Electronic Supplementary Material (ESI) for ChemComm.
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Electronic Supplementary Information

Enzymatically self-assembled DNA patch for enhanced blood coagulation

Dajeong Kim,[a] Jieun Kim[a] and Jong Bum Lee*[a]

[a]Department of Chemical Engineering, University of Seoul, 163 Seoulsiripdaero, Dongdaemun-gu, Seoul, South Korea

*Corresponding author. E-mail: jblee@uos.ac.kr

Experimental section

Synthesis of self-assembled DNA film via the enzymatic method

All linear DNAs were purchased from Integrated DNA Technologies (IDT) with sequences listed in Table S1. Circular DNAs for generating repeated TB aptamers were first prepared as previously reported.[1] Briefly, 3 μM of phosphorylated linear DNA and primer DNA were mixed in nuclease-free water and heated to 95 °C for 2 min followed by a gradual cooling process to 25°C for 1 h. To ligate the nick of the circular DNA, the mixture was incubated overnight at room temperature (RT) with 0.03 U/μl of T4 DNA ligase (Promega) in ligase buffer (300 mM Tris-HCl (pH 7.8), 100 mM $MgCl_2$, 100 mM dithiothreitol, and 10 mM adenosine triphosphate).

For the cRCA, 0.5 μM of circular DNAs (from linear DNA 1, 2 or 3, 4) were mixed with 2 mM of deoxyribonucleotide solution (Thermo Scientific), reaction buffer (100 mM Tris-HCl,

20 mM $(NH_4)_2SO_4$, 8 mM dithiothreitol, 20 mM $MgCl_2$), and 1 U/µl of Phi29 DNA polymerase (Lucigen) in nuclease-free water. Followed by incubation of the mixture at 30°C for 0, 1, 4, or 20 h for the enzymatic reaction, a lid of the tube was opened to induce evaporation-induced self-assembly (EISA) of DNA strands at 30°C overnight.[2] The resultant was washed with nuclease-free water for several times.

To prepare the pre-labeled DNA film, cy5-dCTP (10 µM, JenaBioscience) was incorporated into cRCA process. For the post-stained DNA film, DNA film generated via cRCA for 20 h was stained with GelRed (Biotium) for 10 min and washed with nuclease-free water. After overnight dehydration, the GelRed-DNA film was visualized by a d camera. The DNA film prepared by cRCA for 4 h was placed on a wafer and analyzed by mini-SEM (SEC, SNE-3200M) to obtain high-magnification SEM images.

Fabrication of DNA patch

To load TB on DNA film, 5 µg/ml of TB from human plasma (Sigma Aldrich) was mixed with a single DNA film for 4 h in nuclease-free water with 5 mM KCl. To visualize the binding of TB with DNA film in a sequence-specific manner, fluorescently labeled TB was prepared using 5-TAMRA protein labeling kit (AnaSpec). 10 µg/ml of TAMRA-TB was incubated with a single cy5-DNA film at RT for 4 h. The resulting TAMRA-DNA patch and CTL patch were washed and analyzed by fluorescence microscopy (Nikon, Eclipse Ti-U).

To quantify the loaded amount of TB, the supernatant solution was collected after the incubation with 1.25, 5, or 20 µg/ml of TB. Using the micro BCA protein assay kit (Thermo Scientific), the amount of TB was measured by a micro-plate reader (BioTek, Synergy HT).

Analysis of the activity of TB

To analyze the enzymatic activity of DNA patch, TB activity assay kit (AnaSpec) was used. Briefly, 50 µl of substrate solution was mixed with 50 µl of sample solutions containing the blank solution, free TB (0.2 µg or 0.6 µg), CTL patch, or DNA patch (0.2 µg or 0.6 µg of TB). Fluorescence intensity of the solution was measured every 10 min by the micro-plate reader and increased intensity revealed the cleavage of fibrinogen by TB. For the cRCA reaction time-dependent activity test, DNA patches were prepared by 0, 1, or 4 h cRCA reaction and CTL patch was prepared by 4 h cRCA reaction followed by EISA and incubated with TB (1 µg/ml).

Coagulation test with human plasma

Anti-coagulant human plasma was purchased from Sigma Aldrich. 10 µl of plasma was dropped on the slide glass and mixed with DPBS, free TB solution (0.6 µg), or DNA patch (0.6 µg of TB) with fibrinogen substrate solution at room temperature. After 10 min, fluorescence images were further obtained using fluorescence microscopy and the pixel intensity (gray value) was analyzed by ImageJ software. To assess the clot generation, 10 µl of plasma was mixed with DNA film, free TB solution (0.6 µg), or DNA patch (0.6 µg of TB) at room temperature for 10 min. The size of aggregates was calculated with their width, length, and height from three different samples. Given that the actual body temperature for the blood coagulation is higher than the room temperature where the TB activity is higher, the alleviated activity would lead to the larger clot combined with the DNA patch than soluble TB in realistic condition.[3-7]

Thermostability of DNA patch

For the thermostability test, 4 h DNA patch was stained with GelRed for 10 min. After the several washing step with nuclease-free water containing 5 mM KCl, DNA patch was incubated in 300 µl of PBS for 24 hours and observed at predetermined time intervals.

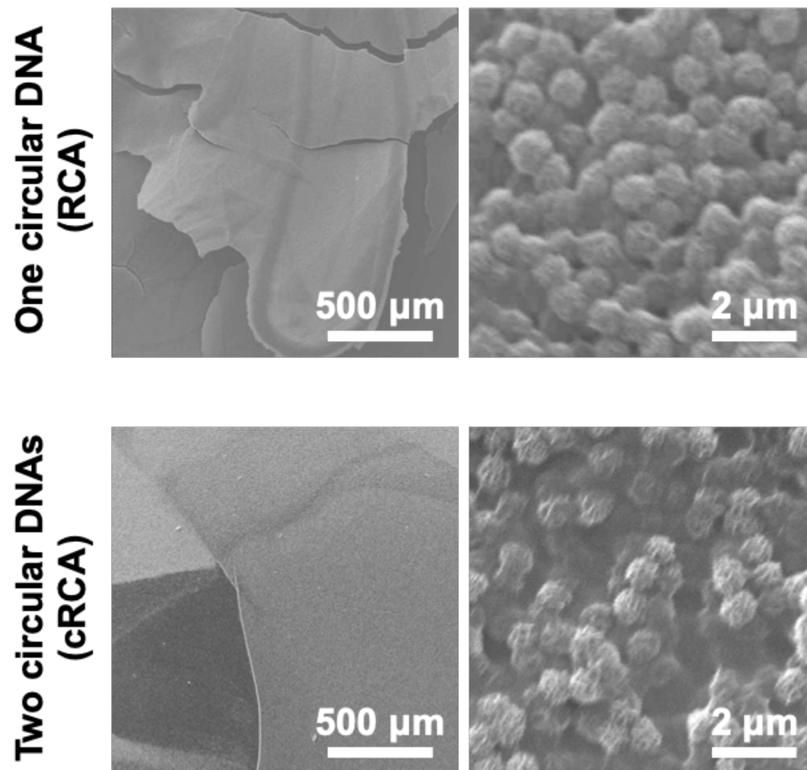

Figure S1. SEM images showing the DNA films generated from one circular DNA or two circular DNAs. For the generation of DNA film from one circular DNA, circular DNA2 (final concentration: 1 μM) was employed in the amplification step.

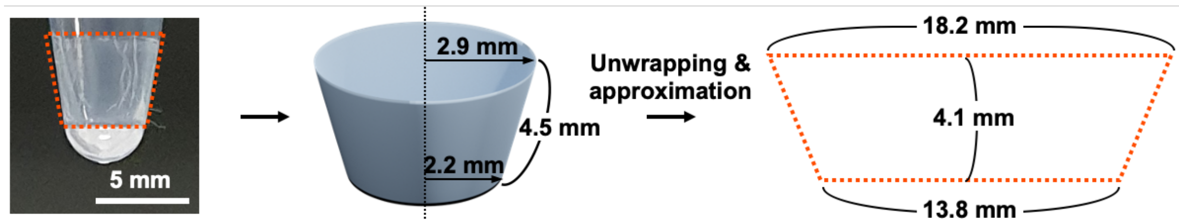

Figure S2. Measurement of the size of dehydrated DNA film in the tube for the CTL patch and DNA patch with the digital image. The size of unwrapped film was approximately represented as a trapezoid with 18.2 × 13.8 × 4.1 mm (top length (a) × bottom length (b) × height (h)).

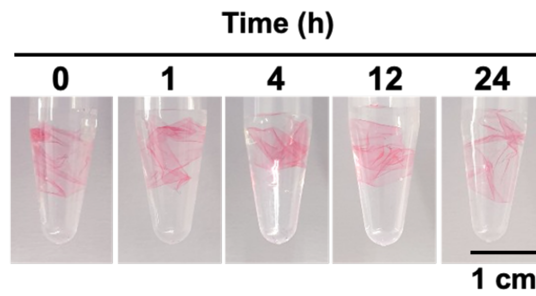

Figure S3. Stability of DNA patch in PBS solution. DNA patch exhibited a high stability at 37°C condition for 24 h, suggesting its suitable thermostability at around skin temperature.

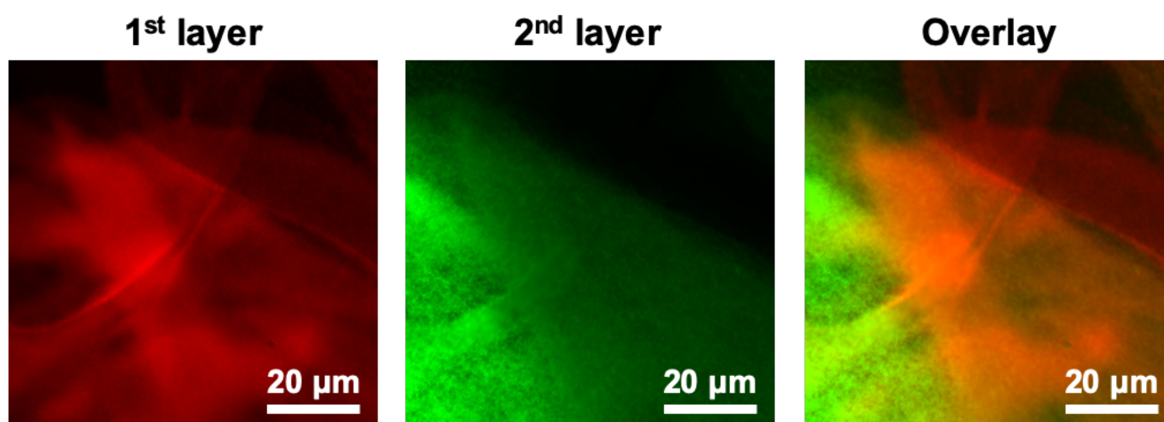

Figure S4. Fluorescence microscopy images of multilayered DNA films. Two separate DNA films were prepared by staining with Gel Red or SYBR Green and sequentially stacked on a slide glass. After drying, the multilayered DNA films were observed by fluorescence microscopy.

Table S1. Sequences of linear DNA sequences for generating CTL patch and DNA patch. Red regions indicate hybridization sites with each primer DNA. Green regions are complementary to the aptamer sequences that bind to thrombin on heparin or fibrinogen binding sites. Underlined regions reveal hybridization sites for corresponding linear DNA.

	DNA strands (bp)	Sequences (5' - 3')
For CTL patch and DNA patch	Primer DNA 1 for linear DNA 1 (22)	AAC ATA ATG TCA CTA TAG GGA T
	Linear DNA 1 for aptamer 1; heparin-binding site (92)	/Phosphate/ ATA GTG ACA TTA TGT TGA TGG TAA GTC ACC CCA ACC TGC CCT ACC ACG GAC TCT CTA TGT TGA TGG TAA TCG CTA TCT AGA GGC ATA TCC CT
	Primer DNA 2 for linear DNA 2 (22)	CTA GAG GCA TAT CCC TAT AGT G
	Linear DNA 2 for aptamer 2; fibrinogen-binding site (92)	/Phosphate/ AGG GAT ATG CCT CTA GAT AGC GAT TAC CAT CAA CAT AGA GAA ACC AAC CAC ACC AAC CAA AGA AAT GAT TAC CAT CAA CAT AAT GTC ACT AT

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