Supplementary Materials

Comparison of Modifications of Cellulose for the Extraction and Elution of DNA †

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A) Full List of Materials and Experimental Details of Methods

1.1. Materials and Apparatus

The following materials were used in this study: Whatman® no. 1 cellulose paper; ddH_2O; sodium hypochlorite (NaClO), 2,2,6,6-Tetramethylpiperidine 1-oxyl, 2,2,6,6-Tetramethyl-1-piperidinyloxy (TEMPO, 99%, Sigma-Aldrich, 426369-5G); potassium bromide (KBr, 99%, Merck, SAAR5041500EM, 500g); N-N'-ethyl-carbodiimide hydrochloride (EDC, 98%, Fluka Analytical, 03540-5G); N-Hydroxysuccinimide (NHS, 98%, Aldrich Chemistry, 130672-25G); Poly-L-Lysine hydrobromide (PLL, mol wt. 1000-5000, Sigma-Aldrich, P0879-100mg); hydrochloric acid (HCl, 32%, Merck); NaOH (97+%, Merk, 221465), LUDOX ®HS-40 colloidal silica (40 wt.% suspension in H_2O, Sigma Aldrich, 420816-1L); Triethoxymethylsilane (MTES, 99%, Sigma Aldrich, 175579-50G); Tetraethyl orthosilicate (TEOS, 98%, Sigma Aldrich, 131903-250ML); nitric acid (HNO_3, 70%), Ethanol (> 99%, Chemistry Department, Rhodes University); Guanidine Hydrochloride (99%, Sigma, G3272-100G); Deoxyribonucleic acid sodium salt from salmon testes (Sigma, D1626). Tris (Merk, 10708976001), EDTA (≥ 98.5%, Merk), MES (Sigma, M3671), Acetic acid (≥ 99%, sigma-Aldrich, A6283), (GelRed® Nucleic Acid Stain 10000× Water (Merk, SCT123), SeaKem® LE Agarose (Whitehead Scientific, 50004), Quick-Load® 1 kb Extend DNA Ladder (New Englans Biolabs, N3239S), Nuclease free water (Qiagen, 129115). Qubit™ dsDNA BR Assay kit (Invitrogen, Q32850).

The following apparatus was used in this study: Qubit 4 fluorometer (Invitrogen), NanoDrop Lite Plus Spectrophotometer (ThermoFisher Scientific, NDLPLUSGL), Gel Doc™ EZ Imager (Bio Rad), Vortex mixer (Lasec), Elmasonic S10H (Elma Schmidbauer GmbH, 90W), PowerPac™ Basic (Bio-Rad), Sub-Cell® GT (Bio Rad).

The following buffers were created: MES (15 mM), adjusted to pH 5.6 using NaOH pellets; Tris (10 mM) adjusted to pH 8 using HCl (10M, 32%); 1×TAE (40 mM Tris, 20 mM acetic acid, 1 mM EDTA (adjusted to pH 8 using NaOH pellets), pH 8.3).

For electrophoresis, 2% $^{w}/_{v}$ agarose gels were made using 1 g agarose, 50 mL TAE buffer and 5 μL of 10000× GelRed®. Electrophoresis proceeded at 100 V for 30 min in TAE buffer. Visualization was conducted by Gel Doc™ EZ Imager using UV transillumination.

1.2. Methods

1.2.1. Preliminary salmon sperm DNA extraction using cellulose

Salmon sperm DNA was sonicated to produce a range of differently sized fragments [20]. Salmon sperm double stranded DNA was dissolved in ddH2O to achieve a concentration of 20 ng/μL, assayed using the Qubit 4 fluorometer (Invitrogen). A 1.1 mL solution of this stock was suspended in ddH_2O in the bath ultrasonicator and sonicated (P: 90W; IP:20). At set time intervals (0, 5, 10, 15, 20, 30, 40, 50, and 60 min), 100 μL of the sample was removed and frozen at −20°C overnight. Samples were thawed and electrophoresed and visualized.

For subsequent experiments, a 450 ng/μL salmon sperm DNA sample was created and fragmented using 60 min ultrasonication, as detailed above.

1.2.2. Cellulose Paper Treatments

Whatman® no. 1 filter paper was cut into rectangles of 7 × 9cm, each weighing ± 0.58 g. Both unmodified and modified forms of cellulose were used for subsequent studies.

1.2.2.1. Unmodified cellulose

Each paper was rinsed 5 consecutive times with ddH_2O, then dried in the oven for 30 min at 50°C. It was subsequently stored in a dry environment until ready for use.

1.2.2.2. Poly-L-Lysine Cellulose Treatment

TEMPO-Oxidized Cellulose (TOC) was created by weighing 0.58 g of cellulose paper and adding to 100 mL of distilled water in a beaker. It was shaken using a rotary shaker for 10–15 min. Sequentially, the following reagents were added to the cellulose: NaClO solution (8 mmol/g cellulose), KBr (0.1157 g), and TEMPO (0.016 g), and the reaction shaken continuously for 2.5 h. The TOC was washed by repeatedly rinsing (5 times) with distilled water [16]and dried in the oven for 1 h at 55°C.

A 6 × 8.5 cm piece (0.5 g) of dried TOC was added to 10 mL of MES (15 mM) adjusted to pH 5.6 with NaOH and placed on a shaking table. Sequentially, 2.5 g of EDC, 1.5 g of NHS, and 22.5 mg of PLL-HBr were dissolved in the MES buffer containing the TOC. The pH was adjusted to 5.6 using small amounts of HCl. The reaction was allowed to proceed for 24 h at room temperature with continuous shaking. After the reaction, the paper was dried in the oven at 55°C for 1 h before the paper was stored in a dry environment until ready for use [16].

1.2.2.3. Silica Cellulose Treatment

The silica solution was prepared by mixing 43.39 mL TEOS and 46.14 mL MTES (methyltrietoxysilane, ABCR, 97%) with 10.16 mL colloidal SiO2 nanoparticles suspension (Ludox-4S, Aldrich, aqueous suspension 40 wt%, particle size 20 nm, pH 9). The solution was stirred for 30 min, after which 0.29 mL of concentrated nitric acid HNO3 (VWR, 65%) was added to 100 mL of solution. Finally, 0.02 mL absolute ethanol was added to the solution to give a final silica concentration of 120 g/L, with 64 g/L of colloidal particles. The final molar ratio of the silica sol was TEOS/MTES/SiO2 = 0.4/0.46/0.14. The sol was deposited on the cellulose (pre-washed with ddH_2O and dried) by dip-coating for 1 min and further cured for 30 min in the oven at 55°C. After the incubation, the dipstick was washed with ddH_2O, as used above, to remove excess silica particles. The cellulose paper was dried for 1 h in the oven at 55°C, before being stored in a dry environment until ready for use [18].

1.2.2.4. Guanidine Cellulose Treatment

Cellulose was modified to produce TOC as detailed in Section A1.2.2.2 of this Supplementary Material.

A 7 × 9 cm section of TOC was suspended in 5 mL of nanopure water. 0.25 g of guanidine hydrochloride was dissolved in 7.5 mL of 0.8 M NaOH solution. The guanidine hydrochloride solution was added dropwise to the cooled cellulose. The mixture was shaken in the ice bath for 3 h, then at room temperature for an additional 12 h. The cellulose paper was washed four times with ddH_2O and dried at 55°C for 1 h [17].

1.2.3. Cellulose Dipstick Analysis

From each of the modified and unmodified cellulose surfaces created in Section A1.2.2 of this Supplementary Material, 0.5 cm × 1 cm rectangles were cut and attached to notched pipette tips to create dipsticks. Three separate dipsticks were created and used for evaluation of their capability to bind and subsequently elute the fragmented salmon sperm DNA created in Section S1B -Figure S2 of this Supplementary Material.

Each dipstick was dipped 15 times for 1 s each in a 100 µL sample containing salmon sperm DNA (sonicated for 60 min, conc ± 450 ng/µL), followed by dipping 5 further times in a 200 µL Tris (10mM, pH8) wash buffer for 1 s each and finally 15 separate 1 s dips in 100 µL of nuclease-free H_2O. Samples were subsequently stored at −20°C for further analysis [14].

Samples were electrophoresed on 1% w/v agarose gels containing 1× GelRed® for 30 min at 100 V prior to visualization. Gel was analyzed using ImageJ to calculate densitometry/DNA yield as a % brightness per lane.

B) Results—Figures

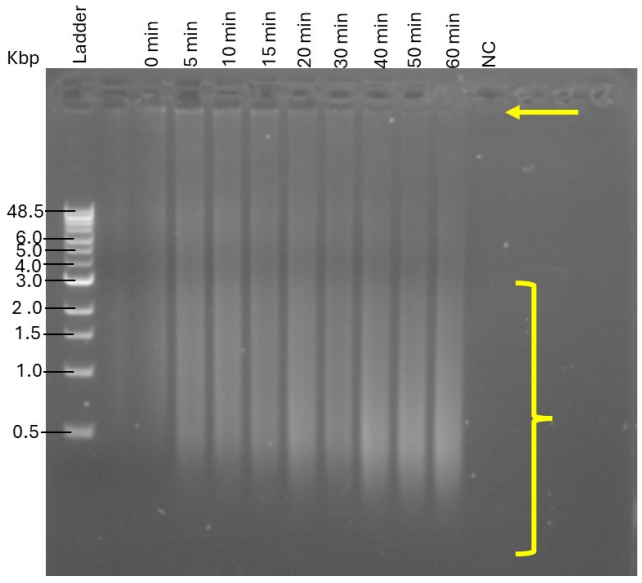


Figure S1. Salmon Sperm Sonicated for Different Time Intervals. 1% agarose gel run for 35min at 100V. Lanes: HR DNA Ladder, 0 min of sonication, 5min of sonication, 10min of sonication, 15min of sonication, 20 min of sonication, 30min of sonication, 40min of sonication, 50min of sonication, 60min of sonication, negative control (NC—ddH_2O sample).

Increased sonication of the sample resulted in the increased production of lower-range molecular weights (yellow bracket), accompanied by a decrease in high-range molecular weights (yellow arrow), which can be seen in Figure 1, as reported by others [20].

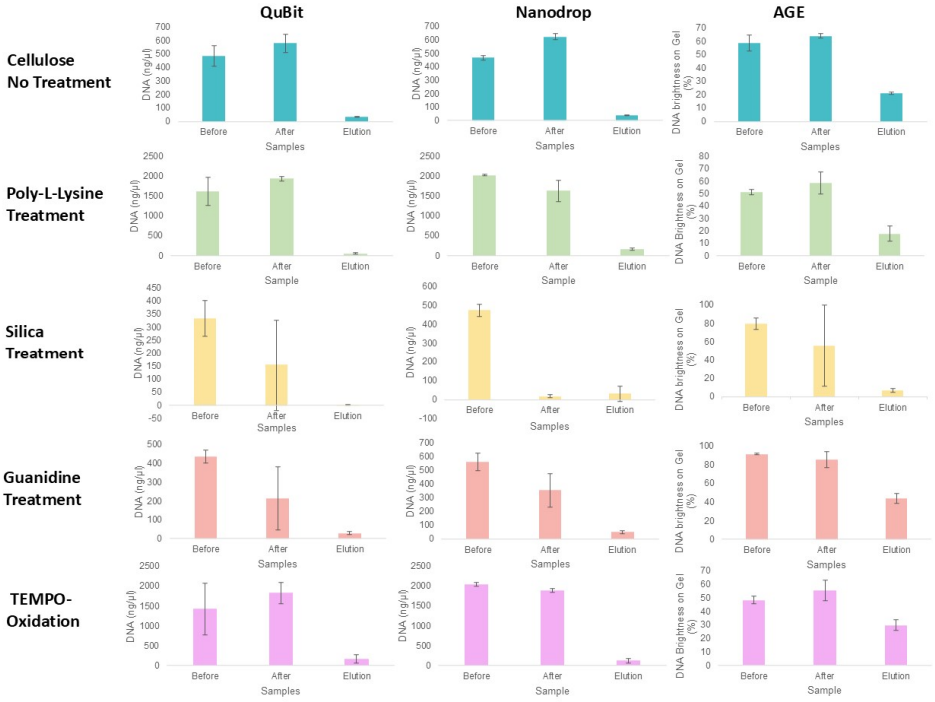


Figure S2. DNA Quantification of the Different Cellulose Dipstick Tests Using Different Methods. The Qubit 4 fluorometer (ng/µL), the Nanodrop (ng/µL) and agarose gel electrophoresis (AGE) were used as different methods to quantify the amount of DNA in the samples: 1. before the dipstick was dipped in the sample, 2. after the dipstick was dipped in the sample, and 3. once the DNA was eluted from the dipstick. This was performed for each of the cellulose treatments where N = 3.

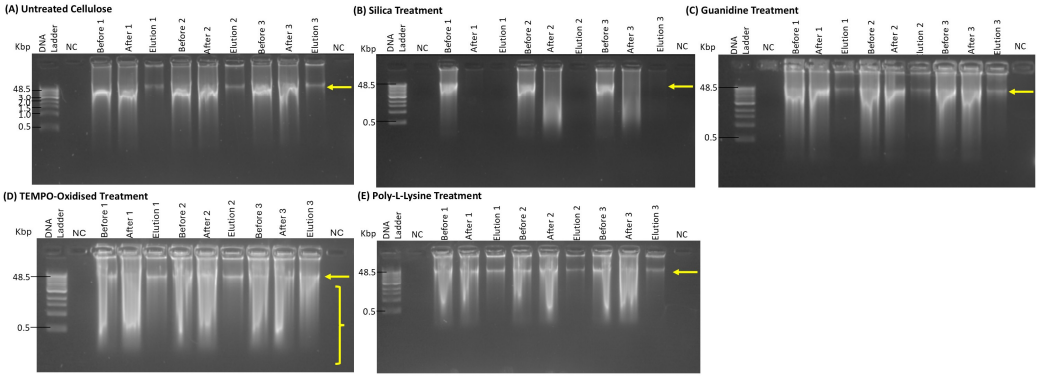


Figure S3. Representative examples of AGE densitometry analysis of size-dependent DNA binding and/or elution by the tested cellulose modification dipsticks. Samples were electrophoresed on 1% w/v agarose gels containing 1× GelRed for 30 min at 100 V prior to visualization. A. Untreated cellulose. B. Silica-treated. C. Guanidine-treated. D.TEMPO-oxidized-treated. E. Poly-L-Lysine (PPL)-treated.

Lane annotations: high range DNA Ladder, negative controls (NC—ddH_2O sample), sample before cellulose dip, sample after cellulose dip, and a sample taken after elution.