Intelligent, Biodegradable, and Self-Healing Hydrogels Utilizing DNA Quadruplexes

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S.T. Conceptualization: Supporting; Investigation: Lead; Supervision: Supporting; Writing – original draft: Supporting; Writing – review & editing: Supporting
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EXPERIMENTAL PROCEDURES

Synthesis of L4.6k-dG4 (before deprotection)

I. Coupling
Polyethylene glycol (PEG4,600) (3.65 g, 0.79 mmol) was placed in a 300 ml three-necked round-bottom flask, and was coevaporated with dry acetonitrile (AN, 10 ml) for three times. Similarly, 1H-tetrazole (0.67 g, 9.55 mmol, 3 eq. of OH in PEG) was coevaporated in a 200 ml two-necked round-bottom flask by using dry AN (15 ml) for three times. Dry AN solution of PEG4,600 (10 ml) was added to 5′-DMT-dG-3′-(β-CE) (N, N-iPr2)-phosphoramidite (2.0 g, 2.38 mmol 1.5 eq. of OH of PEG) and 1H-tetrazole, both dissolved 20 ml in dry AN, by syringes through the rubber septum, and the solution was stirred under N₂ at room temperature for 1 h. The solution was concentrated on a rotary evaporator, and was added dropwise to 300 ml of ice-cooled diethyl ether with vigorous stirring. The product was recovered as white powder after filtration, which was washed with ether and was dried under vacuum.

II. Oxidation
After coupling, the product was dissolved in 30 ml of dry AN. Ice-cooled 70% tert-butyler hydroperoxide in water 2 ml was added to the solution, and the mixture was stirred for 30 min. The solution was added dropwise to 300 ml of ice-cooled diethyl ether with vigorous stirring. The product was recovered as white powder after filtration, which was washed with ether and was dried under vacuum. The product was analyzed with ¹H-NMR by using JEOL JNM-ECS 400 in CDCl₃. Typical crude yield is 4.27 g (88.6%).

III. Detritylation
After oxidation, the product was dissolved in 80 ml of 3wt% TCA in dichloromethane (TCA/DCM), and was stirred at 0°C for 30 min. The solution was concentrated on a rotary evaporator into half volume. The solution was then added dropwise to 500 ml of ice-cooled diethyl ether. Filtered precipitate was washed thoroughly with ether, and was dried under vacuum. This process was repeated until the orange color disappears. The final product was recovered as white powder after filtration, which was washed with ether and dried under vacuum.

The above process (I–III) was repeated 4 times except for the final detritylation (III) step. Typical overall yield is 5.12 g (73.4%).
De protection of phosphate groups and nucleobases

Protected L4.6k-dG4 (250 mg) was dissolved in 10 ml of 28% ammonium hydroxide/40% methylamine (AMA), and stirred for 2 hours at room temperature. The solution was allowed to stand for 10 min at 50°C, and then cooled to room temperature. After 10 ml of milliQ water was added, the solution was stirred for another 30 min. A Glen-Pak DNA purification cartridge (3 g, Glen Research) was washed with 2×10 ml of AN followed by 20 ml of milliQ water. The sample was loaded on the cartridge, and the cartridge was washed with 2×10 mL of 5% AN. Detritylation was done by passing 2×10 mL of 2% TFA in water. The cartridge was washed twice with 10 ml of milliQ water. Then 10 ml of 0.25 M LiOH was passed twice to neutralize phosphodiesters, and the cartridge was washed with 10 ml of milliQ water six times. The final product was eluted using 50% acetonitrile (2×10 ml). After the elute was evaporated, the product was dissolved into 10 ml of methanol with heating. The solution was added dropwise to 100 ml diethyl ether under vigorous stirring for reprecipitation. The product was recovered as white powder after filtration, which was washed with ether, was freeze dried under vacuum. The final product was identified with ¹H-NMR in D₂O, and was stored at -20°C. Typical yield is 117 mg (57.3%).

Preparation of DNA quadruplex gel

Macromonomer stock solution (25wt%) was prepared by dissolving 250 mg of L4.6k-dG4 in 750 µl of sterilized water. For preparation of 10wt% hydrogel, 93.4 µl of milliQ water, 40 µl of 1 M Tris/HCl (pH 7.0), and 88.8 mg of the stock solution were first mixed, and then 5 µl of 4 M KCl (or 4 M NaCl) was added dropwise to the mixture with stirring (see Supplementary Movie S1).

Measurements of phase transition temperatures

A vial containing DNA quadruplex gel was heated in a water bath at a desired temperature for 10 min. The vial was then inverted outside the bath to determine "flow" (= sol) or "no flow" (= gel) within 30 seconds. The process was repeated with a temperature increment of 1°C per step until 5°C above T_{sol}.

Measurements of rheological properties

A cylindrical DNA quadruplex gel prepared in a mold with 195 µl of 11wt% L4.6k-dG4 solution in 0.2 M Tris/HCl and 5 µl of 4 M KCl was subjected to temperature-dependent measurement of the storage modulus (G') and the loss modulus (G") on a dynamic rheometry (Thermo Fisher HAAK MARS 40 Rheometer). A solvent trap was used to prevent evaporation of the solvent. The hydrogel
was put on a 35-mm parallel plate, and an 8-mm jig was applied on the gel with a constant force of 0.05 N. The plates were heated at 1.5 °C/min. The data were collected under controlled stress (4.0 dyn/cm²) and a frequency of 1.0 rad/s.

**Preparation of gel beads and a string**

For preparation of 10wt% macromonomer solution, 43.4 µl of milliQ water, 40 µl of 1 M Tris/HCl (pH 7.0), 50 µL of 100 µM Rose Bengal solution, and 88.8 mg of the 25% stock solution were mixed. The mixture was then added by a syringe equipped with 26G needle to 0.2 M Tris/HCl (pH 7.0) containing 20 wt% PEG4.6k and 1 M KCl to form gel beads (dropwise) and a string (injection with a rapid stroke. See Supplementary Movie S2).

**Enzymatic digestion of gel beads**

For enzymatic digestion test, 10 µl of macromonomer solution containing fluorescent polystyrene beads was added dropwise to 10×PBS(-) containing 20wt% PEG4.6k to prepare the substrate gel beads. These gel beads were transferred to 1×PBS(-) in a glass vial with or without 5 U/µl phosphodiesterase II (PDE II) in the presence of 20wt% PEG4.6k. The vial was incubated at 37 °C for 3 days.

**Sequence-selective dissolving of gel beads**

As a macromonomer solution, 400 µl of 20 wt% X20k-dG3toe in 0.2 M Tris/HCl ([DNA] = 22 mM), and 2 µl of fluorescent polystyrene beads dispersion (PSI Polysciences, Inc. FLUORESBRITE, PLAIN RED 0.5 µm) were mixed. Then, 10 µl of this solution was added dropwise to 0.2 M Tris/HCl (pH 7.0) containing 20wt% PEG4.6k and 1 M KCl to prepare a gel bead. Totally three gel beads were prepared, and the beads were transferred to vials containing 0.2 M Tris/HCl (pH 7.0), 20wt% PEG4.6k, and 1 M KCl, without DNA strand, or with 5.5 mM mismatched DNA, or with 5.5 mM matched complementary DNA. The vials were incubated at 25˚C for 12 hours.

**Self-healing test**

Cylindrical DNA quadruplex gels (d = 8 mm, h = 3 mm) were prepared by mixing 20 µl of 10×PBS(-) with 180 µl of 11wt% LA4.6k-dG4 solution in 0.2 M Tris/HCl and 25 µM Bromophenol Blue (BPB) or Rose Bengal (RB) in a mold. These gels were taken out from the mold, and cut by a razor into two pieces. Pieces in blue and red were aligned to touch with each other on a glass dish and put back into the mold. The system was kept under room temperature for 3 days.
**Preparation of hydrogel strata**

Cylindrical DNA quadruplex gel \((d = 8 \text{ mm}, \ h = 3 \text{ mm})\) in red as the first layer of strata was prepared by mixing \(15 \mu l\) of \(10\times\text{PBS(-)}\) and \(135 \mu l\) of \(11\text{wt}\%\ \text{L4.6k-dG4 solution in 0.2 M Tris/HCl}\) and \(25 \mu M\ \text{RB}\) in a mold. The second transparent layer was then prepared by adding \(90 \mu l\) of \(11\text{wt}\%\ \text{L4.6k-dG4 solution in 0.2 M Tris/HCl}\) and \(10 \mu l\) of \(10\times\text{PBS(-)}\) on the first layer. Then, \(10 \mu l\) of \(10\times\text{PBS(-)}\) and \(135 \mu l\) of \(11\text{wt}\%\ \text{L4.6k-dG4 solution in 0.2 M Tris/HCl}\) and \(25 \mu M\ \text{BPB}\) were mixed on the second layer to form the third blue layer (see Supplementary Movie 3). The resulting cylindrical gel was taken out from the mold, sliced into a thin piece, and kept at room temperature on a slide glass for 2 days.

**CD measurement**

Circular dichroism (CD) and UV-Vis absorbance (Abs) spectra of \(0.1 \text{wt}\%\ \text{L4.6k-dG4 solution in 0.2 M Tris/HCl and 0.1 M KCl}\) were collected using a Jasco J-1100/1500 spectropolarimeter equipped with a Peltier temperature controller. CD/Abs spectra at \(25^\circ\text{C}\) were collected from 220 to 320 nm with 1 nm steps and standard sensitivity. Scan rate was set to 20 nm/min with a 1 second integration time. A total of three scans were collected and averaged.
Scheme S1. Synthetic route of DNA-PEG-DNA conjugates via High-Efficiency Liquid Phase (HELP) synthesis.
Figure S1. $^1$H-NMR spectra of each intermediates for L4.6k-dG4 synthesis.
Figure S2. (a) $^1$H-NMR spectra of L4.6k-dG3 and X10k-dG3 in D$_2$O. (b) $^1$H-NMR spectra of L4.6k-dG4, L10k-dG4, X10k-dG4, and X20k-dG4 in D$_2$O.
Figure S3. $^1$H-NMR spectrum of X20k-dG3toe in D$_2$O.
Figure S4. (a) Temperature-dependent phase diagram for K⁺-triggered gelation (100 mM) as a function of conjugate concentrations (0-15wt%). Apparent CGCs were 5wt% for L4.6k-dG3, 4wt% for X10k-dG3, and 3wt% for L4.6k-dG4, L10k-dG4, X10k-dG4, and X20k-dG4, respectively. (b) Temperature-dependent phase diagram for Na⁺-triggered gelation (100 mM) as a function of conjugate concentrations (0-15wt%). Apparent CGCs were 7wt% for L4.6k-dG3, 6wt% for L10k-dG4, and 4wt% for X10k-dG3, L4.6k-dG4, X10k-dG4, and X20k-dG4, respectively. (c) Temperature dependence of storage (G’) moduli for 10wt% DNA quadruplex gels in 0.2 M Tris/HCl (pH 7.0) containing 0.1 M KCl. The G’ of L4.6k-dG3 at 25°C is 8.45 Pa. (d) Temperature dependence of storage (G’) moduli for 5wt% DNA quadruplex gels in 0.2 M Tris/HCl (pH 7.0) containing 0.1 M NaCl. The G’ of at 10°C L4.6k-dG3 is 231.8 Pa.
Figure S5. Repetitive sol-gel transitions of 10wt% L4.6k-dG4/Na⁺ (100 mM).
**Table S1.** Phase transition temperatures ($T_{sol}$) of DNA quadruplex gels (0-15wt%) in 0.2 M Tris/HCl (pH 7.0) containing 0.1 M KCl or NaCl.

|          | 5wt%                      | 10wt%                     | 15wt%                     |
|----------|---------------------------|---------------------------|---------------------------|
|          | $T_{sol}$, K$^+$ (˚C)     | $T_{sol}$, Na$^+$ (˚C)    | $T_{sol}$, K$^+$ (˚C)     | $T_{sol}$, Na$^+$ (˚C)    |
| L4.6k-dG3| 22                        | n.d.*                     | >70                       | 21                        | >70                       | 40                        |
| X10k-dG3 | 56                        | 21                        | >70                       | 50                        | >70                       | >70                       |
| L4.6k-dG4| >70                       | 46                        | >70                       | 59                        | >70                       | >70                       |
| L10k-dG4 | 49                        | n.d.*                     | >70                       | 42                        | >70                       | 50                        |
| X10k-dG4 | >70                       | 46                        | >70                       | 58                        | >70                       | >70                       |
| X20k-dG4 | 59                        | >70                       | >70                       | 53                        | >70                       | >70                       |

*No gelation was observed even at 5˚C.

**Table S2.** Storage (G') moduli of DNA quadruplex hydrogels (5 and 10wt%) in 0.2 M Tris/HCl (pH 7.0) containing 0.1 M KCl or NaCl at 25˚C.

|       | $G'_5$, K$^+$ (Pa) | $G'_10$, K$^+$ (Pa) | $G'_10$, Na$^+$ (Pa) |
|-------|--------------------|---------------------|----------------------|
| L4.6k-dG3 | 0.437              | -                   | 6.881                |
| X10k-dG3 | 843.5              | -                   | 2.502                |
| L4.6k-dG4 | 2,134              | 6.095               | 3,922                |
| L10k-dG4 | 1,543              | -                   | 2,228                |
| X10k-dG4 | 3,202              | 11,110              | 6,418                |
| X20k-dG4 | 965.2              | -                   | 2,199                |

**Table S3.** Phase transition temperatures ($T_{sol}$) of 10wt% DNA quadruplex gels in 0.2 M Tris/HCl (pH 7.0) containing 0.1 M NaCl.

|          | L4.6k-dG3 | L4.6k-dG4 | L4.6k-dG5 |
|----------|-----------|-----------|-----------|
| $T_{sol}$ (˚C) | 21        | 42        | 23        |
Figure S6. (a) CD spectra of 0.1wt% L4.6k-dG4 solutions in 0.2 M Tris/HCl (pH 7.0) with or without 0.1 M KCl or NaCl at 25°C. (b) CD spectra of 0.1wt% various macromonomer solutions in 0.2 M Tris/HCl (pH 7.0), 0.1 M KCl at 25°C. (c) CD spectra of various 0.1wt% macromonomer solutions in 0.2 M Tris/HCl (pH 7.0), 0.1 M NaCl at 25°C.
Figure S7. (a) Photographs of DNA quadruplex gels prepared by using 10×PBS(-) or 5×E-MEM. (b) Photographs of DNA quadruplex gels prepared by using one third the volume of various body-related fluids.
Figure S8. (a) Emission spectra of liquid phase after enzymatic digestion of fluorescent L4.6k-dG4/Na\(^+\) gel bead with or without PDE II in PBS(-). (b) Emission spectra of liquid phase fluorescent leakage from L4.6k-dG4/K\(^+\) gel bead with 500 nm fluorescent polystyrene beads. (c) Emission spectra of liquid phase fluorescent leakage from L4.6k-dG4/K\(^+\) gel bead with 100 nm fluorescent polystyrene beads. (d) Emission spectra of fluorescent leakage from L4.6k-dG4/K\(^+\) gel bead with 50 nm fluorescent polystyrene beads. No background (i.e. buffer solution) subtraction was done for (b)-(d). No background (i.e. buffer solution) subtraction was done for (b)-(d).
Figure S9. (a) Enzymatic digestion assay using L4.6k-dG4/Na+ gel beads. Fluorescent DNA quadruplex gel beads were prepared with 10 µl macromonomer solution, and immersed in PBS(+) with or without enzymes. (b) Emission spectra of liquid phase after enzymatic digestion assay of fluorescent L4.6k-dG4/Na+ gel bead with or without enzymes in PBS(+).
Figure S10. Emission spectra of liquid phase after sequence selective gel-to-sol transition of fluorescent X20k-dG3toe/K⁺ gel bead.
Table S4. Melting temperatures ($T_{m}$) determined by $\theta$ at 260 nm of 0.1 wt% macromonomer solutions in 0.2 M Tris/HCl (pH 7.0) containing 0.1 M KCl or NaCl.

|          | $T_{m,K}$ (°C) | $T_{m,Na}$ (°C) |
|----------|----------------|-----------------|
|          | 10$\rightarrow$90°C | 90$\rightarrow$10°C | 10$\rightarrow$90°C | 90$\rightarrow$10°C |
| L4.6k-dG3 | 53.5           | 26.4            | 43.8           | 37.3            |
| X10k-dG3  | 56.4           | 27.8            | 49.0           | 29.7            |
| L4.6k-dG4 | 61.9           | 34.5            | 59.2           | 31.6            |
| L10k-dG4  | 64.8           | 37.4            | 44.7           | 34.9            |
| X10k-dG4  | 63.5           | 39.7            | 65.1           | 30.8            |
| X20k-dG4  | 58.2           | 31.3            | 60.4           | 46.2            |

Table S5. Phase transition temperatures ($T_{sol}$) of L4.6k-dG3/K$^+$ gel without or with 20wt% PEG4.6k.

|          | $T_{sol}$ (°C) |
|----------|----------------|
|          | 5wt% | 6wt% | 10wt% | 15wt% |
| W/O PEG4.6k | 20   | 42   | >70   | >70   |
| With 20wt% PEG4.6k | 40   | 45   | >70   | >70   |