Bioinspired hybrid DNA/dendrimer-based films with supramolecular chirality

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SUPPLEMENTARY INFORMATION

A. ADDITIONAL FIGURES AND TABLES WITH RESULTS

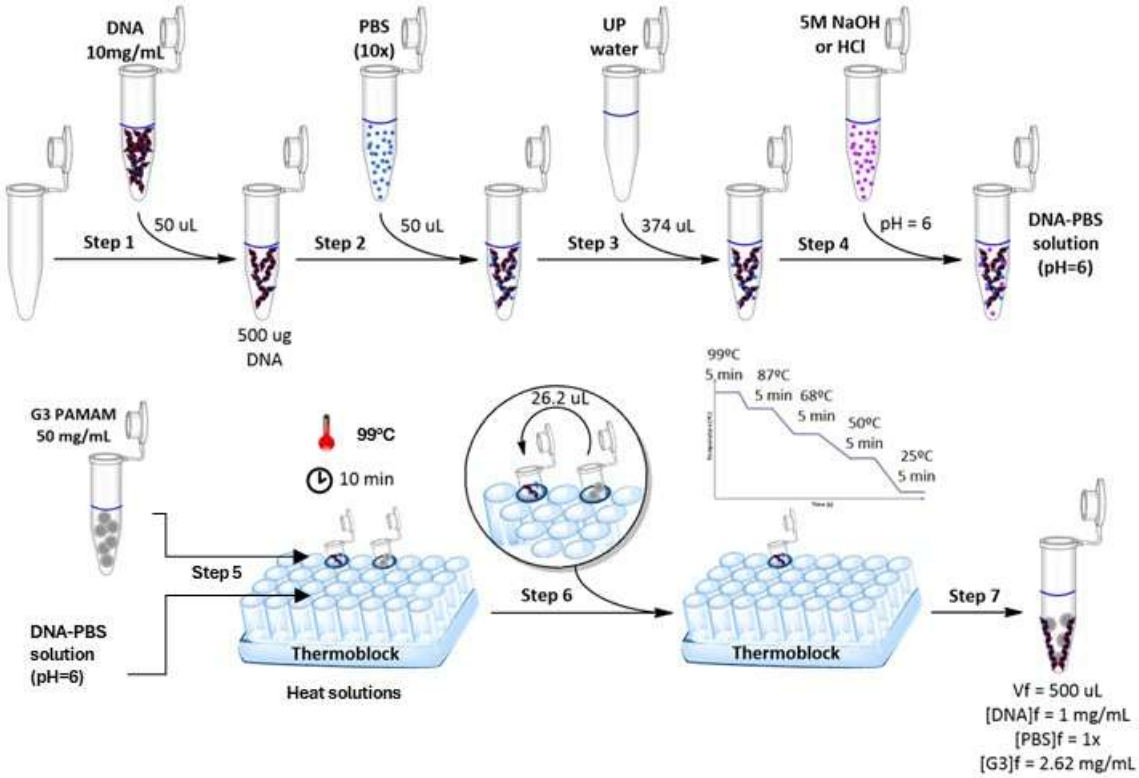

Figure S1. Scheme of the optimized experimental protocol to prepare DNA/dendrimer films. The preparation of DNA/G3 PAMAM dendrimers at a N/P ratio of 4 is shown as example.

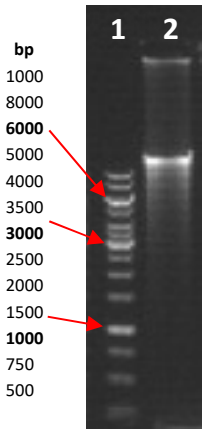

Figure S2. Agarose gel electrophoresis experiment for the determination of the base pair length and molecular mass of the dsDNA from salmon testes. DNA was shown to have a molecular mass of 7.8×10^6 Da (≃12 kbp). Lanes: (1) GeneRuler™ 1 kb DNA Ladder; (2) native DNA from salmon testes.

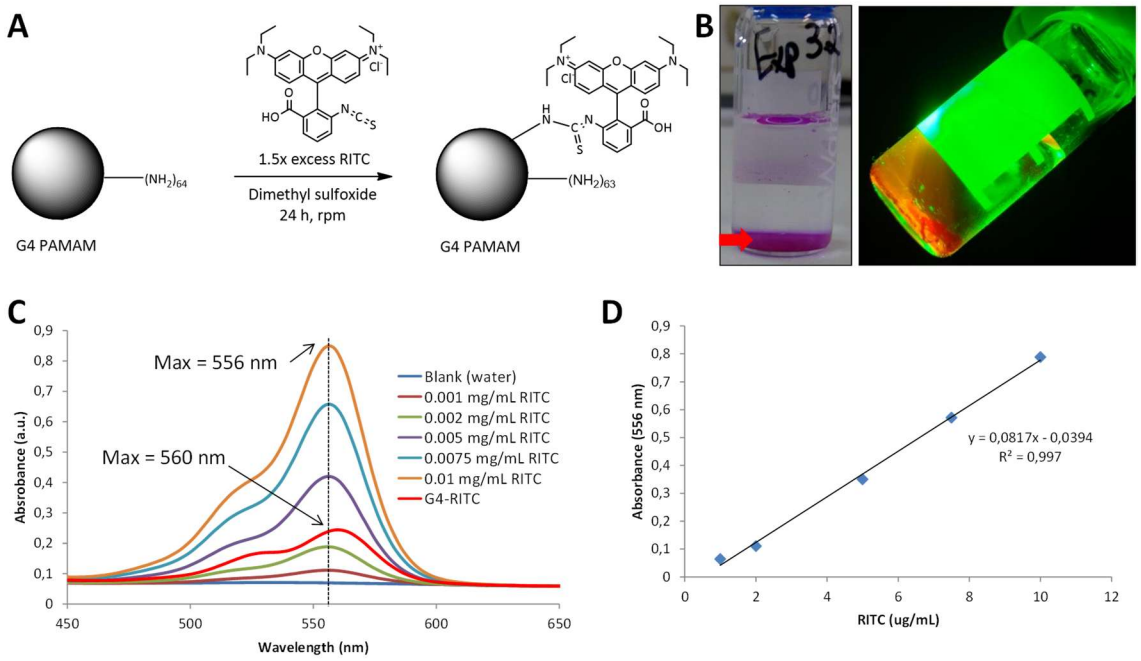

Figure S3. Functionalization of dendrimer with rhodamine B isothiocyanate (RITC). (A) Scheme of the reaction used to prepare RITC-labelled G4 PAMAM dendrimers. (B) Stained DNA film (pink) at the bottom of a vial (indicated by a red arrow) under green light (filter: 510-540 nm) showing the characteristic bright orange-red fluorescent emission of rhodamine. (C) Absorbance spectra of RITC standard solutions and of the final product, RITC-labelled G4 PAMAM dendrimers; a red shift (bathochromic shift) in the maximum absorbance wavelength confirms successful conjugation. (D) RITC calibration curve was used to calculate the RITC:dendrimer ratio (the result was 1.01). The desired degree of functionalization was minimal to avoid significantly altering the properties of the dendrimer.

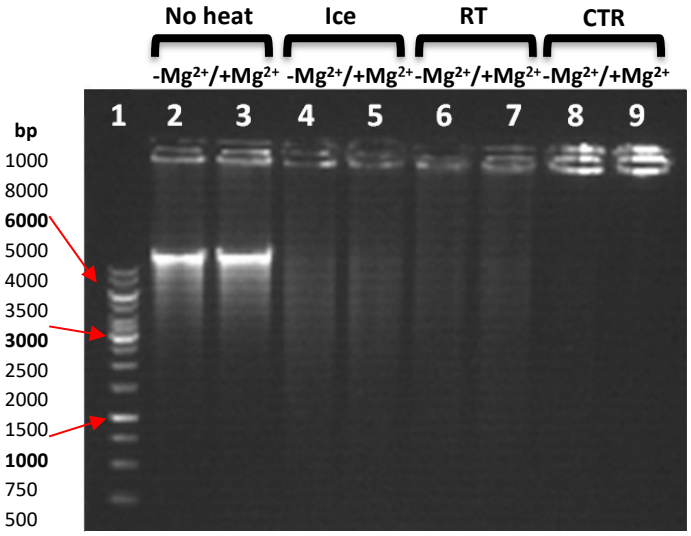

Figure S4. Investigation of the effect of the presence of magnesium ions on DNA hybridization, essential for film formation, using agarose gel electrophoresis with ethidium bromide to detect the presence of dsDNA. Lanes: (1) 1 kb DNA ladder; (2) DNA (not heated) in PBS and (3) in PBS +Mg^{2+}; (4) DNA after heating and cooling on ice in PBS and (5) in PBS +Mg^{2+}; (6) DNA after heating and cooling at RT in PBS and (7) in PBS +Mg^{2+}; (8) DNA after heating and cooling using the CTR in PBS and in (9) PBS +Mg^{2+}. Although the addition of Mg^{2+} impaired film formation, agarose gel electrophoresis assay did not show any change in DNA hybridization under the different cooling conditions. Thus, Mg^{2+}-induced shielding of DNA's negative charge and decreased interaction with the dendrimers was observed.

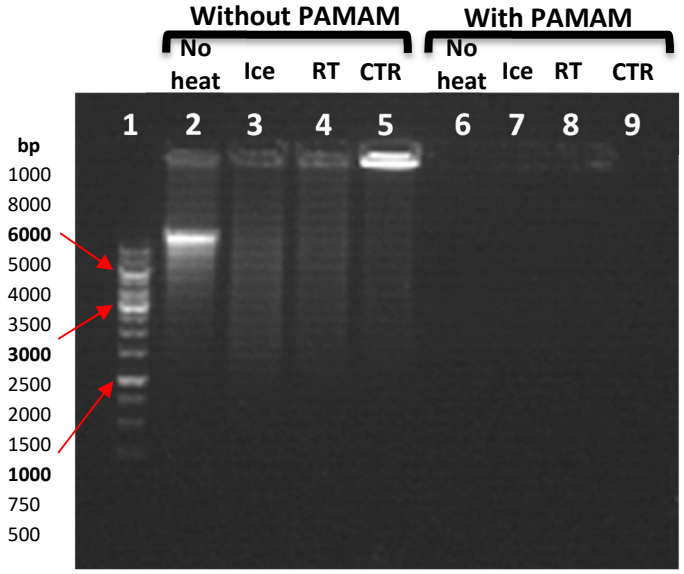

Figure S5. Investigation of the effect of the cooling program on the mechanism of film formation using agarose gel electrophoresis assays with ethidium bromide to detect the presence of dsDNA. Lanes: (1) GeneRuler™ 1 kb DNA ladder; (2) native DNA (not heated); (3) DNA heated and cooled on ice; (4) DNA heated and cooled at room temperature (RT); (5) DNA heated and cooled using the CTR; (6) DNA (not heated) + G5 PAMAM (N/P=4); (7) DNA heated + G5 PAMAM (N/P=4, cooled on ice); (8) DNA heated + G5 PAMAM (N/P=4, cooled at RT); and (9) DNA heated + G5 PAMAM (N/P=4, cooled using the CTR). With film forming conditions (CTR, lane 5), dsDNA was detected and did not migrate in the gel due to simultaneous hybridization and crosslinking. With dendrimer, under the same condition (lane 9), no dsDNA was detected due to condensation.

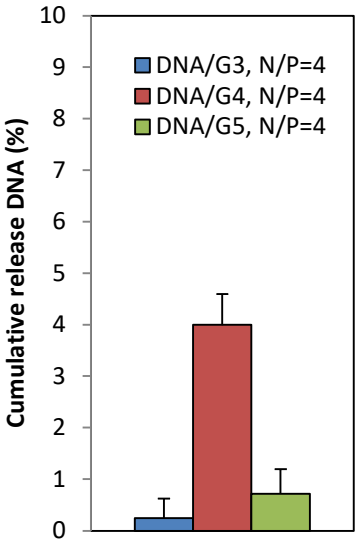

Figure S6. DNA released from films exposed to a PBS solution containing 10% (v/v) foetal bovine serum (FBS) at 37°C (percentage of the initial DNA amount incorporated in the films, mean ± s.d., n=3).

Table S1. Zeta potential (ZP) of DNA/dendrimer films produced. DNA films were prepared with PAMAM dendrimers of different generations (G3, G4 and G5) at N/P ratio of 4. The pH of the solution used for the measurement is indicated. Results are represented as mean ± S.D. (n=3).

	pH	ZP (mV)
DNA/G3 (N/P=4)	5.15 ± 0.03	24 ± 5
DNA/G4 (N/P=4)	5.32 ± 0.01	22 ± 5
DNA/G5 (N/P=4)	5.66 ± 0.06	22 ± 12

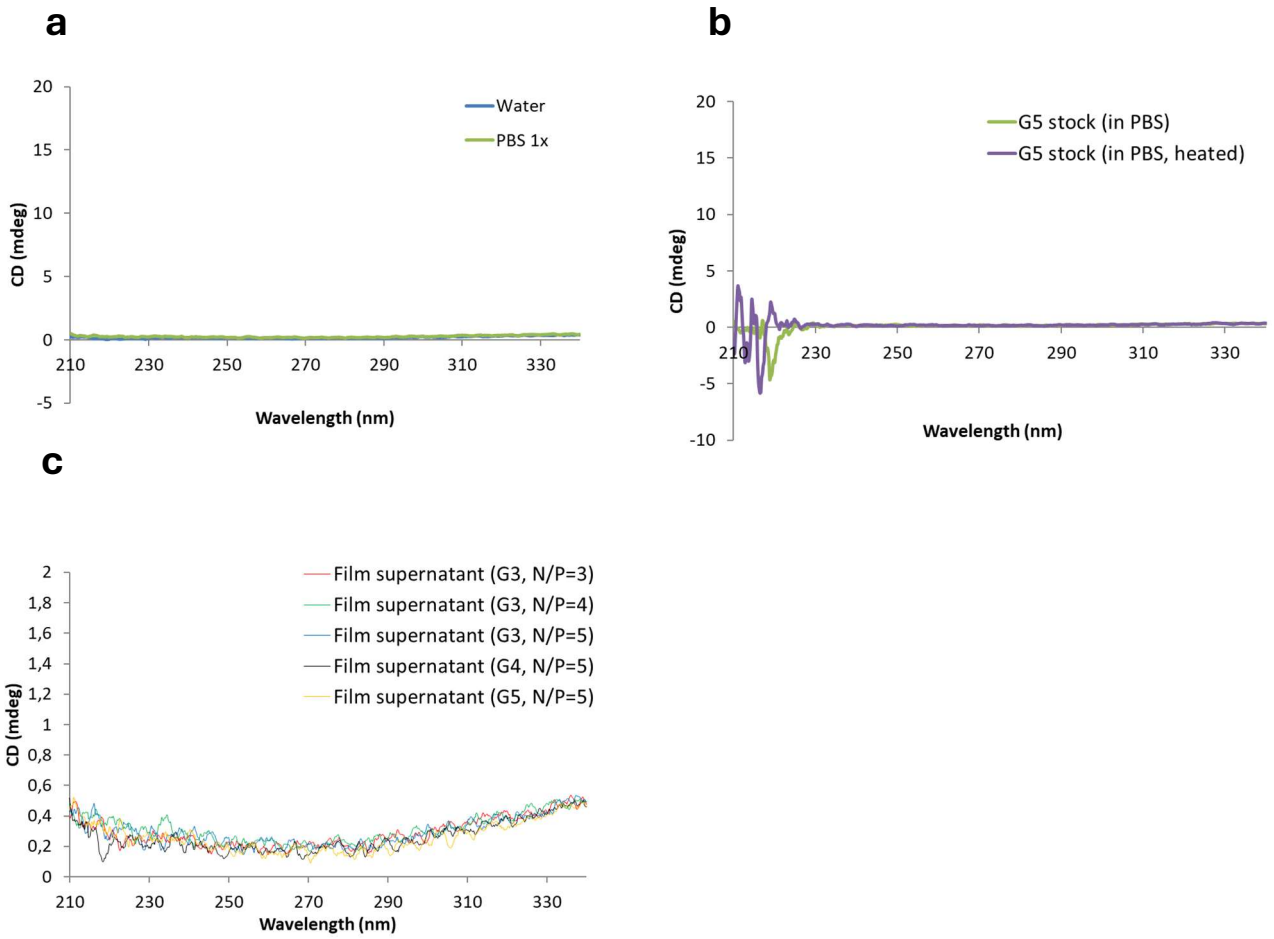

Figure S7. Circular dichroism (CD) spectra for control experiments. CD spectra of (a) water and PBS; (b) G5 PAMAM dendrimer at 50 mg/mL in PBS, before and after heating; (c) supernatant after DNA film preparation, with G3 (N/P = 3, 4 and 5), G4, and G5 PAMAM dendrimer (N/P = 5) for comparison. Note that the y-axis scale differs in (c), being much lower.

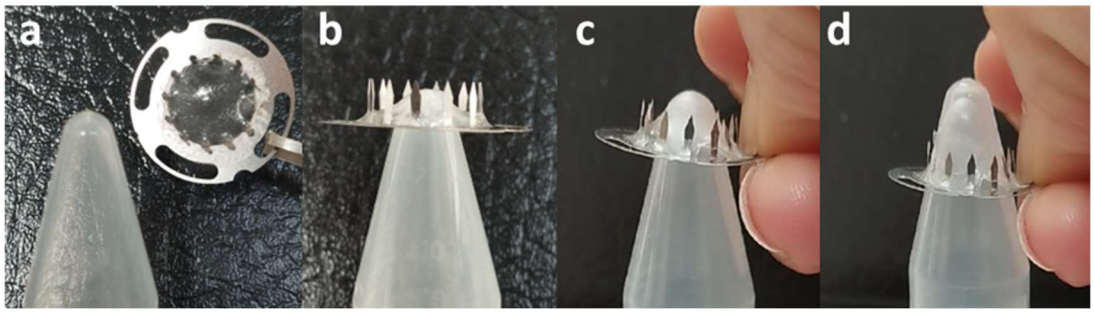

Figure S8. Visualization of films' behaviour under stress. Images of air-dried DNA/dendrimer films (a) on membrane grip sample holder; (b) and (c) with increased pressure; and (d) until rupture (both the elastic and plastic regimes were exceeded).

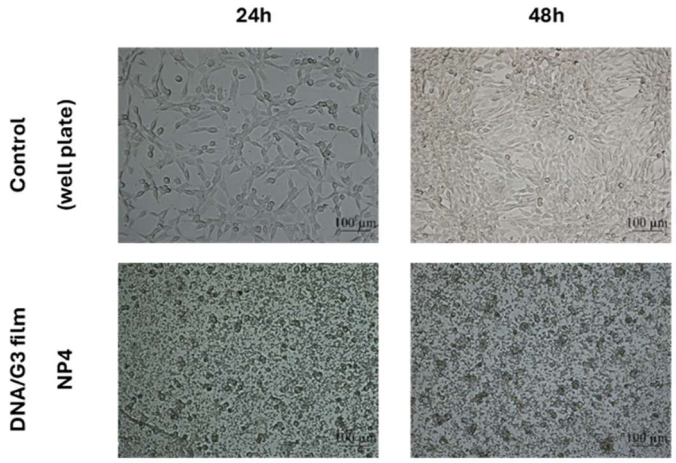

Figure S9. Optical microscopy images of NIH 3T3 cells cultured on the top of a DNA/dendrimer-based film prepared with G3 dendrimers at an N/P ratio of 4 (the scale corresponds to 100 µm).

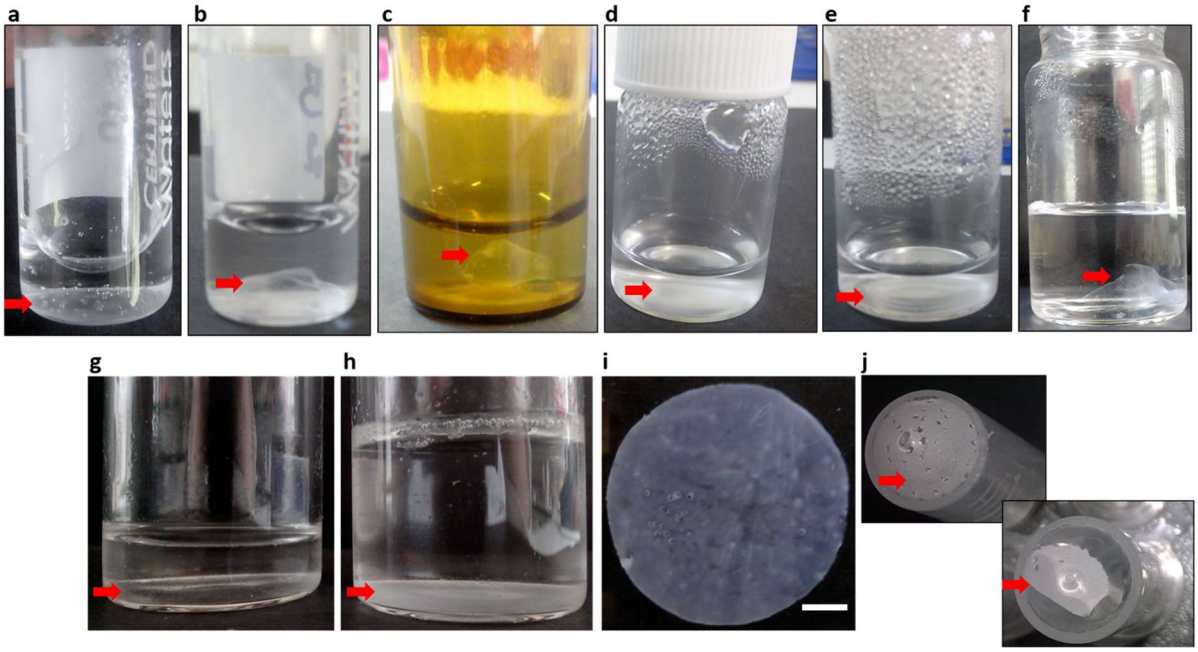

Figure S10. Scale-up experiments for DNA/dendrimer films prepared with G5 PAMAM dendrimer at a N/P ratio of 5. Experiments were made using flat bottom glass flasks with different bottom surface areas at a DNA concentration of 472 µg/cm^2: (a) 1.06 cm^2, (b) 1.06 cm^2 (after detachment), (c) 2.00 cm^2 (after detachment), (d) 3.76 cm^2, (e) 5.90 cm^2, (f) 5.90 cm^2 (after detachment). Experiments were also made using flasks with the same bottom surface area but at different DNA amount: (g) 5.90 cm^2 (472 µg/cm^2), and (h) 5.90 cm2 (1416 µg/cm^2). Lyophilized films: (i) film having 1416 µg DNA/cm^2 (after detachment and lyophilization), and (j) films on the bottom of microcentrifuge tubes (top - film attached to the tube, bottom - detached and curled film). The arrows are pointing to the films on the bottom of the flasks. Scale bar on (i) is 0.5 cm.

B. Experimental section

Reagents and materials

Poly(amidoamine) (PAMAM) dendrimers of generations 1, 2, 3, 4, and 5 (G1, G2, G3, G4, and G5 PAMAM) with an ethylenediamine (EDA) core and terminal amine groups (-NH_2), dissolved in methanol, were purchased from Dendritech Inc. (USA). Deoxyribonucleic acid sodium salt from salmon testes (Sigma-Aldrich, D1626), with a G-C content and T_m of 41.2% and 87.5°C (determined in a 0.15M NaCl and 0.015M sodium citrate solution), respectively, was used. Branched poly(ethyleneimine) (PEI) (0.8 and 25 kDa), linear PEI hydrochloride (10 kDa), poly(allylamine hydrochloride) (56 kDa), chitosan oligosaccharide lactate (5 kDa) and protamine from salmon (4.2 kDa, grade IV, histone-free) were also obtained from Sigma-Aldrich. All additional reagents and media were purchased from Sigma-Aldrich unless stated otherwise.

Preparation of dendrimer, DNA and buffer stock solutions

All solutions were prepared with ultrapure water (UP H_2O, resistivity 18.2 MΩ·cm) from the Milli-Q® Direct 8 water purification system (Merck Millipore) and subsequently filtered using a 0.20 µm cellulose acetate filter (VWR). Methanol was removed from the acquired PAMAM solutions by dialysis against water for 3 days, using pre-treated regenerated cellulose dialysis membranes (Spectrum® Labs) with the appropriate MWCO for each generation: 0.5-1 kDa, 1 kDa, 3.5 kDa, 6-8 kDa and 12-14 kDa, followed by lyophilization in a FreeZone 4.5 Liter Console Freeze Dry System (Labconco®). Stock solutions of each PAMAM and DNA were prepared at concentrations of 50 mg/mL and 10 mg/mL, respectively. The DNA solution was vortexed and left for 24 h at 4°C to ensure complete dissolution, resulting in a highly viscous solution. One hundred millilitre of a 10x PBS stock solution was prepared using 8.00 g NaCl (FisherScientific), 0.20 g KCl, 0.24 g KH_2PO_4 and 1.44 g $Na_2HPO_4{\cdot}12H_2O$ (all three from Panreac). Magnesium-containing Dulbecco's PBS was obtained from Sigma-Aldrich (D5773). The Tris-EDTA buffer stock solution (10x TE) was prepared at 100 mM Tris and 10 mM EDTA-Na (FisherScientific) and adjusted to pH = 7.5 with HCl (FisherScientific).

Determination of the molecular mass of DNA

The base pair number of the DNA was determined by agarose gel electrophoresis. Briefly, 2 µL of a DNA aqueous solution at 1 µg/µL was mixed with 14.7 µL of DNAse/RNAse-free water (GE Healthcare Life Sciences), followed by 3.3 µL of 6x Blue Loading Buffer (Fermentas) to obtain a final volume of 20 µL. Subsequently, 10 µL of this mixture was loaded onto a 1% (w/v) agarose gel in 1x TAE (48.4 g Tris base, 1.14 mL glacial acetic acid, and 2 mL of 0.5 M EDTA, pH = 8, all from Fisher Scientific), containing 0.06 µL of 10 mg/mL ethidium bromide (EtBr, MPBio) per mL of gel. A DNA fragment size standard, GeneRuler™ 1 kb DNA ladder (Fermentas), was loaded according to the manufacturer's instructions. The electrophoresis was performed using an EC Minicell Primo EC-320 horizontal gel system (Thermo Scientific) at 75 V for 30 - 45 min and documented using a GelDoc™ EQ Imaging System Model 170-8060 (BioRad). The DNA base pair size was determined using the DNA ladder migration standard curve.

Established (optimized) protocol for DNA/dendrimer film preparation

An optimized protocol for gel preparation in microcentrifuge tubes was established. To start, 50 µL of the DNA stock solution (500 µg of DNA) were added to 2 mL microcentrifuge tubes (SC - SuperClear™, VWR), corresponding to 472 µg of DNA per cm^2 of the tube bottom surface area. Next, considering the desired final volume of 500 µL and an intended final DNA concentration of 1 mg/mL, 50 µL of 10x PBS solution (final concentration of 1x) were added, along with the adequate volume of UP H_2O, accounting for the volume of the dendrimer solution that would later be added, which depended on the desired N/P ratio. The pH of the prepared DNA solution (DNA + PBS + UP H_2O) was then adjusted to 6.00 ± 0.05 using the necessary amount of 5 M NaOH (Merck) or 6 M HCl. The adjustment was carried out using a SympHony

SB21 pH meter (VWR) equipped with a LoT406-M6-DXK puncture pH electrode (Metter Toledo) designed to fit into microcentrifuge tubes. Between measurements, the electrode was always rinsed with distilled water, followed by ethanol, and dried. The pH-adjusted DNA mixture (tubes closed) and dendrimer stock solution (containing enough volume for all the desired N/P ratios) were heated at 99°C for 10 min using a ThermoStat™ Plus thermoblock (Eppendorf). The necessary volume of dendrimer was then added to the heated DNA solution, mixed, and homogenized by "up and down" fluid motion using the micropipette. After mixing, the tubes were allowed to cool using the thermoblock, with the following controlled cooling temperature ramp (CTR) program: 99°C (5 min) → 87°C (5 min) → 68°C (5 min) → 50°C (5 min) → 24°C (5 min) → room temperature. The presence of the film was confirmed visually and by gently touching it with a micropipette tip. Once formed, the films could be detached from the bottom of the tube as a single piece.

Confirmation of the presence of dendrimers in the films

To confirm the presence of dendrimers in the films, G4 PAMAM dendrimers labelled with fluorescent rhodamine B isothiocyanate (RITC) (G4-RITC) were used for film preparation. For this functionalization, lyophilized G4 dendrimers (163.90 mg; 0.115 µmol) were first dissolved in 4.5 mL DMSO (ThermoScientific). A 1.5x molar excess of RITC (9.94 mg; 0.173 µmol) was also dissolved in 0.5 mL of DMSO. The RITC solution was then added dropwise to the dendrimer solution and left to react for 24 h under vigorous stirring, at room temperature (RT). Dialysis was performed for 3 days against water using a 6-8 kDa MWCO dialysis membrane, followed by 3 days of freeze-drying of the RITC-labelled dendrimer. The ratio of RITC molecules per dendrimer was determined to be 1.01 through a RITC standard calibration curve in water using a UV-vis spectrometer (Lambda 25, PerkinElmer®). A film was then prepared using the establish protocol (500 µg of DNA, pH= 6 and 1x PBS) and the RITC-G4 dendrimer at a N/P of 5 in a clear glass vial, with heating and cooling stages performed in an incubator. Finally, the film supernatant was removed, and the film was washed with 1 mL of UP H_2O. The film was then kept in 1 mL of fresh UP H_2O. The colour change was visually detected, and the fluorescence emission was confirmed by placing the vial with the labelled film under red light (510-560 nm) using an Eclipse TE 2000E fluorescence inverted microscope (Nikon Instruments).

Confirmation of the presence of DNA in the films

To visually confirm the presence of DNA in the films, a film was prepared as previously described (500 µg of DNA, pH= 6 and 1x PBS) with G4 dendrimers, at a N/P ratio of 5. After film formation, the supernatant was removed, and the film was washed with 1 mL of UP H_2O and stained with 1 mL of a 20 µM solution of ethidium bromide (EtBr) in H_2O for 24 h, protected from light. After staining, the film was rinsed with water to remove excess EtBr. The colour change was observed, and fluorescence emission was confirmed by placing the vial with the EtBr-stained film under blue light (330-380 nm) using the fluorescence inverted microscope.

Quantification of the incorporation of DNA in the films

To quantify the DNA incorporation efficiency during film preparation, multiple sets of films were prepared as previously described (500 µg of DNA, pH= 6 and 1x PBS) using G3, G4 and G5 dendrimers, and PEI 25kDa, at an N/P ratio of 5. The DNA content in the freshly prepared films was indirectly quantified by subtracting the initial amount of DNA used to prepare the film from the DNA quantified in the supernatant. For this, 300 µL of each supernatant was transferred to a microcentrifuge tube and diluted to a final volume of 1 mL with UP H_2O (the blank consisted of 300 µL of 1x PBS and 700 µL of UP H_2O). Absorbance was measured at 260 nm, using a Lambda 25 UV-vis spectrometer (PerkinElmer®), considering n=5.

Evaluation of the impact of the experimental parameters on film formation

To evaluate the impact of several experimental conditions on the formation of the hybrid DNA films, several parameters were tested, namely: the maximum temperature applied, the ionic composition of the dsDNA solution, the cooling program, the DNA concentration, the initial pH of the dsDNA solution and the N/P

ratio, the dendrimer generation and the use of other polymers with varying molecular structure and weights. Film formation was confirmed visually, requiring the film to be mechanically resistant enough to be detached from the bottom of the tube in one piece.

a. Maximum heating temperature

Several sets of DNA solution were prepared according to the established protocol, with each set heated separately until different maximum temperatures, namely 25°C, 50°C, 68°C, 87°C, and 99°C. After adding the appropriate amount of G5 dendrimer to reach the desired N/P ratio, the mixtures in the microtubes were allowed to cool to room temperature following the established controlled temperature ramp.

b. Ionic composition of the dsDNA solution

Different solutions were tested to assess how variations in ion concentration and composition of the medium would affect film formation, namely ultrapure water, 0.15M NaCl, Tris-ethylenediaminetetraacetic acid (Tris EDTA) buffer (10 mM Tris-HCl, 1 mM EDTA), PBS solution (137 mM NaCl, 2.7 mM KCl, 1.8 mM KH_2PO_4, and 4 mM Na_2HPO_4, without Ca^{2+} and Mg^{2+}) and magnesium containing PBS (0.5mM Mg^{2+}-PBS). Following the established protocol and using the G5 dendrimer at N/P=4, 50 µL of the DNA stock solution was added to the microtubes, followed by 50 µL of the different 10x concentrated solution (replacing the standard 10x PBS solution) and the appropriate volume of UP H_2O, to achieve final concentrations of 0.15M NaCl, 1x TE or 1x PBS. The pH value was then adjusted to 6.00 ± 0.05 using 5 M NaOH (Merck) or 6 M HCl and the samples were heated. The heated G5 dendrimer solution was added, and the mixture was allowed to cool following the established controlled temperature ramp.

c. Cooling program

The optimized protocol for film formation was generally used, except that different cooling procedures were assayed, namely: tubes were immersed in ice (0°C); tubes were left to cool at room temperature (RT); and tubes were cooled under a controlled temperature ramp (CTR: 99°C (5 min) → 87°C (5 min) → 68°C (5 min) → 50°C (5 min) → 24°C (5 min) → RT). G5 dendrimers were used in these studies at N/P= 4.

d. Electrophoresis assays (to study the effect of the presence of magnesium ions and the effect of the cooling program)

Several sets of DNA solutions, each with multiple replicates, were prepared according to the established protocol (without the addition of dendrimers). One set was prepared with the standard PBS solution and the other with the Mg^{2+}-PBS solution. Briefly, solutions containing 2 µg of DNA in 1x PBS (with or without Mg^{2+}) were prepared to a final volume of 16.7 µL. These solutions were then heated at 99°C for 10 min and subjected to different cooling processes: on ice (0°C), at room temperature (RT) or using the controlled temperature ramp (CTR). Additionally, samples that did not undergo the heating/cooling steps (no-heat controls) and those subjected to film-forming conditions with the addition of G5 PAMAM at N/P=4 (dendrimer control) were also considered. Afterward, 3.3 µL of Blue Loading Buffer was added to each sample to obtain a final volume of 20 µL. From each of the prepared solutions, 10 µL was loaded into a 1% (w/v) agarose gel, and electrophoresis was carried out and documented as previously described.

e. Initial pH of the DNA solution and N/P ratio

The optimal initial pH of the DNA solution (DNA + PBS + UP H_2O) was evaluated by adjusting it to different values (5.00, 6.00, 7.00 and 8.00 (± 0.05)) using HCl (6M) or NaOH (5M) solutions. The optimized protocol for film formation was followed using G3, G4 and G5 dendrimers at different N/P ratios (1 to 10).

f. DNA concentration

The effect of different DNA concentrations on film formation was evaluated using G5 PAMAM dendrimers. DNA solutions at final concentrations of 0.2, 0.6, 1.0 and 2.0 mg/mL were prepared and the optimized protocol to form films was used.

g. Dendrimer generation (low generation dendrimers)

The effect of dendrimer generation on film formation was evaluated using G1 and G2 dendrimers at different N/P ratios, ranging from 0 to 10. The optimized protocol to form films was used.

h. Other cationic polymers

The following polymers were used: linear poly(ethylenimine) (10 kDa), branched poly(ethylenimine) (0.8 kDa, similar to G1 PAMAM, and 25 kDa, similar to G5 PAMAM), linear poly(allylamine) hydrochloride (56 kDa), linear chitosan oligosaccharide lactate (5 kDa), and protamine from salmon (4.2 kDa). All stock solutions were prepared at a concentration of 50 mg/mL and allowed to dissolve for 24 h at 4°C. Film preparation was performed following the optimized protocol at an N/P ratio of 4.

Characterization of DNA/dendrimer-based films

a. Stability over time

The stability of films prepared with G3, G4, and G5 dendrimers, at a N/P ratio of 4, was studied in PBS solution (pH= 7.4, after 3.5 months) and in PBS solution containing 10% (v/v) of foetal bovine serum (pH= 7.4, after 3 weeks). Films prepared with 500 µg of DNA were prepared and their content in DNA was determined as previously described (by reading the absorbance at 260 nm in the supernatant). Then, after discarding the supernatant, films were washed with 1 mL of filtered UP H_2O and immersed in 1 mL of PBS solution or PBS+FBS solution. The films were then placed inside the incubator at 37°C. Blanks without films were also prepared and kept in the same conditions. After the designated period, the absorbance of the solution was measured at 260 nm, and the amount of released DNA was determined. The percentage of DNA released was calculated based on the initial DNA content of the films (n=5).

b. Stability under extreme pH variation

The stability of the films was evaluated under extreme alkaline and acidic conditions. To do this, several films were prepared using DNA and G5 PAMAMs (N/P ratio 3 to 5). Briefly, two sets of films were prepared in 2 mL microcentrifuge tubes, one for testing extreme alkaline conditions and the other for testing extreme acidic pH conditions. Then, 10 µL of 1 M NaOH or 10 µL of 1 M HCl was added to the corresponding set tubes and changes were recorded. For imaging purposes, two additional films were prepared (G5 dendrimer, N/P ratio 4), washed with 1 mL of UP H_2O, and placed on glass coverslips. Afterwards, 10 µL of 6 M HCl was added to one of the films, and 10 µL of 5 M NaOH was added to the other. These films were observed under an inverted microscope starting from the moment of addition of the acid or the base (see the **Movie** in ESI).

c. Zeta-potential (ZP) measurements

To determine the surface zeta-potential of the films, thicker and more handling-resistant films were prepared. Accordingly, DNA/dendrimer-based films (using G3, G4, and G5 PAMAM dendrimers at N/P ratio of 4) were prepared in Ø =2.2 cm glass flasks using a DNA density of 1416 µg/cm^2. The solutions were heated in an incubator at 99°C for 1 h, then left to cool at 15°C/h to RT. Surface ZP measurements were conducted using an ElectroKinetics Analyzer (EKA, Anton Paar). The films were positioned and immobilized in the sample holder using warm paraffin wax at low temperature, ensuring it was not too hot to avoid any DNA film alterations. The wax was injected into the holder, filling the gap between the film and the holder to provide a solid, flat surface. The prepared sample was then positioned in the equipment, and a poly(methylmethacrylate) (PMMA) clamping cell was placed on top of the film, with the microchannels facing it. A 1 mM KCl solution (pH = 5.5 ± 0.2) was used to measure two ZP values for each film (left and

right flow direction), at a pressure of 200 mbar and temperature of 25°C. Measurements were also performed with a PMMA block as a standard reference for calibration. Three measurements were recorded for each sample, with results presented as mean ± S.D. (n=3).

d. Circular dichroism (CD) measurements

For circular dichroism (CD) measurements, DNA/dendrimer films (using G3, G4, and G5 PAMAMs at N/P ratios of 3, 4, and 5) were prepared according to the optimized protocol in 2 mL microcentrifuge tubes. The films were removed from the tubes, placed in a cuvette with detachable windows (QS, 0.1 ± 0.005 mm path length, 26 µL, Hellma), and then transferred to a J-815 CD spectropolarimeter coupled with a Peltier-Type Cell (PTC)-423S (Jasco Inc.). Measurements were performed at 20°C, from 340-200 nm (0.2 nm increments), standard sensitivity, DIT 0.2 s, 50 nm/min speed, and 9 accumulations, without baseline correction.

e. Scanning electron microscopy (SEM)

SEM analysis was performed using lyophilized hybrid films. Several films using G3 at a N/P ratio of 4 were prepared on glass coverslips with a diameter of 1.3 cm, at a DNA density of 472 µg per cm^2. After cooling, the films were washed twice with 2 mL of filtered UP H_2O and lyophilized. After gold-sputter coating, images were recorded using a FEG-SEM equipment (JEOL 7001F) at accelerating voltages of 15 - 20 kV under high vacuum. Magnification ranged from 100x to 10,000x. Films were also analysed using a benchtop SEM (Phenom ProX) without gold coating, with a charge-reduction sample holder and an accelerating voltage of 10 kV.

f. Elasticity measurements

Films were prepared with G4 (N/P=4) and G5 (N/P=3, 4, and 5) dendrimers, in triplicate, using glass flasks with a bottom surface area of 3.76cm^2 and 1416 µg of DNA/cm^2 (this corresponded to three-fold the DNA amount indicated in the standard protocol, in order to obtain films that were easier to handle). The films were prepared as described bellow (scale-up of the films), washed three times with UP H_2O and kept at 4°C in UP H_2O until analysis. Then, they were transferred into the membrane grip sample holder, left to airdry, and their shear storage modulus (G') was measured using a dynamic non-destructive non-contact mechanical analyser (ElastoSens Bio™, Rheolution Instruments). The G' was measured at 25°C, with the average of measurements taken over 120 seconds recorded (data acquisition every 5 seconds). Results are presented as mean ± s.d., with n = 3.

g. Cytocompatibility evaluation

Cytocompatibility studies were performed using DNA films prepared with G3 dendrimers, at a N/P ratio of 4, prepared in glass coverslips. For that, the glass coverslips (Ø= 1.3 cm) were previously cleaned in 1M HCl at 50 - 60°C for 4h, washed with filtered UP H_2O, sonicated for 30 min and finally autoclaved. Then, the glass coverslips were placed in glass sample flasks (Ø= 2.74 cm) and the films were prepared using 472 µg/cm^2 DNA as described below (scale-up of the films). After removal of the supernatants, the films were washed twice with 2 mL of DNAse/RNAse-free water (Panreac AppliChem). The film-containing coverslips were then transferred into 24-well plates (Nunc) and washed with PBS prior to cell seeding. NIH 3T3 fibroblast cells (DSMZ, Germany) were then seeded on the top of the films at a density of $5x10^5$ cells/well, in a final volume of 1 mL of complete DMEM (medium supplemented with 10% FBS and 1% Antibiotic-Antimycotic solution, all from Gibco). Cells were incubated at 37°C, in a humidified atmosphere containing 5% CO_2. Control experiments were done in wells without coverslips. After 24h and 48h-post seeding, the cell cultures were observed in an TE2000 Nikon Eclipse inverted microscope (100x final magnification), and cell viability was evaluated using a metabolic activity assay, namely the resazurin reduction assay. Briefly, after 24h- and 48h-post seeding, the cell culture medium in each well was replaced by 1 mL of fresh complete DMEM containing 10% (v/v) of a 0.1 mg/mL resazurin solution, and cells were further incubated for 3 h. Finally, 100 µL of the cell culture media in each well were removed and placed in an opaque 96-well plate. Resorufin

fluorescence (λ_{ex}= 530 nm, λ_{em}= 590 nm) was measured using a microplate reader (Victor^3 1420, PerkinElmer). Results are presented as mean ± s.d., with n = 4.

Scale-up of the films

Although microcentrifuge tubes were used in the initial film preparation process, the ability to obtain scale up film production is important for potential practical applications. The feasibility of producing larger surface areas and thicker hybrid DNA/dendrimer films was further explored. Scaling-up experiments were conducted in glass vials with progressively increasing flat bottom surface area (1.06 cm^2, 2.00 cm^2, 3.76 cm^2 and 5.90 cm^2) using G5 PAMAM at N/P ratio of 5. The initial DNA content was controlled by adjusting the volume of reagent solutions in proportion to the available flask bottom surface area for film deposition, while maintaining a constant concentration and a ratio of 472 µg of DNA per cm^2. For thicker films, the ratio was increased to 1416 µg of DNA per cm^2, tripling the volume of each of the component. In this setup, the incubator (UM200-Memmert GmbH or T6030-Heraeus model) was set to an initial temperature of 100°C with a cooling rate of 15°C/h. Film formation was confirmed visually and by detaching it from the bottom of the vial in one piece.