2 alternate, generating pulsatile pressure in point A (Figure S1C). A more detailed explanation is provided in refs. 27 and 29 of the main body.

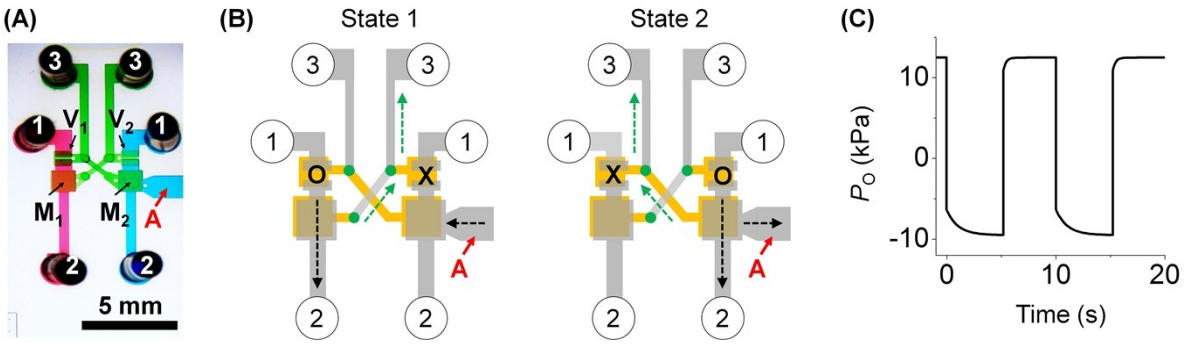

Figure S1. Autonomous generation of pulsatile pressure. The numbers shown in (A) and (B) correspond to the port numbers. The arrows with dotted lines in (B) indicate the movements of main (black) and control (green) liquids. The two liquids do not meet because of the middle layer. The symbols O and X denote the opened and closed states of the valves, respectively. Point A in (A) and (B) is the place where pulsatile pressures applied to the pump unit are produced. (A) Photograph of the oscillator unit. V_1 and V_2 are the valves, and M_1 and M_2 are flexible membranes. (B) Self-control of the opened and closed states of the valves of the oscillator unit. The periodic change of the valve states generates pulsatile pressure pulses at point A. (C) Theoretical pressure is generated at point A.

II. On-chip amplification of nucleic acid

Reagents

DNA oligonucleotides were chemically synthesized (Bionics, Seoul, Korea) and purified with polyacrylamide gel electrophoresis (PAGE). Detailed sequences are shown in Table S1. The T4 DNA ligase for the ligation of the dumbbell padlock DNA and the four dNTPs (viz., dATP, dGTP, dCTP, and dTTP) were purchased from Takara Korea Biomedical Inc. (Seoul, Korea). The Phi29 DNA polymerase was purchased from New England Biolabs (Ipswich, MA, USA). Sybr Green was purchased from Sigma-Aldrich Korea (Yongin, Korea). The pooled normal human plasma was obtained from Innovative Research (Novi, MI, USA).

Circularization of dumbbell padlock DNA

To prepare the pre-ligated dumbbell padlock DNA, the ligation reaction was performed in a 10-μL mixture containing 2.5 μL of 5'-phosphorylated dumbbell padlock DNA (500 nM), 0.5 μL of T4 DNA ligase (175 U), and 1 μL of 10 × T4 ligase reaction buffer (660 mM Tris-HCl, 66 mM $MgCl_2$, 100 mM DTT, 1 mM ATP, pH 7.6) and 6 μL of distilled water. The dumbbell padlock DNA was heat-denatured at 85 °C for 3 min and cooled to room temperature for 7 min. After the T4 DNA ligase and ligation buffer were added, the mixture was then incubated at 25 °C for 1 h.

Rolling circle amplification for detection of miDNA21

To detect miDNA21 (analogue of miRNA21 with same sequence), 52 μL of the rolling circle amplification (RCA) reaction mixture—containing ligated dumbbell padlock DNA (50 nM), 1x phi29 polymerase buffer (50 mM Tris-HCl, 10 mM $MgCl_2$, 10 mM $(NH_4)2SO_4$, and 4 mM DTT, pH 7.5)—along with 100 μg/mL bovine serum albumin, a mixture of dNTPs (each at a concentration of 0.6 mM), 500 U of phi29 polymerase, and 5 nM or 1 nM of miDNA21 was added to the blood solution (for a total volume of 400 μL).

Gel electrophoresis and fluorescence detection

We used agarose gel electrophoresis; specifically, we took 10 μL sample out of the 120 μL of RCA reaction mixture in the device (i.e. amplification unit). Then, the sample (10 μL) was mixe with 6×loading dye (2 μL), and the resulting sample was loaded onto 1.5% agarose gel for electrophoresis with 100 V for 30 min. For the positive and negative controls, RCA was also

performed in a reaction mixture (120 µL) containing 22 µL of the pooled normal human plasma with and without miDNA21 (5 nM). The RCA products were visualized under UV illumination after being stained with ethidium bromide for 10 min. The ladder we used was the 1 kb marker with the range of 1–10 kb (purchased from Takara, Japan).

For the visual detection of Sybr Green fluorescence generated by intercalation, 50 µL of reaction mixtures containing 10 µl of RCA product and 1X Sybr Green were prepared and photographs of the reaction mixtures in a PCR tube were taken using a PowerShot A640 digital camera (Canon Inc., Tokyo, Japan) under UV illumination. To measure the fluorescence of the Sybr Green, 50 µL of the reaction mixtures were transferred to quartz cuvettes of a fluorescence spectrophotometer (Model Cary Eclipse, Agilent Technologies, Santa Clara, CA, USA). Instrumental setups for the fluorescence measurements were as follows: λ_{ex}= 485 nm with a slit width of 10 nm; λ_{em}= 490-720 nm with a slit width of 10 nm; and a PMT voltage of 600 V.

Table S1. Sequence of oligonucleotide. The blue and underlined segments denote miDNA binding and the stem forming site, respectively. Further, P and nts denote phosphorylation and nucleotides, respectively.

Oligonucleotide (nts)	Sequence* (5′ → 3′)
miDNA21 (22)	TAGCTTATCAGACTGATGTTGA
Dumbbell padlock (73)	p-CGAAATCCCTAACCCTAACCCTAACCCTATTTCGGCAGTGTCCTTTTCAACATCAGTCTG CCCTTTTCACTGC

III. Experimental setup for on-chip amplification of nucleic acid

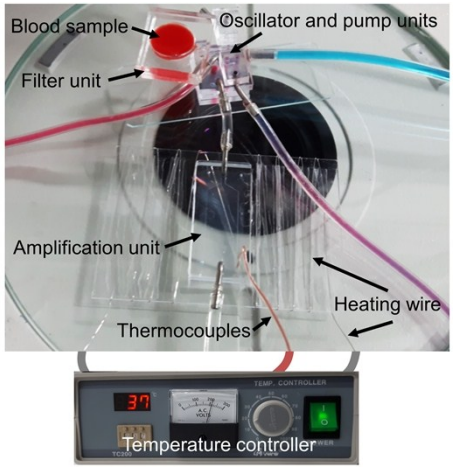

Figure S2. Experimental setup for the on-chip amplification of nucleic acid. The amplification unit was bonded to aslide glass with Ni/Cr wire heater. The temperature controller measures the temperature of the amplification unit with a K-type thermocouple and applies heat through the wire heater to maintain the temperature of 37 °C.

IV. Calculation of plasma recovery

In this section, we provide a detailed explanation on how we calculated the plasma recovery shown in Fig. 6E of the main text. The hematocrit is defined by the following equation: (plasma volume)/(volume of the mixture of whole blood and PBS solution). For example, 30 % hematocrit means that the plasma volume is 3/10 of the mixture volume. In the original whole blood, we measured a hematocrit level of 50 % without the addition of PBS solution. In other words, the cell to plasma volume ratio is 1:1 for the original whole blood. This volume ratio is retained even after dilution upon the addition of PBS solution. Thus, if the hematocrit level is 30 % and the mixture volume that corresponds to the loading volume (V_L) is 400 μL, the plasma volume is 120 μL (0.3×400 μL) and the cell volume is 120 μL. Hence, the volume of the PBS solution in the mixture is 160 μL (400–120–120 μL).

We assumed that once the plasma to PBS solution volume ratio in V_L is set, the volume ratio will not change in the extraction volume (V_E). For example, for a hematocrit level of 30 %, the plasma to PBS solution volume ratio is 120:160 in V_L. When V_E is 91 μL, the plasma volume in the V_E is 39 μL ((91×120)/(120+160) μL). The plasma recovery is defined by the following equation: (extracted plasma volume at the device output)/(original volume of blood plasma at the device input). Thus, the plasma recovery for 30 % hematocrit is found to be 32.5 % ((39/120)×100). For 15 % hematocrit, the plasma recovery can be calculated in the same manner.