Hydrogel functionalization with DNA aptamers for sustained PDGF-BB release

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Supporting Information

Materials and Methods

Reagents. *N*-ethyl-*N*-(3-diethylaminopropyl)carbodiimide (EDC), *N*-hydroxysuccinimide (NHS), Tween 20, ammonium persulfate (APS), N,N,N′,N′-tetramethylenediamine (TEMED), and a premixed solution of acrylamide and bis-acrylamide (40%; 29:1) were purchased from Fisher Scientific (Suwanee, GA). The anti-PDGF-BB aptamer (5′-/Acrydite/GC GAT ACT CCA CAG GCT ACG GCA CGT AGA GCA TCA CCA TGA TCC TG-3′) and its control aptamer (5′-/Acrydite/GC GAT ACT CCA CAG CTG ACG GCA CGG TAA GCA TCA CCA TGA TGT CC-3′) were purchased from Integrated DNA Technologies (Coralville, IA). The 10-nt tail sequence is marked in blue. Recombinant Human PDGF-BB was purchased from R&D Systems (Minneapolis, MN). Bovine serum albumin (BSA) was purchased from Invitrogen (Carlsbad, CA). Human PDGF-BB ELISA

development kit was purchased form PeproTech (Rocky Hill, NJ). Diammonium 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) was purchased from Sigma-Aldrich.

Secondary structure prediction. The secondary structures of the aptamers were generated with the program RNAstructure version 4.6 (http://rna.urmc.rochester.edu/rnastructure.html). Of the secondary structures generated, the most stable ones with the lowest free energies were presented.

Surface plasmon resonance analysis. The molecular interaction between PDGF-BB and the aptamers were studied by SPR spectrometry (SR7000DC, Reichert Analytical Instrument, Depew, NY). PDGF-BB was immobilized onto a sensor chip via amide synthesis. An aqueous solution of NHS (0.0115 g/ml) and EDC (0.038 g/ml) was flowed over the sensor chip at a flow rate of 30 μL/min. After the EDC/NHS-mediated activation of the biochip surface for 10 min, 10 μg/mL of PDGF-BB solution (pH 8.5) was flowed over the chip surface for protein immobilization. Prior to the molecular recognition analysis, the sensor chip was equilibrated for 30 min with a phosphate-buffered saline (PBS) solution containing 0.05% Tween 20. During the binding analysis, 500 nM of binding solution containing either anti-PDGF-BB aptamer or its control was flowed over the biochip at 30 μL/min. The biochip was regenerated with 1 M of NaCl. The binding analysis was repeated twice to confirm the reproducibility. The equilibrium dissociation constant (K_D) was calculated with direct curve fitting of the sensorgrams using the software provided by the manufacturer.

Hydrogel synthesis. To prepare aptamer-functionalized hydrogels, PDGF-BB was incubated with the aptamer at a molar ratio of 1:625 in 50 μL of PBS (pH 7.4) for 30 min. The mixture was then added into a 10% of acrylamide/bis-acrylamide solution to prepare 1000 μL of solution. After gentle mixing of the acrylamide solution, 1 μL of APS solution (0.43 M)

and 1 μL of TEMED solution (20%) were added sequentially. 1000 μL of solution was immediately transferred into a 1.0 mL cylindrical mold made from a syringe. The polymerization was carried out for 3 h at room temperature. The molar ratio of aptamer to acrylamide monomer was fixed at ~ 1:2,800,000. Native hydrogels were prepared with the same protocol except the addition of the aptamer. The hydrogels were cut into small pieces (50 μL/each) for a releasing study experiment.

Examination of aptamer incorporation. Gel electrophoresis has been used to examine the efficiency of oligonucleotide incorporation into hydrogels because free oligonucleotides could be removed from the hydrogels during electrophoresis [1]. Therefore, we performed two gel electrophoresis experiments to examine aptamer incorporation. In the first experiment, 50 μL polyacrylamide hydrogels were subjected to gel electrophoresis for 90 min with a Bio-Rad Sub-Cell GT agarose gel electrophoresis system, stained with ethidium bromide for 30 min, and finally incubated in 1.5 mL PBS buffer on a shaker for another 30 min to remove free unintercalated ethidium bromide. In the second experiment, we first synthesized a piece of native polyacrylamide gel with loading wells. After the preparation of polyacrylamide solutions with acrydited aptamers or non-acrydited aptamers, we then transferred the polyacrylamide solutions into the loading wells. The final gels were subjected to vertical nucleic acid electrophoresis with a Bio-Rad Mini-PROTEAN tetra cell and stained by ethidium bromide. Gel images were captured with a Bio-Rad GelDoc XR system (Hercules, CA). The intensities of free-aptamer bands and aptamer-functionalized hydrogels were analyzed with the Quantity One software (Bio-Rad, Hercules, CA) and used as an indicator to qualitatively determine the percentage of free DNA aptamers that were unincorporated into the hydrogel network.

Rheology characterization. The storage and loss moduli of hydrogels were measured with an AR-G2 rheometer (TA Instruments, New Casstle, DE). The gels were prepared as circular discs of 20 mm in diameter and 1.5 mm in thickness. The samples were placed between two plates covered by a humidity chamber. The temperature was set at 37 °C for all experiments. The gap was adjusted until the normal force reached 0.3 N. To confirm that the measurement was in the linear viscoelastic regime, a stress sweep was performed by varying the oscillation stress from 0.01 to 1000 Pa at a fixed frequency of 6 rad/s. Frequency sweep experiments were performed from 0.6 to 90 rad/s at 1 Pa oscillation stress.

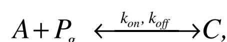
Sustained release study. For each type of hydrogel, we prepared 14 hydrogel samples. The samples were incubated at 37 °C in a 1.5 mL centrifuge tube containing 450 µL of release medium. The release medium was a mixture of PBS and 0.5%BSA. The shaking rate was 70 RPM. At the predetermined time points (1, 6, 12, 24, 48, 96, and 144 hr), two hydrogel samples were taken out of the tubes to stop the release. At the end of the release experiments, native hydrogels were minced and PDGF-BB was eluted from the gels to calculate the total amount of PDGF-BB. The released PDGF-BB was quantified by human PDGF-BB ELISA.

ELISA analysis. ELISA microplate was coated with 100 µL of anti-hPDGF-BB antibodies (0.5 µg/mL in PBS) at 4 °C for overnight. The wells were washed by 300 µL of washing buffer (0.05% tween 20 in PBS) for 4 times. The wells were subsequently blocked by 1% BSA in PBS for 2 hours and then were washed by 300 µL of washing buffer for 4 times. Then, 100 µL of releasing samples and standard proteins solutions, reconstituted in a diluent (0.1% BSA and 0.05% tween 20 in PBS), were incubated in the coated wells for 2 hours before being discarded. The wells were washed with 300 µL of washing buffer for 4 times. 100 µL of biotinylated antibodies (0.25 µg/ mL in the diluent) were added into the

wells and incubated for 2 hours. The wells were washed with 300 μL of washing buffer for 4 times. 100 μL of avidin-HRP conjugate were added into the wells and incubated for 30 min. After discarding avidin-HRP conjugate, the wells were washed with 300 μL of washing buffer for 4 times. 100 μL of substrate were added and incubated for 30 minutes. Subsequently, absorptions at 405 and 650 nm were recorded by Synergy HT Multi-Mode microplate reader (BioTeK, Winooski, VT). The amount of PDGF-BB release was calculated by subtracting absorbance at 650 nm from absorbance at 405 nm. All experiments were performed in duplicate.

Modeling of the sustained release of PDGF-BB

The experimental determination of PDGF-BB release was also quantified by a reaction-transport model. There are four species concentrations involved in the process: the aptamer in the gel domain (A), the aptamer–protein complex in the gel (C), the unbound PDGF-BB in the gel (P_g), and the released PDGF-BB in the solution (P_s). The association/dissociation reaction is



$$A + P_g \xleftrightarrow{k_{on},\, k_{off}} C,$$

where k_{on} and k_{off} are the corresponding rate constants. We propose the following reaction-transport equations to determine the bulk transient species concentrations:

$$\lambda V_g \frac{dP_s}{dt} = hS\left(\frac{1}{1+at}\right)\left[P_g(t) - P_s(t) - P_g(\infty) + P_s(\infty)\right] , \tag{1a}$$

$$\frac{dP_g}{dt} = -k_{on} A P_g + k_{off} C - \lambda \frac{dP_s}{dt} , \tag{1b}$$

$$\frac{dA}{dt} = -k_{on} A P_g + k_{off} C , \tag{1c}$$

$$\frac{dC}{dt} = k_{on} A P_g - k_{off} C \ , \tag{1d}$$

where λ is the volume ratio between the solution phase and the gel phase (V_s/V_g), h is the initial apparent mass transfer coefficient at the gel-solution interface, S is the total interfacial area, t is time, and $1/(1+at)$ is an empirical correction to accommodate the anomalous transport of proteins from the hydrogel, and the difference between $P_g(\infty)$ and $P_s(\infty)$ accounts for the partition effect. Using the conservation relations for aptamers and PDGF-BBs shows that

$$y_1 = A_0 + C_0 = A(t) + C(t) \ , \quad y_2 = P_0 + C_0 = P_g(t) + C(t) + \lambda P_s(t) \ , \tag{2}$$

where subscript 0 indicates the initial conditions in the gel phase, and y_1 and y_2 are the initial amount of aptamers and PDGF-BBs prior to the reaction. From the conservation relations, the four governing equations (1a) to (1d) can be simplified to two equations for the protein concentration in the solution and the aptamer–PDGF-BB complex in the hydrogel:

$$\frac{dP_s}{dt} = \left(\frac{hS}{\lambda V_g (1+at)} \right) \left[-(1+\lambda) P_s - C + C(\infty) + (1+\lambda) P_s(\infty) \right] \ , \tag{3a}$$

$$\frac{dC}{dt} = k_{on} (y_1 - C)(y_2 - \lambda P_s - C) - k_{off} C \ , \tag{3b}$$

where the corresponding initial conditions are $P_s(0) = 0$ and $C(0) = C_0$. The proposed model (3a) and (3b) are coupled nonlinear differential equations and can be solved by direct numerical integration with time. The partition coefficient Q is given by the expression: $Q=P_s(\infty)/P_g(\infty)$. The rate constants k_{on} and k_{off} (or the equilibrium constant $K_D=k_{\mathrm{off}}/k_{\mathrm{on}}=A_0P_0/C_0$) are experimentally determined using SPR. The initial amounts of aptamer and PDGF-BB, y_1 and y_2, and the volume ratio λ are control parameters. The initial

condition C_0 and steady state conditions $P_s(\infty)$ and $C(\infty)$ can be calculated based on the equilibrium conditions at the initial and final equilibrium end states.

The values of parameters used in the model were determined by experiments or curve fitting of the free diffusion (i.e., protein release from the native hydrogel): (i) for the free diffusion experiment using the native hydrogel, the initial protein concentration $y_2 = 8\times10^{-10}$ M, the apparent transport coefficient $hS/\lambda V_g$~0.01 h^{-1}, a~0.05 h^{-1}, $P_g(\infty) = 0.1099y_2$, $P_s(\infty) = 0.0989y_2$, and the partition coefficient $Q = 0.9$; (ii) for the sustained release experiment using control aptamer hydrogel, the initial aptamer concentration $y_1=5\times10^{-7}$ M, the measured reaction rate constants $k_{on} = 2.986\times10^8$ $M^{-1}h^{-1}$ and $k_{off} = 64.799$ h^{-1}, the calculated initial equilibrium conditions before protein release are $C_0 = 0.697y_2$, $P_g(0) = 0.303y_2$, $P_s(0) = 0$, and at the end state $C(\infty) = 0.20198y_2$, $P_g(\infty) = 0.0877y_2$, $P_s(\infty) = 0.07893y_2$; (iii) for the sustained release using anti-PDGF-BB aptamer hydrogel, the measured reaction rates reduce to $k_{on} = 2.41\times10^8$ $M^{-1}h^{-1}$ and $k_{off} = 6.12$ h^{-1}, indicating a weaker dissociation of aptamer PDGF-BB complex, and the initial and end equilibrium conditions are $C_0 = 0.9516y_2$, $P_g(0) = 0.0484y_2$, $P_s(0) = 0$, $C(\infty) = 0.6837y_2$, $P_g(\infty) = 0.03476y_2$, and $P_s(\infty) = 0.03128y_2$. The solution-to-gel volume ratio is $\lambda = 9$ for all experiments. Note that $hS/\lambda V_g$ and a are estimated from the first experiment using a native hydrogel and later used by experiment (ii) and (iii). No fitting parameters were used in experiments (ii) and (iii).

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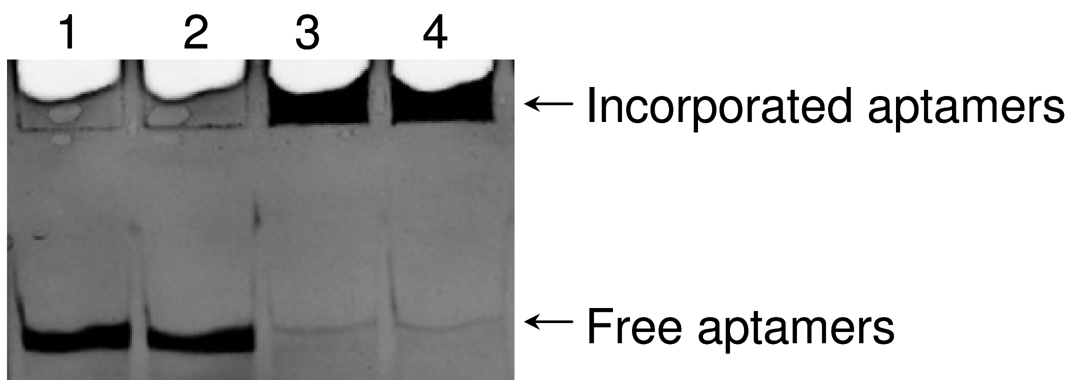

Supplementary Figure 1. Qualitative analysis of aptamer incorporation. Lanes 1 and 2: gels containing non-acrydited aptamers; lane 3 and 4: gels functionalized with acrydited aptamers.

Reference:

1. S. Venkatesh, J. Wower and M. E. Byrne, *Bioconjugate chemistry*, 2009, 20, 1773-1782.