Deoxyribonucleic acid polymer nanoparticle hydrogels Supplementary Information

Robert H. T. Bagley and Samuel T. Jones*

Materials and Methods

Materials were purchased from Sigma Aldrich and Alfa Aesar unless stated otherwise. The TM-50 grade colloidal silica possesses silica nanoparticles of 22 nm average size with sodium serving as the counter-ion as a 50 wt% stock in deionised water. Rheometry testing was performed using a DHR-2 Hybrid Rheometer (TA Instruments, Inc) except for the step-strain rheometry study that was performed on a AR-G2 Stress Controlled Rheometer (TA Instruments, Inc.). The cycles were performed with a Peltier Plate attachment and a 20mm stainless steel parallel plate geometry. Dye release fluorescence readings were recorded using a FLUOstar® Optima microplate reader (BMG Labtech). The measurement type was fluorescence. Samples were loaded using COSTAR 96 well plates. Endpoint settings were 20 flashes per well. Optic settings were an A-492 excitation filter and an empty emission filter. General settings include a 0.5s settling time, a bidirectional reading direction horizontal left to right and top to bottom and a temperature of 25°C.

Safety Information

All substances used in this work are not hazardous according to Regulation (EC) No. 1272/2008.

Manufacture of Gels

The gel consisted of 25 wt% LUDOX® and 5 wt% Deoxyribonucleic acid from fish sperm (74782). Firstly, A DNA stock solution of 25 wt% DNA from fish sperm in 0.5M TRIS buffer solution was made. This was left for several hours until a dark brown liquid formed. To a vial was added LUDOX® TM-50 colloidal silica (50 wt% in deionized water, 1.25 mL) and deionised water (750 µL). The mixture was agitated until uniform before adding DNA stock solution (500 µL). The mixture was agitated again until uniform. A light brown liquid was formed. This was left for 2 hours, after which a light brown gel formed. Samples were left overnight before performing further studies to ensure gel stability.

Qualitative Inverted Vial Study

Samples of various quantities of DNA and silica were tested, ranging from 1-10 wt% DNA and 5-25 wt% silica. These were categorised based on gel strength following vial inversion and gentle tapping on a benchtop. Figure S1 maps the quantitative gel strength according to DNA and silica quantity. This served as a guide for the study in the main article. Sample categorisation was based on gel strength:

1. Gel - sample remained at the base of the vial after inversion and after tapping on the benchtop.
2. Weak Gel - sample remained at the base of the vial after inversion but dislodged after tapping on the benchtop.
3. Very Weak Gel - sample did not remain at the base of the vial after inversion, but displayed viscosity.
4. No Gel - sample was fully liquid and ran down to the neck of the vial upon inversion.

For the main article study, Samples A, B and C were prepared following the protocol in the manufacture of gels section except for using the component volumes shown in Table 1. After leaving the mixtures overnight, each vial sample was inverted and lightly tapped on a benchtop. Photos were taken shortly after.

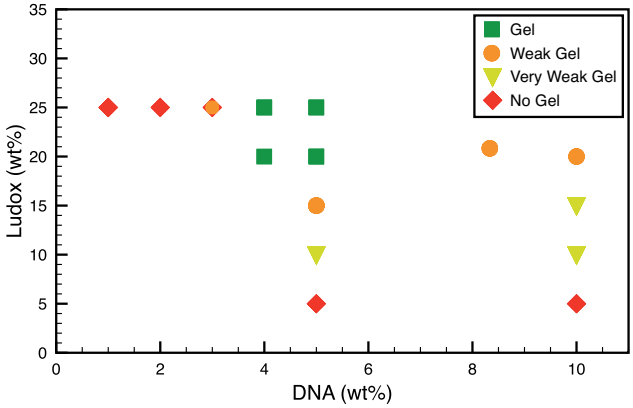

Figure S 1: Qualitative results of initial inverted vial experiment.

Sample	Constituent					
	LUDOX® TM-50 colloidal silica		DNA solution		DI H2O	
	Vol. (mL)	Wt%	Vol. (mL)	Wt%	Vol. (mL)	Wt%
A	1.25	25	0.3	3	0.95	72
B	0.75	15	0.5	5	1.25	80
C	1.25	25	0.5	5	0.75	70

Table 1: Quantities of DNA PNP hydrogel constituents.

Rheometry Studies

Strain-dependant studies were performed in a logarithmic sweep from 0.1 - 100% oscillation strain at 25°C and angular frequency 10 rad s^{-1}. Frequency-dependant studies were performed in a logarithmic sweep from 0.1 - 100 rad s^{-1} angular frequency at 25°C and oscillation strain 2%. The step-strain study consisted of four successive cycles of 40 seconds at 500% strain followed by 800 seconds at 0.5% strain at 25°C and angular frequency 10 rad s^{-1}.

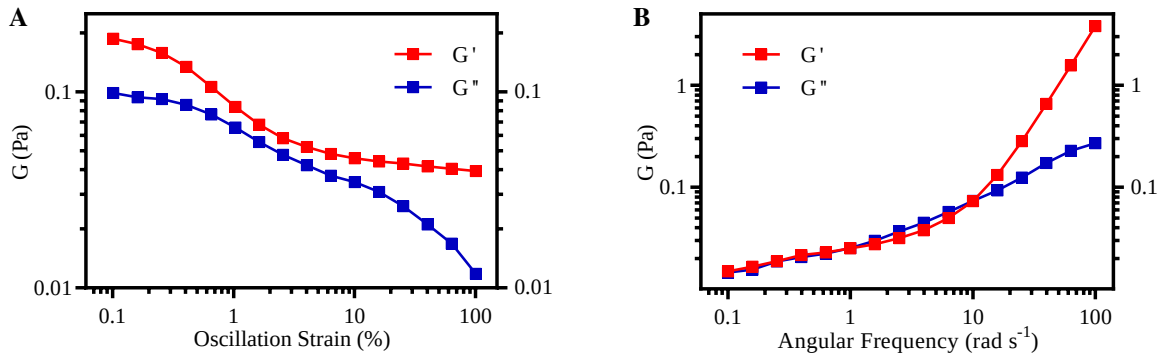

Figure S 2: Rheometry data of Sample B with 15 wt% LUDOX® and 5 wt% DNA. A) strain-dependant cycle, B) frequency-dependant cycle.

Dye Release Study

The gel was made as follows: a stock solution of bovine serum albumin labelled with fluorescein isothiocyanate in deionised water of concentration 1 mg/mL was prepared. The total volume of each gel was 2.5 mL. To a small vial was added LUDOX® TM-50 colloidal silica (1.25 mL). Next was added bovine serum albumin-fluorescein isothiocyanate conjugate dye stock solution (550 µL) and deionised water (200 µL). The mixture was agitated until uniform. Finally, DNA stock solution prepared from the manufacture of gels section (500 µL) was added. The mix was agitated until fully uniform and left to gelate overnight.

For the release study, 1 mL deionised water was added gently to the vial, forming a layer on top of the gel. The vial was left for 10 hours, after which time 500 µL of the top layer was carefully extracted, so as not to disturb the gel, and added to a light-proof vial and stored in a dark place. The vial was then topped up with 500 µL of deionised water. This process was repeated at various time points (Table 2). 150 µL of each of the collected aliquots were taken for fluorescence recordings.

Time (h)	10	24	48	58	72	146	219	248	359	383	407	486
Fluor. (a.u)	0.477	0.658	0.557	0.465	0.425	0.623	0.614	0.490	0.470	0.426	0.465	0.511
Cum. Fluor. (a.u)	0.477	1.135	1.692	2.157	2.582	3.205	3.819	4.309	4.779	5.205	5.670	6.181

Table 2: Fluorescence readings for aliquots at time intervals ranging from 10 to 486 hours.

For the Ritger-Peppas plot, M_∞ was calculated as the hypothetical fluorescence reading if the total mass of dye added to the gel (0.55 mg) was dissolved in the aliquot volume added to each well of the plate (150 µL). This gives a concentration of $3.\dot{6}$ mg/mL. This concentration gave a reading of 4.5 a.u, the maximum achievable value for the plate reader. Therefore, this concentration quenches the machine.

To obtain an accurate value for M_∞, a serial dilution study was conducted. A stock solution of bovine serum albumin labelled with fluorescein isothiocyanate (BSA-FITC) in deionised water of concentration $3.\dot{6}$ mg/mL was prepared. 225 µL of stock solution was added to the first well of a 96-well plate. 75 µL of deioinised water was added to the next seven wells. 75 µL of stock solution from the first well was extracted and added to the second well. The contents of the second well was drawn up and down using a pipette to give a uniform mixture. 75 µL of the contents of the second well was extracted and added to the third well. The process of mixing and extracting was repeated until the final well, where 75 µL was extracted and discarded. The well plate was then taken for fluorescence readings.

Concentration (mg/mL)	$3.\dot{6}$ (11 / 3)	11 / 6	11 / 12	11 / 24	11 / 48	11 / 96	11 / 192	11 / 384
Fluorescence (a.u)	4.5	4.5	2.881	1.404	0.795	0.447	0.304	0.221

Table 3: Fluorescence readings for aliquots from serial dilution.

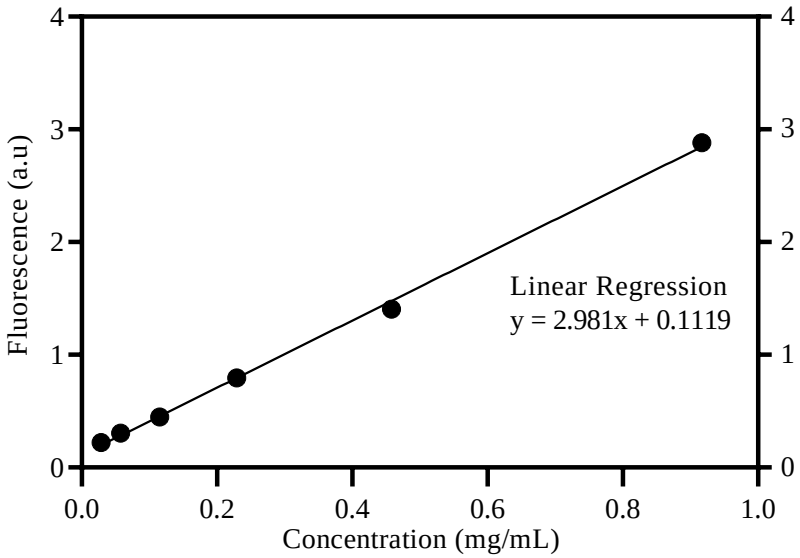

Figure S 3: Plot of fluorescence against aliquot concentration of BSA-FITC with simple linear regression.

Concentrations of 11/3 and 11/6 mg/mL produced readings of 4.5 a.u, indicating quenching, and were discounted from the graph plot. Simple linear regression with 95% confidence intervals gave the equation $y = 2.981x + 0.1119$. This was used to calculate the theoretical fluorescence value at concentration $3.\dot{6}$ mg/mL :

$$M_\infty = 2.981(3.\dot{6}) + 0.1119 = 11.04 \text{ a.u}$$

Now the data for the Ritger-Peppas plot can be obtained. Firstly, the $\log_{10}$ of the time intervals was calculated and plotted on the y axis. Secondly, the percentage release rate was calculated by dividing the cumulative fluorescence at time t by M_∞ multipied by 100. The $\log_{10}$ of $100^* M_t / M_\infty$ was plotted on the x axis.

Time (h)	10	24	48	58	72	146	219	248	359	383	407	486
log(t)	1	1.380	1.681	1.763	1.857	2.164	2.340	2.394	2.555	2.583	2.610	2.687
Cum. Fluor. (a.u)	0.477	1.135	1.692	2.157	2.582	3.205	3.819	4.309	4.779	5.205	5.670	6.181
Release Rate (%) $100*M_t/M_\infty$	4.320	10.279	15.324	19.535	23.384	29.026	34.587	39.024	43.281	47.139	51.350	55.978
log(Release Rate)	1	1.380	1.681	1.763	1.857	2.164	2.340	2.394	2.555	2.583	2.610	2.687

Table 4: Ritger-Peppas plot data collection.

DNase Study

A 25 wt% stock solution of deoxyribonucleic acid from fish sperm in DNase I reaction buffer (Invitrogen) was made. The total volume of each gel was 2.5 mL. The first sample was the non-DNase control. It was made by adding to a small vial LUDOX® TM-50 colloidal silica (1.25 mL), deionised water (750 µL) and DNA stock solution (500 µL). The second sample contained DNase. It was made by adding to a small vial LUDOX® TM-50 colloidal silica (1.25 mL), DNase (10 mg dissolved in deionised water (750 µL), and DNA stock solution (500 µL). The mixes were agitated until fully uniform and placed in an incubator at 37°C for 24 hours. Both vials were inverted and lightly tapped on the benchtop before taking a photo and conducting rheometry experiments.