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DNA data storage in electrospun and melt-electrowritten composite nucleic acid-polymer fibers

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ABSTRACT

Incorporating biomolecules as integral parts of computational systems represents a frontier challenge in bio- and nanotechnology. Using DNA to store digital data is an attractive alternative to conventional information technologies due to its high information density and long lifetime. However, developing an adequate DNA storage medium remains a significant challenge in permitting the safe archiving and retrieval of oligonucleotides. This work introduces composite nucleic acid-polymer fibers as matrix materials for digital information-bearing oligonucleotides. We devised a complete workflow for the stable storage of DNA in PEO, PVA, and PCL fibers by employing electrohydrodynamic processes to produce electrospun nanofibers with embedded oligonucleotides. The on-demand retrieval of messages is afforded by non-hazardous chemical treatment and subsequent PCR amplification and DNA sequencing. Finally, we develop a platform for melt-electrowriting of polymer-DNA composites to produce microfiber meshes of programmable patterns and geometries.

1. Introduction

Data centers are responsible for about 2 % of global greenhouse gas emissions [1], with their electricity consumption exhibiting an exponential growth trend. It is, therefore, necessary to explore alternative information storage media that are both space- and energy-efficient. Many data storage technologies exist beyond conventional magnetic data storage, including optical storage, cloud-based solutions, and holographic storage methods. Notably, encoding information into deoxyribonucleic acid (DNA) molecules is emerging as a potential frontier in data storage technology due to its durability, data density, resiliency, and low maintenance cost. Drawing a parallel with cloud storage, prospective technologies include DNA data storage [2] and barcoding in plants [3] and cells [4,5], which could be envisioned as ubiquitous methods for disseminating information. In the realm of material science, the 'DNA-of-things' (DoT) data storage architecture is evolving towards materials endowed with immutable memory [6]. Recent advancements have shown optimized digital data storage on a dual-plasmid system in *E. coli* using genetic editing tools, improving storage density and allowing in vivo data rewriting [7,8]. This approach could move DNA storage closer to "warm storage", where data can be retrieved at a reasonable speed. DNA molecules are expected to store data at a density of up to a theoretical maximum of 215 petabytes (millions of gigabytes) per gram of material, far above conventional storage media [9]. Next-generation sequencing technologies, such as nanopore sequencing, have made reading this information more accessible, with current sequencing speeds reaching up to 450 base pairs per second (bp/s) [10].

Translating digital data into DNA requires the conversion of binary code, represented by 0s and 1s, into the four nucleotide bases - adenine (A), cytosine (C), guanine (G), and thymine (T) - that make up DNA strands [11]. Leveraging the customizable nature of DNA synthesis, it is possible to encode digital information into specific DNA sequences using various algorithms [12], such as the Goldman scheme [9] or the DNA Fountain [13]. Such encoding techniques offer an information-dense storage medium that can surpass magnetic and optical storage alternatives by approximately six orders of magnitude [4].

For DNA to serve as a storage system, a dry medium where oligonucleotides can be safely stored and retrieved is necessary. As shown with DNA recovered from fossils thousands of years old, the integrity of the molecule can be preserved at ambient temperature when stored in inorganic matrices [14]. Reported methods for DNA preservation are generally focused on "cold storage" (i.e., long-term) and encompass techniques such as sugar-coating, filter paper [15], encapsulation within silica particles [6], and magnetic nanoparticles [16]. Although these

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techniques enhance the half-life of DNA, they often compromise DNA density and ease of data recovery, frequently necessitating harsh chemical conditions for the retrieval process. Therefore, there is still a need to improve materials for DNA-based data storage, allowing, among others, the release of DNA under mild conditions, which would move DNA storage closer to the "warm"-type storage.

Synthetic polymer fibers offer an alternative matrix material for encapsulating DNA molecules. Solution electrospinning (SE) is an electrohydrodynamic process used to produce micro- and nanofibers from a variety of polymer materials. A high voltage causes a fluid jet to emerge from a viscous solution, creating a Taylor cone. This results in fibers of sizes ranging from several nanometers to micrometers in diameter [17]. Electrospun fibers can enable the stable and accessible encapsulation of nucleic acids. Previous works have shown the encapsulation of pure genomic DNA [18], and the incorporation of λ-DNA [19]. These electrospun fibers have primarily been used as a medium for DNA and drug release in the biomedical field, made to be easily dissolved under specific conditions whilst maintaining the integrity of the molecules [20]. However, using electrohydrodynamic processes to produce electrospun fibers for DNA digital data storage has not been explored to date. In contrast to DNA encapsulation following state-of-the-art procedures in pure SiO_2 particles, fibers can biodegrade, thereby facilitating access to embedded DNA without using expensive or hazardous reagents, such as phosphines or hydrofluoric acid. Electrospun fibers can provide stable and accessible encapsulation of nucleic acids by producing DNA and polymer blends that are non-toxic, environmentally friendly, and do not require energy or costly maintenance.

Melt-electrowriting (MEW) is an alternative electrohydrodynamic process that combines a melted polymer with the controlled movement of the printing nozzle to create two-dimensional patterns, usually of millimeter dimensions, through a printing process. MEW involves melting a thermoplastic polymer and using high voltages to deposit the molten polymer as fibers with diameters in the micrometer range. This versatile technique has been previously used to create scaffolds with predictable fiber diameters in the micrometer range for tissue engineering and biomedical applications [21]. Melt-electrowriting requires high temperatures to melt the precursor material, which can pose stability and integrity challenges for biomolecules like DNA, which is known to degrade at 130 °C [22]. However, thermoplastic polymers of low melting points, ideally well below 100 °C, can facilitate the synthesis of melt-electrowritten DNA-polymer composite fibers.

In this work, we incorporate digital information-bearing oligonucleotides into various polymer fibers and demonstrate a complete DNA data storage and retrieval workflow. We employ solution electrospinning and melt-electrowriting to produce polymer-DNA composite nano and microfibers of diverse geometries. Further, we show the release of DNA and the full recovery of the stored digital messages.

2. Results and discussion

2.1. DNA encapsulation in polymer fibers via solution electrospinning

We devised a method for storing DNA in polymer fibers, following the workflow shown in Fig. 1a. First, DNA was added to a polymer solution, followed by an electrospinning step. The resulting fiber meshes were collected on an aluminum sheet and extracted with tweezers for further analysis (shown in SI-1).

To test the feasibility of our DNA storage method, we first used a standard genomic DNA (salmon sperm) to encapsulate large amounts in electrospun poly(ethylene oxide) (PEO) nanofibers. The DNA was mixed with the polymer before the fiber was synthesized. DNA/PEO electrospun fibers were obtained from an aqueous mixture containing 0.2 % (w/w) dsDNA and 10 % (w/w) PEO (400 kDa). We carried out a morphological characterization of the fibers with optical microscopy, scanning electron microscopy (SEM) images, and atomic force microscopy (AFM) presented in Fig. 2, showing fiber diameters of 103 ± 65 nm (SI-2). To confirm the presence of DNA in electrospun fibers, we incorporated an intercalating DNA fluorescent dye (SYBRSafe) before the electrospinning process. The fibers were observed under UV illumination with a fluorescence microscope, see Fig. 2b. A control measurement without DNA but with the same amount of SYBRSafe did not show significant fluorescence, indicating successful DNA embedding in the polymer fibers (SI-3). We note that the DNA was double-stranded genomic DNA, and thus much longer than short single-stranded oligonucleotides that we employed for data storage experiments.

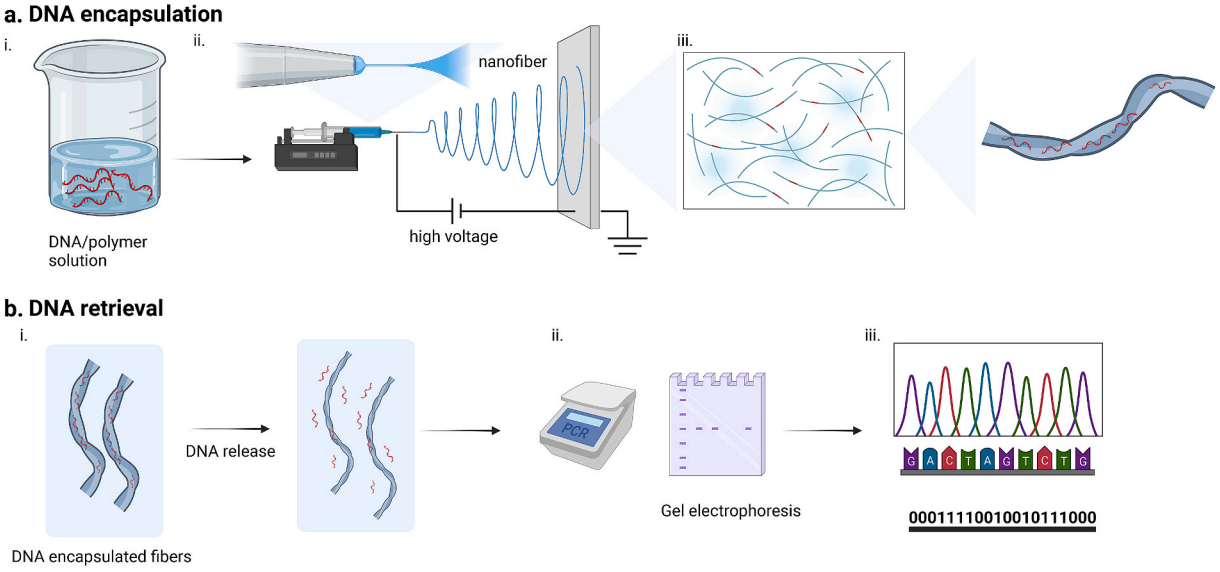

Fig. 1. a) DNA encapsulation process through solution electrospinning (SE) i) Polymer-DNA mix is prepared by DNA and dissolved polymer. ii) Experimental apparatus for fiber production, consisting of high voltage applied to a spinneret, syringe pump, and collector. iii) Electrospun fiber mesh and single fiber embedded with oligonucleotides. b) Retrieval and sequencing of oligonucleotides encapsulated inside fibers. i) DNA encapsulated fibers undergo a degradation process (e.g. heat, pH, sonication) to release oligonucleotides into solution. ii) Retrieved oligonucleotides are amplified by PCR, and products are analyzed by gel electrophoresis. iii) Recovered samples are sequenced and decodified.

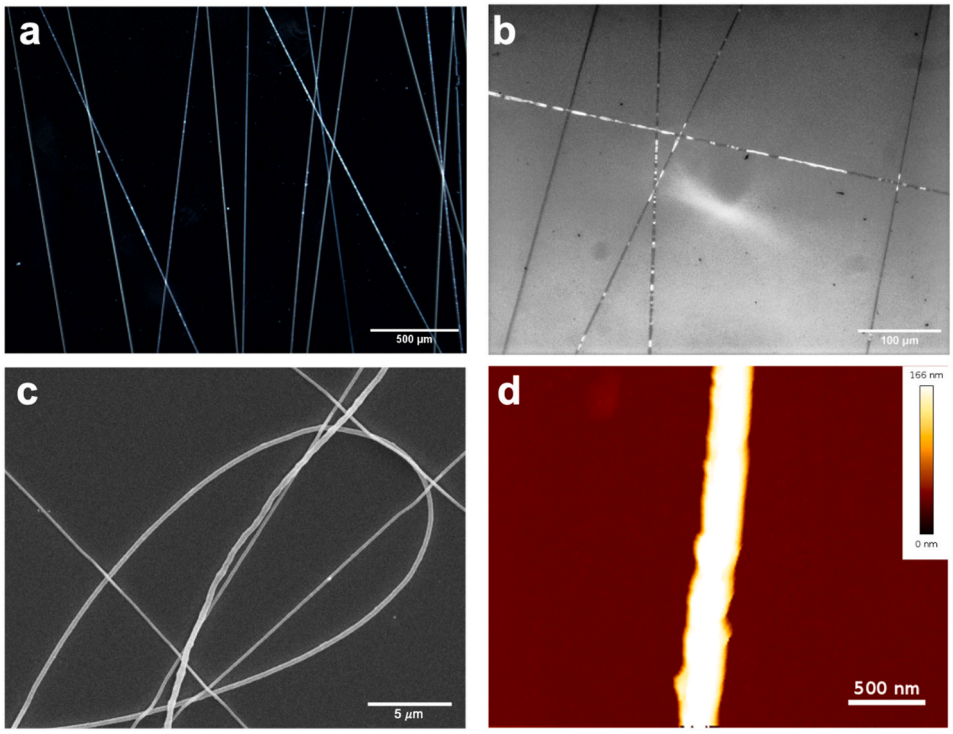

Fig. 2. Optical characterization of 10 % PEO - DNA (at 0.003 % (w/w)) hybrid electrospun fibers, collected on a microscope glass slide and observed with (a) dark-field microscopy, (b) fluorescence microscopy, (c) SEM, and (d) AFM. The DNA used for the fluorescence experiment in (b) was double stranded, genomic DNA at 0.2 % (w/w) and marked with SYBRSafe. Short single-stranded DNA was used for the other experiments.

2.2. *Controllable retrieval of DNA from electrospun nanofibers*

As shown in Fig. 1b, the encapsulated DNA is retrieved by immersing the fiber mesh, or randomly selected parts of it, into a suitable elution buffer. The DNA elution over time was measured by UV absorption, without heating or stirring, at a wavelength of 260 nm, which is the spectrophotometric analysis commonly used to quantify DNA [23]. The maximal absorbance corresponding to the complete DNA release after fiber dissolution was measured after heating the solution to 80 °C for 1 h , followed by 10 min of sonication. The release of DNA from PEO fibers is significant within the initial minutes of measurement, as seen in Fig. 3. This observation is consistent with the well-known high solubility of PEO in water, which can be attributed to its hydrophilic nature arising from the presence of ether groups in its repeating units [24]. While we demonstrate that the retrieval of DNA from polymer fibers does not require complex chemistry, this method does not offer precise control over the rate of message recovery. One evident parameter for DNA release rate modulation is the intrinsic solubility of the polymer.

In light of this, we carried out analogous experiments with two alternatives: poly(vinyl alcohol) (PVA, 146–186 kDa) and poly(caprolactone) (PCL, 45 kDa). We prepared polymer/DNA blends and produced DNA-encapsulated electrospun fiber meshes. PVA/DNA and

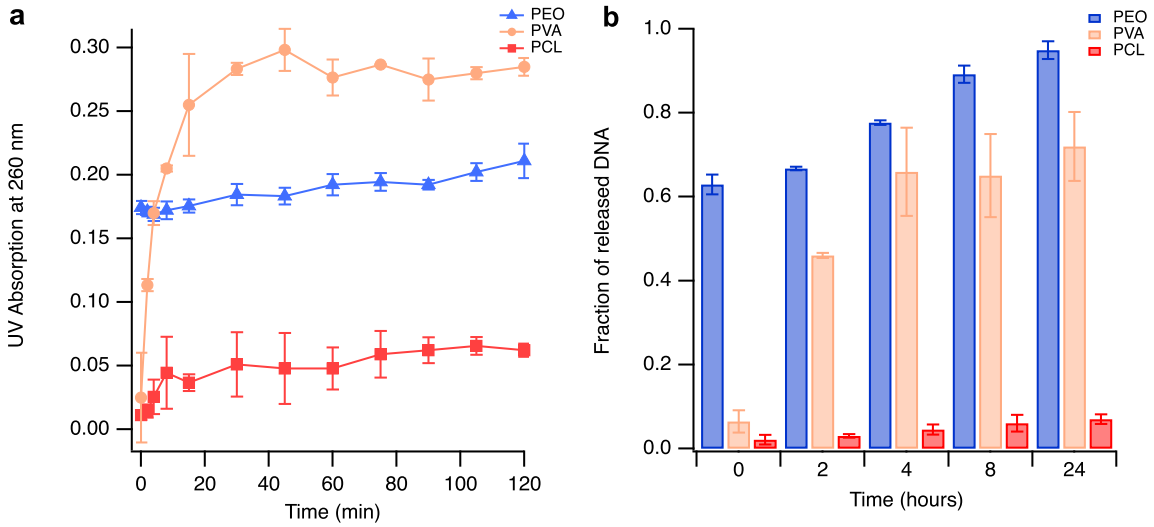

Fig. 3. a) UV–Vis spectroscopy peak absorbance at 260 nm as a function of time for PEO, PVA, and PCL electrospun fibers in solution. b) Fractional release of DNA over time for PEO, PVA, and PCL fibers.

PCL/DNA fibers were then dissolved in water to measure their release kinetics of DNA (Fig. 3a). Due to the variation in fiber amounts when transferring electrospun fibers, the total amount of DNA differs from one experiment to the other. We, therefore, normalized the DNA concentration by fitting a first-order rate release model $Q = Q_0(1 - \exp(-\kappa t))$ to extract maximum plateau values Q_0 and release rates κ (shown in SI-5). Fig. 3b shows the obtained normalized absorption peak at 260 nm in the first 24 h for all three polymer fibers.

As we hypothesized, the difference in the DNA release kinetic is largely dependent on the solubility of the polymer of choice. PEO releases the DNA almost immediately (more than 60 % at t = 0), and while it is more gradual in the case of PVA, we still observe an important passive release of DNA, already reaching over half of the total DNA content in 2 h. However, less than 10 % of the DNA is released from PCL even after 24 h.

The findings are consistent with the theoretical Hildebrand solubility parameters (δ) [25,26]. Solutions with similar δ parameters are typically miscible. Accordingly, the solubility parameters of PEO ($\delta = 22$ MPa$^{1/2}$) and PVA ($\delta = 30.5$ MPa$^{1/2}$) are proximate to that of water ($\delta = 47.8$ MPa$^{1/2}$) Further, the δ parameter of PCL ($\delta = 19.2$ MPa$^{1/2}$) aligns more closely with acetone ($\delta = 19.7$ MPa$^{1/2}$), in agreement with our experimental observations.

A finer tuning of the kinetics is afforded by chemical modification. We crosslinked poly(vinyl alcohol) (PVA) with glutaraldehyde (GA), which was reported to enhance its water resistance and modulate the release of DNA. (Hemi)-acetals are formed when the glutaraldehyde reacts with the hydroxyl groups present in polyvinyl alcohol, thus providing intra- and intermolecular covalent bonds [27]. We observed that crosslinked PVA slowed down the release of DNA by 50 % (SI-6). Still, the release is significantly faster than from PCL. We performed contact angle measurements to compare the water resistance of electrospun PVA fibers, crosslinked PVA fibers, and PCL fibers (SI-7). We observed no significant difference in water-resistance between the electrospun crosslinked PVA fibers and the non-crosslinked ones. In contrast, the PCL fibers exhibited high hydrophobicity, indicated by a contact angle near 100°. This makes PCL electrospun fibers better suited for storage in high-humidity conditions.

2.3. Encoding and retrieval of message-loaded oligonucleotides

We encoded a text file of 16 bytes (“Nanogune”) into DNA bases by first converting it to binary and mapping bit pairs to bases (A:01, T:11, G:10, C:00), as detailed in SI-8. The DNA message sequence, named “Mezu” hereafter, includes the encoded message with the forward primer and the complementary to the reverse primer at the 5′ and 3′ ends, respectively, allowing for PCR amplification. Message duplication within the sequence (total of 64 bases) is employed to aid decoding and minimize sequencing errors in short DNA strands. The two message iterations are separated by a 4-base sequence (CGCC) encoding a bar space that serves as the starting point of both the second iteration and the decoding process.

Electrospun nanofibers containing the message oligonucleotide “Mezu” were successfully synthesized using the previous method with PEO and PCL. The produced mesh of nanofibers was carefully collected into an Eppendorf tube and supplemented with 200 μL of deionized water or acetone, respectively, for dissolution. The obtained solution underwent PCR amplification and was subsequently evaluated using agarose gel electrophoresis. Fig. 4 shows bands corresponding to PCR products from DNA extracted from PEO and PCL fibers alongside negative controls (i.e., without the DNA template and fibers without DNA). Successfully amplified PCR products appear as distinct bands on the gel, whereas samples that contained no DNA do not show any bands. PCL fibers were successfully recovered using water and acetone. The signal of the amplified product was stronger after the acetone treatment, indicative of initially higher amounts of eluted DNA as the fibers were fully dissolved.

Amplified PCR products extracted from PEO and PCL fibers were sequenced with the Sanger method. As illustrated in Fig. 5, the results demonstrate a successful alignment between the PCR products and the reference sample sequence. Although the Sanger method’s inherent limitations prevent the sequencing of the initial bases—typically the first 40 to 60 nucleotides— the encoded message “Nanogune” was still accurately deciphered. This was achieved by reverse decoding the DNA sequence in the second iteration of the message back to its binary format after the space marker. Detailed information on the decoding process and the corresponding code is provided in SI-8. The results demonstrate that DNA is stable throughout the solution electrospinning fiber formation and PCR amplification steps, and we can retrieve the encoded

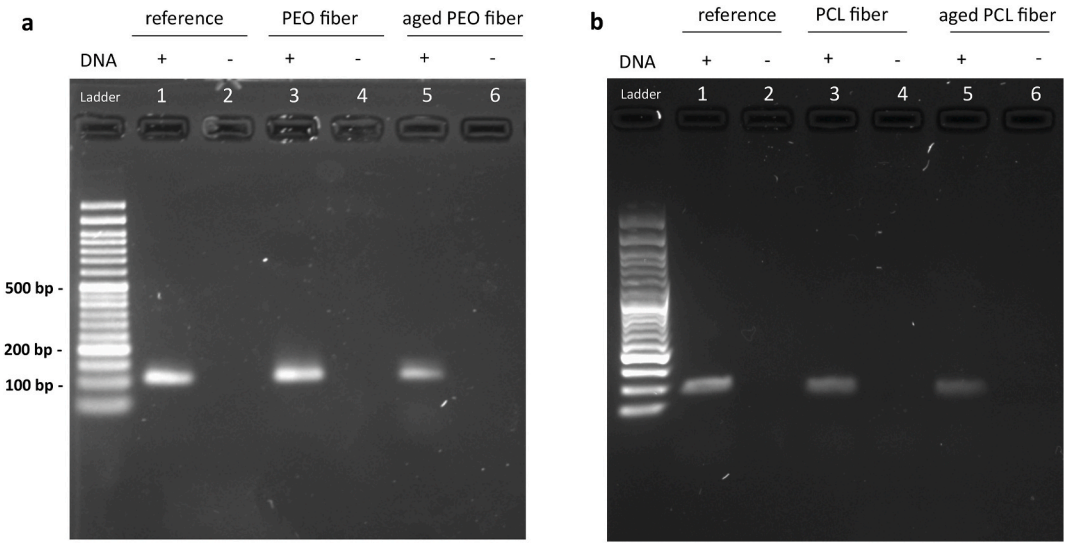

Fig. 4. DNA recovery and amplification. Agarose gel electrophoresis after PCR amplification of (a) PEO-DNA fibers and (b) PCL-DNA fibers. In each gel, showing the original reference DNA oligonucleotide (lanes 1 and 2), the retrieved oligonucleotide in the fiber prior to aging (lanes 3 and 4), the retrieved oligonucleotide in the fiber after aging (lanes 5 and 6). Amplified PCR products appear as distinct bands on the gel when the oligonucleotide message (reference) was present in the samples (marked as +), whereas samples that contained no DNA but were subjected to the same amplification protocol do not show any bands (marked as -).

Fig. 5. DNA sequencing and decoding of the message. The PCR samples run on the message DNA alone or in PEO and PCL fibers after dissolution or aging were subjected to Sanger sequencing. The resulting sequences and chromatograms were aligned to show message retrieval and integrity in the second iteration of our encoded message (underlined in the alignment). Highlighted in grey or red are the non-identified bases at the beginning and end of the sequences. The grey background on the traces shows the statistical reliability of the base call. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

message undamaged without errors.

2.4. Accelerated aging tests

To further assess our preservation method efficiency, we studied the DNA digital storage capabilities of the electrospun fibers on longer time scales. Amongst all the degradation mechanisms DNA molecules face, hydrolysis is the most prominent one [28], and as such, we need to determine whether the fibers can protect DNA from moisture and oxygen. Dry fibers, prepared according to the procedure described above for solution electrospinning, were subjected to high humidity and temperature in open Eppendorf tubes and on microscope slides inside an enclosed chamber. High humidity was produced using a saturated aqueous sodium chloride solution in an open Petri dish. The samples were left undisturbed until the relative humidity reached 80 %, as measured by a hygrometer (Testo 174H) placed inside the chamber. After the humidity stabilized, the samples were placed inside and left overnight. Subsequently, they were kept in an oven at 85 °C for an additional 24 h. The integrity of the deposited fibers was analyzed by optical microscopy, and the retrieved DNA from Eppendorf tubes was then characterized by PCR, analyzed by gel electrophoresis, and finally sequenced using the Sanger method, as described above.

The accelerated test degraded the PEO fibers, as seen in the micrograph in SI-9, while the PCL fiber maintained most of the original structure, with bead-like structures forming along the fibers, which can be attributed to the polymer degradation. However, in both cases, the integrity of the message was preserved, showing similar gel electrophoresis and sequencing results in Figs. 4 and 5. Moreover, we compared the lifetime of DNA in fibers to the oligonucleotide in its dry state. Accelerated aging was performed at 65 °C and 85 °C for both lyophilized DNA and DNA encapsulated in PCL fibers. The samples then underwent PCR and were analyzed by agarose gel electrophoresis (SI-9) to estimate the concentration of DNA left intact for PCR after high humidity and temperature treatment. The gel analysis reveals that although both conditions demonstrate a decline in DNA concentration at higher temperatures, the decay is more pronounced when the DNA is left dry. This indicates that DNA is more stable under PCL fiber conditions than without any additives. The aging tests show that the composition for data storage in polymer fibers is robust and provides protection from environmental factors.

2.5. Melt-electrowritten PCL/DNA microfibers

Melt-electrowriting (MEW) is a high-resolution biofabrication approach that combines electrospinning with 3D printing. Contrary to solution electrospinning, the polymer is dry and free of solvent (apart from some traces of water). The polymer is melted and kept at a precisely controlled temperature to adjust the viscosity. Applying voltages in the kV range shapes the melt into fibers with diameters in the micrometer range. To test the feasibility of melt electrowriting of DNA-polymer composites, we first mixed 0.003 % (w/w) DNA in PCL dissolved at 15 % (w/w) in acetone, following the same preparation steps as for solution electrospinning. The solution underwent a drying process to obtain PCL/DNA composite powder, which was then fed into a pneumatic extruder. PCL and DNA/PCL composite MEW fibers were created with an in-house MEW system (NovaSpider [29]). This apparatus utilizes controlled pneumatic pressure-driven extrusion at 0.2 bar via a metal nozzle onto a motorized collection platform. A 6 mm working distance was maintained, with −7 kV applied to the extrusion nozzle. The grounded collection plate was an aluminum surface. The PCL/DNA powder was heated to 100 °C. To avoid damage to DNA, a lower printing temperature of 95 °C was used, which still allowed for the melting of PCL. Fibers were produced in a controlled rhomboid shape (115 mm × 58 mm), following a G-code program (SI-10). The entire experiment was also carried out with a pure PCL sample as a negative control. The following experiments are based on the workflow established for solution electrospinning. Half of the rhomboid mesh (Fig. 6) was dissolved in acetone and amplified using the PCR protocol described previously. The amplified product was observed by gel electrophoresis (Fig. 6d) and sequenced, successfully recovering the encoded message (Fig. 6e), highlighting that under harsh, non-toxic conditions, the integrity of the message was preserved in the fibers.

3. Conclusion

Our work establishes a novel comprehensive DNA-polymer digital storage workflow, including the encoding of information, storage within polymer fibers, using controlled retrieval, and decoding via sequencing. We demonstrate DNA storage in a variety of polymers (PEO, PVA and PCL). At this stage of the study, the release rate of DNA can be modulated to a certain extent by the solubility of the polymer, by the solvent, and by chemical cross-linking of the polymer chains, still leaving room for further improvement and more precise control of DNA release. Among them, PCL fibers exhibit the most favorable water-resistance properties, which are beneficial for long-term “cold” DNA data storage, where data is not expected to be accessed frequently or immediately.

Our platform enables the design of polymer fiber-based DNA storage systems that can be modified for specific applications. We have demonstrated the printability of DNA-containing fibers using a melt-electrowriting apparatus to produce meshes of desired geometry with micrometer precision. We envision 3D melt-electrowriting and solution electrospinning of polymer fibers as production tools for substrates for DNA digital data storage.

In contrast to DNA encapsulation in pure SiO_2 particles, electrospun and melt-electrowritten polymer fibers can be made biodegradable and provide stable and accessible encapsulation of nucleic acids without the need for complex and hazardous chemical treatments. These fibers, comprising nucleic acid, are stable, especially under high humidity conditions, which usually cause the most damage to DNA. Therefore, we consider them suitable for storing digital data. Advantageously, the mechanical properties of the bulk polymer are readily translated to the fiber, thus allowing also for flexible materials. We believe that the introduction of functional groups will open many more options to finely tune properties. Further research in this direction could pave the way for a wide range of applications. Looking forward, we envisage this platform will enable a variety of polymer-DNA composites for medium (“warm”) and long-term (“cold”) DNA storage for different use cases. As DNA synthesis and sequencing of long and stable oligonucleotides become more accessible, using polymer fibers for high information storage density will become an affordable and accessible solution to the global challenge of exponentially growing data storage demand.

4. Materials and methods

4.1. Encoding and oligonucleotide design

A text file of 16 bytes is encoded into DNA bases by first converting it to binary and mapping bit pairs to bases (A:01, T:11, G:10, C:00). The oligonucleotide includes the encoded message “Nanogune” with a forward primer and the complementary to the reverse primer at the 5′ and 3′ ends , respectively, allowing for PCR amplification. Message duplication within the sequence is employed to aid decoding and minimize sequencing errors in short DNA strands. The two message iterations are separated by a 4-base sequence which encodes a bar space (in bold) and also serves as the starting point for the message.

Forward primer: TCCTCTACAGAGAGCAGGTT.

Reverse primer: ACGCAACTTCGAGACAGGTA.

Message: TCCTCTACAGAGAGCAGGTT**CGCC**ACTGAGCAAGTGAG TTAGATATAAAGTGAGAA**CGCC**ACTGAGCAAGTGAGTTAGATATA AAGTGAGAATACCTGTCTCGAATTGCGT.

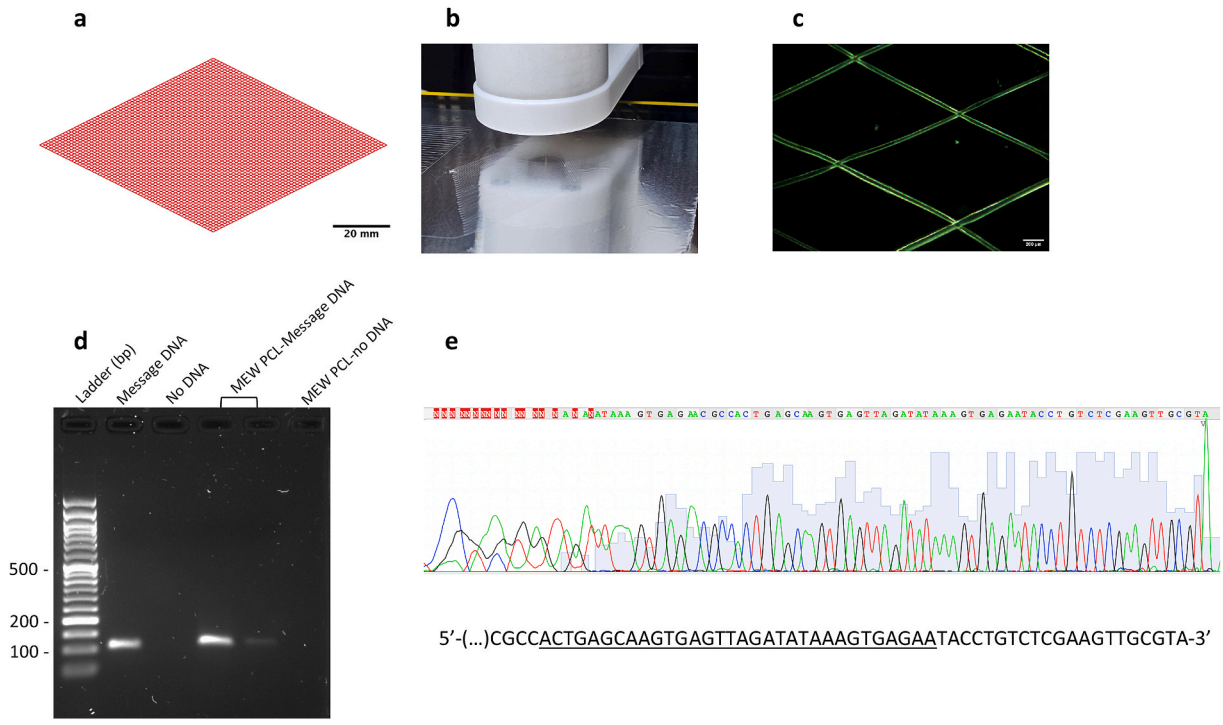

Fig. 6. Melt-electrowriting of DNA-loaded PCL and message retrieval. PCL mixed with the message oligonucleotide "Mezu" DNA is melt-electrowritten (MEW) (a) into a rhomboid shape collected on an aluminum foil (b). Dark-field optical microscopy (c) shows the controlled deposition of the fibers. After dissolution in acetone, we can specifically recover the DNA using PCR (d) and carry out Sanger sequencing (e) with the encoded message (underlined in the sequence.)

4.2. Chemicals

Deionized water was supplied from a Millipore system, continuously controlled for 18 MΩ cm and < 10 ppm TOC (total organic content). PCR primers (forward and reverse) and oligonucleotides containing the encoded message were purchased from Integrated DNA Technologies (IDT) and received in lyophylised form after standard desalting. All oligonucleotides were resuspended in deionized water to obtain a 100 μM stock and kept at 4 °C. Deoxyribonucleic acid, low molecular weight from salmon sperm (CAS:100403-24-5), Polyethylene oxide (PEO, Mw 400,000 Da, CAS:25322-68-3), Polyvinyl alcohol (PVA, Mw 146,000–186,000 Da, CAS: 9002-89-5) and Polycaprolactone (PCL, Mw 45,000 Da) were purchased from Sigma-Aldrich in dry form. The crosslinker Glutaraldehyde Grade I at 70 % in water (CAS: 111-30-8) and the surfactant Triton X-100 were purchased from Sigma-Aldrich. The DNA gel stain SYBRSafe was purchased from Invitrogen (Thermo Fischer Scientific).

4.3. Polymer/DNA mixture preparation

PEO/DNA mixture A homogeneous PEO/DNA mixture was prepared by combining 100 mg of PEO and 10 μL of message oligonucleotide at a concentration of 100 μM in water, resulting in final concentrations of 10 % (w/w) PEO and 0.0034 % (w/w) of DNA. The procedure was repeated without the addition of DNA to provide a control. For thorough mixing, the solution was left on a magnetic stirrer overnight at ambient temperature.

PVA/DNA mixture A PVA/DNA mixture was prepared with 80 mg of PVA and 10 μL of message oligonucleotide at 100 μM in deionized water, resulting in final concentrations of 8% (w/w) PVA and 0.0034 % (w/w) of DNA. Furthermore, Triton X-100 surfactant was introduced at a final concentration of 0.25 % (w/w) to minimize the surface tension, thereby facilitating fiber formation. The prepared mixture was subsequently left on a magnetic stirrer overnight for optimal mixing. For samples with cross-linked PVA, glutaraldehyde was added at 2% (w/w) to the polymer solution and stirred at ambient temperature overnight before the addition of DNA.

PCL/DNA mixture A PCL/DNA mixture was prepared with 1 g of PCL dissolved in 5.6 g of acetone for a final concentration of 15 % (w/w). This mixture was sonicated at a moderate temperature of 40 °C for an hour to ensure a uniform blend. Post-sonication, DNA was added at the same concentration as employed for the other two mixtures.

4.4. Electrospinning

The solution electrospinning (SE) experimental setup comprises a high-voltage power supply, a syringe pump, a Hamilton glass syringe with a metallic needle, and an aluminum sheet acting as a grounded collector (SI-1). A 5 mL Hamilton glass syringe was filled with 2 mL of the polymer/DNA mixture for electrospinning. The syringe with the needle was placed horizontally on the pump, and the needle tip pointed towards the collector. The flow rate was set to 0.35 mL h^{-1}. PEO/DNA and PVA/DNA fibers were electrospun at a positive voltage of +10 kV and PCL/DNA at +15 kV. The electrospinning process was carried out at ambient temperature in air. The fibers were collected on an aluminum foil placed 12 and 20 cm away from the tip of the needle, respectively. In addition, a microscope glass slide served as a secondary collection surface for subsequent optical characterization. Following collection, approximately 100 mg of the electrospun fibers were gathered with the aid of a pair of precision tweezers and subsequently transferred into an Eppendorf microtube for storage. All samples were preserved under controlled temperature conditions. For crosslinking of PVA, glutaraldehyde was added at 2% (w/w) to the polymer solution and stirred at ambient temperature overnight before the addition of DNA, adapted from the protocol in Ref. [30].

4.5. DNA passive release study with UV spectroscopy

After adding 200 μl of water to the electrospun fibers, considered time point 0, a sample of 2 μl was taken every 2 h for the first day, and

then at 24 h intervals. For each time point, the absorbance at 260 nm was measured with a Nanodrop spectrometer (Thermofisher) in triplicates. The maximal DNA quantity in each sample was estimated after full fiber dissolution: The fibers underwent heating at 80 °C for an hour, followed by 10 min of sonication before reading the absorbance again. A complete UV–vis spectrum was recorded for each polymer fiber in an aqueous solution, devoid of DNA, to determine the basal level of absorbance at 260 nm (SI-4).

4.6. PCR DNA amplification

The PCR reactions were carried out in the MiniAmpTM Plus thermal cycler (Applied Biosystems, ThermoFisher). Using the OneTaq HotStart, HiFidelity polymerase (New England Biolabs), the reactions were performed on 1 ng of DNA template or 10 μL of fibers-DNA mix, with forward and reverse primers at 0.5 μM final concentration in a reaction volume of 50 μL. The PCR reaction cycle started with an initial denaturation at 94 °C for 30 s, followed by 30 cycles of 10 s denaturation at 94 °C, 30 s annealing at 65 °C, 30 s of extension at 68 °C and a final extension at 68 °C for up to 3 min. PCR products were then analyzed by electrophoresis on 2 % agarose TBE 0.5X gels, pre-stained with SYBRSafe (10000X, Invitrogen) according to the manufacturer's protocol. For each sample, 5 μL was mixed with 1 μL of Orange Loading Dye (6X, Thermofisher) and injected in the gel well for migration at 80 V. We used a 50 bp molecular weight ladder (FastLoad, SERVA) as a reference. Images were recorded with a BioRad UV transilluminator.

4.7. DNA sanger sequencing

PCR products were sent for purification and Sanger sequencing to StabVida (Portugal), alongside the forward primer. Upon completion, StabVida provided chromatogram files containing raw sequence data and the quality of the sequencing results. We analyzed the results with the software 4Peaks [31], extracting the sequences for alignment with the reference ("Mezu" sequence).

4.8. Accelerated aging test

We subjected lyophilized DNA and the dry fibers in open Eppendorf tubes or on a microscope slide to high humidity and temperature in a closed chamber. The high humidity was created using a saturated sodium chloride solution in an open petri dish. The solution was left until it reached 80 % of humidity, as read by a data logger (Testo 174H) placed inside the chamber. Once the humidity was stable, we placed the samples inside and left them overnight before an additional 24 h at 65 °C, 70 °C or 85 °C. We then characterized the integrity of the stored DNA by PCR analysis, gel electrophoresis, and subsequent Sanger sequencing. To quantify DNA degradation, the fluorescence intensities of bands on agarose gel after aging were measured using ImageJ [32]. All samples were subjected to the same PCR protocol. We estimated their DNA concentrations based on fluorescence intensity, using dry DNA at room temperature as a reference. This reference DNA is known to correspond to a concentration of 16 μM, as measured by UV–Vis spectroscopy.

4.9. Melt-electrowriting (MEW)

A PCL and message oligonucleotide "Mezu" DNA mixture was prepared following the same protocol as for solution electrospinning. 2 mL of the solution was dried on a glass petri dish, and the obtained pellet was crushed into a powder. The sample was then directly melted at 100 °C in a pneumatic extruder in the NovaSpider tool. The temperature was set to 95 °C for the deposition of fibers produced from of the melts at −7 kV, 6 mm away from the collector (aluminum foil) at 0.2 bar. Half of the rhomboid-shaped fibers were collected into a tube and dissolved in acetone under sonication at 40 °C for an hour. The negative control consisted of the exact same experiment without DNA.

4.10. Fibers characterization

The fibers collected on a glass slide were characterized by four different instruments/methods. Firstly, we used dark-field microscopy (Leica DFC425) with a HC PLANS 10x/25 objective and a magnification of either 50x or 100x, and environmental scanning electron microscopy (FEI Quanta 250) for optical characterization and estimation of the diameter of the fiber. In the latter, fibers spun on a silicon wafer were sputtered with gold by a Quorum Q150T ES sputter coater (set at 25 mA for 30 s) to obtain a 10 nm thick layer prior to observation in high vacuum. The imaging conditions were 10 kV and a working distance of 8.7 mm. Images were then analyzed by Fiji, using the GIFT macro to determine the fiber diameter (see also SI-3). To determine the presence of DNA in the fibers, we employed fluorescence microscopy (Zeiss). Finally, atomic force microscopy (AFM) measurements were performed with the QI (quantitative imaging) mode of the JPK NanoWizard V (Bruker) with Multi75-5Ai-G cantilevers (75 kHz, 3 N m^{-1}). Three fibers of each sample (with and without DNA) were scanned. The setpoint force was set at 4 nN, with an acquisition speed of 20 μm s^{-1}. For water contact angle measurements, fibers electrospun on aluminum were placed as flat as possible on the KRÜSS G10 machine stage. Droplets of 5 μl were deposited and photographed.

CRediT authorship contribution statement

Diana Soukarie: Investigation, Writing - review & editing, Data curation, Methodology, Visualization. **Lluis Nocete:** Investigation, Software. **Alexander M. Bittner:** Methodology, Resources, Writing - review & editing, Funding acquisition. **Ibon Santiago:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing - original draft, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mtbio.2023.100900.

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