Long-term stabilization of DNA at room temperature using a one-step microwave assisted process

Claudia W. Narvaez Villarrubia¹ · Keyla C. Tumas² · Rajat Chauhan³ · Thomas MacDonald¹ · Andrew M. Dattelbaum¹ · Kristin Omberg¹ · Gautam Gupta⁴

Received: 21 January 2021 / Accepted: 10 March 2021 / Published online: 23 March 2021
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Abstract

Long-term stabilization of DNA is needed for forensic, clinical, in-field operations and numerous other applications. Although freezing ($<-20\text{ }^{\circ}\text{C}$) and dry storage are currently the preferential methods for long-term storage, a noticeable pre-analytical degradation of DNA over time, upfront capital investment and recurring costs have demonstrated a need for an alternative long-term room-temperature preservation method. Herein, we report a novel, fast ($\sim 5\text{ min}$) silica sol–gel preparation method using a standard microwave-initiated polymerization reaction amenable to stabilization of DNA. The method involves use of one chemical, tetramethoxy silane (TMOS) and eliminates the use of alcohol as co-solvent and catalysts such as acids. In addition, the process involves minimal technical expertise, thus making it an ideal choice for resource-challenged countries and in-field applications. The sol–gel is capable to store and stabilize *Escherichia coli* DNA in ambient conditions for 210 days. DNA recovered from the sol–gel showed no significant nucleolytic and/or oxidative degradation, outperforming conventional storage conditions at $-20\text{ }^{\circ}\text{C}$, and reported state-of-the-art technology.

Keywords Silica · Sol–gel · Immobilization · *Escherichia coli* DNA · Room temperature · PCR

1 Introduction

Both short- and long-term storages of DNA samples for subsequent analysis are a major concern for researchers and clinical and forensic laboratories, especially in the resource-challenged countries where novel pathogens often emerge [1–6]. Samples are commonly stored frozen in Tris-EDTA (TE) buffer at $-20\text{ }^{\circ}\text{C}$, $-80\text{ }^{\circ}\text{C}$, or $-196\text{ }^{\circ}\text{C}$ [4, 7–9]. However, liquid-based storage and freeze-thaw cycles cause

sample degradation. During storage sample, loss may result from impurities, interactions between the sample and the storage tube, or shearing during formation of ice crystals [4, 10–12].

Newer technologies have been developed for dry DNA stabilization at room temperature [4, 5, 13–15]. A few commercial products are also available and have been shown to perform comparably to $-20\text{ }^{\circ}\text{C}$ storage, provided that initial DNA concentrations are high and low humidity ($<X\%$ relative humidity) storage conditions are consistently maintained [2, 6, 16, 17]. However, samples still degrade at $-20\text{ }^{\circ}\text{C}$. As noted by Kuzima et al., “The ideal DNA preservation system should work on diluted samples as the availability of high concentration samples cannot be assumed.” [2]. Furthermore, the relatively high cost products needed for the identified commercial technologies may be inaccessible to resource-challenged countries.

Several researchers have reported the use of silica sol–gels for biomolecule encapsulation [18–21]. The sol–gel matrix restricts sample mobility similarly to frozen storage and should provide theoretically similar stabilization. However, traditional sol–gel production involves the use of a hydrolyzing agent (acid or base) and a co-solvent (alcohol) that are not

✉ Kristin Omberg
kristin.omberg@pnnl.gov

✉ Gautam Gupta
gautam.gupta@louisville.edu

¹ Materials Physics and Applications Division, Los Alamos National Laboratory, Los Alamos, NM, USA

² Bioscience Division, Los Alamos National Laboratory, Los Alamos, NM, USA

³ Department of Bioengineering, University of Louisville, Louisville, KY 40292, USA

⁴ Department of Chemical Engineering, University of Louisville, Louisville, KY 40292, USA

amenable to biological materials [1, 19, 22–32]. Herein, we report a novel, fast (~5 min) silica sol–gel preparation method using a standard microwave-initiated polymerization reaction amenable to stabilization of low concentration samples, like DNA. It involves only the use of one chemical, tetramethoxy silane (TMOS), thus making it an ideal choice for resource-challenged countries and in-field applications. The optimum characteristics of the silica sol–gel for DNA storage and recovery by manipulating the P–O–Si interaction of a phosphate from DNA with the silica matrix are achieved by varying precursor concentration, buffer properties, and pH of the starting mixture [13, 18, 33]. Moreover, a desired sample size of silica matrix with DNA stored at room temperature (RT) can be vortexed and directly analyzed by polymerase chain reaction (PCR) techniques or purified by gel electrophoresis to recover the molecular DNA, thus avoiding traditional routes of freeze-thawing, leading towards significant DNA degradation. After 210 days at RT, nearly 100% of the initial sample can be recovered, outperforming the control samples stored conventionally at -20°C . The proposed technology is highly versatile and can preserve other types of biomolecular structures such as RNA and some proteins.

2 Material and methods

TMOS was purchased from Sigma–Aldrich (St. Louis, MO, USA). TE and PBS buffers were purchased from Teknova (Hollister, CA, USA), and TBE buffer was purchased from Fischer BioReagents (Waltham, MA). Quant-iTTM PicoGreen® dsDNA Assay Kit was purchased from Thermo Fisher Scientific (Waltham, MA), and agarose gel was made using SeaKem Agarose (Lonza, Basel, Switzerland). *Escherichia coli* DNA was purchased from Affymetrix (Santa Clara, California, USA). qPCR was performed on an Applied Biosystems® AB 7500 Fast Dx Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA).

2.1 Preparation of quantity standard and DNA control samples

Escherichia coli DNA was quantified using the Quant-iTTM PicoGreen® dsDNA Assay Kit then diluted to 1×10^8 copies/ μl using TE buffer. *Escherichia coli* quantity standards were stored in 30- μl aliquots at 1×10^8 copies/ μl at -20°C .

For control sample and sol–gel sample preparation, *E. coli* DNA was diluted to 2×10^5 and 2×10^3 copies/ μl using TE buffer. DNA test samples were prepared by further diluting 20- μl aliquots of these samples with 180 μl TE buffer, then adding 10 μl 1× phosphate buffer solution.

2.2 Preparation of silica sol–gels

0.25 ml TMOS was mixed with 9.75 ml of TE buffer to produce a 2.5% v:v solution. The solution was transferred to a 50-ml centrifugal tube and covered with parafilm to minimize pressure accumulation during the microwave process. The tube was microwaved for 30 s at 20% microwave power, shaken gently inside a hood to mix for 15 s, microwaved for 10 s at 20% power, shaken gently inside a hood to mix for 15 s, microwaved for 10 s at 20% power, and then left inside a hood to cool for 10 min.

2.3 Encapsulation of DNA in silica sol–gels

Twenty microliters of *E. coli* DNA in TE buffer were added to 180- μl microwaved sol–gel material. 10 μl of 1× PBS were added to induce gelation in some cases. The final sol–gel sample DNA concentrations were 5×10^2 copies/ μl and 4×10^4 copies/ μl for samples from experiment 1, TMOS in TE buffer. The final sol–gel concentration was of 2×10^4 DNA copies/ μl and 2×10^6 DNA copies/ μl for experiment 2, TMOS in PCR-grade water.

2.4 DNA recovery from silica gel

Tubes were vortexed at 2500 rpm for 5 s. At later time points, vortexing was repeated 2–5 times until the sol–gel was liquefied. No further purification was performed; aliquots of the liquefied gel were loaded directly into electrophoretic gels or qPCR plates.

2.5 qPCR

qPCR was performed on an Applied Biosystems® AB 7500 Fast Dx Real-Time PCR System using a published 16S rDNA assay [34]. Primers and probes were suspended in TaqMan GenEx master mix (Thermo Fisher Scientific), and 45 thermal cycles were performed; otherwise, conditions were identical to those previously reported [34]. Each sample comprised 5 μl of *E. coli* DNA in TE buffer or 5 μl *E. coli* DNA in liquefied silica gel.

On each day that qPCR was performed, an *E. coli* DNA quantity standard was thawed, quantified using PicoGreen®, serially diluted in TE buffer, and used to generate an eight-point standard curve (2×10^7 copies/ μl to 2 copies/ μl) for test sample quantitation. Five replicate tubes of each sample were analyzed in quadruplicate, on four separate plates. One replicate sample from each tube was performed on each plate, and each plate was run on a different AB 7500 Fast Dx system. Five no-template controls were included on each plate.

qPCR data were analyzed using the Applied Biosystems SDS software (version 2.0.6, Thermo Fisher Scientific). Autobaseline and a threshold of 0.1 were applied. Data were

imported into Excel (Microsoft®), which was used to calculate average concentrations and average threshold cycle (Ct) with standard deviations for each set of replicates.

2.6 Gel electrophoresis analysis of DNA in silica sol–gels and control samples

For each time point of experiment 2, the 72 samples were run through a 1% agarose gel via gel electrophoresis. The agarose gel was made using SeaKem Agarose dissolved in 1× TBE via microwaving for 2 min. Ethidium bromide (EtBr) was added to the gel, and the gel was placed in a gel box apparatus and had 1× TBE with EtBr added. Each sample mixed with gel loading dye in a 1:5 ratio (sample: loading dye) had 20 µl pipetted into the wells of the gel. Gel electrophoresis was run at 120 V for 90 min. The gel was imaged, and results observed.

3 Results and discussion

Figure 1 a shows the schematic of sol–gel synthesis and recovery of DNA from its matrix. TMOS is mixed with deionized water and, since it is not soluble in aqueous solutions, immiscible phases are observed. A standard microwave oven is used to impart mixing and induce hydrolysis (30 s, 2 min) that results in the formation of $\text{Si}(\text{OH})_4$ without the use of additional chemicals. The hydrolysis reaction generates undesired methanol byproduct that is mostly evaporated by exposing the solution to air for a few minutes. DNA solution (with or without standard buffer) is further added, and condensation reaction continues resulting in the formation of gels that effectively encapsulate the DNA. Figure 1 a shows a schematic of DNA encapsulated in silica matrix. Recovery of DNA is performed by a singular vortexing step to liquefy the sample (Fig. 1a), which is followed by gel electrophoresis or qPCR to establish the quality and quantification of DNA. The process is extremely versatile, inexpensive (time and money), and involves no use of additional acids or alcohols.

The stability and recovery of DNA depended on the porous size of the encapsulating matrix, the DNA concentration, and the interaction of DNA with the matrix at the molecular level (Si–O–P interactions) [3, 5, 12, 13, 18, 33, 35–38]. On the other hand, the physical properties of the silica sol–gel matrix are controlled by the pH, saline content of the aqueous solution, and drying process [22, 24, 25, 27, 29, 39, 40]. Therefore, to obtain an optimum system, we explored four key factors that can impact effective molecular DNA encapsulation and recovery from silica sol–gel matrices: (a) concentration effect of silica, (b) kinetics of gelation (time), (c) catalyst concentration (saline content), and (d) concentration of DNA.

First, the effect of precursor concentration and catalyst was investigated to understand sol–gel matrix formation. Hydrolysis of silica precursor was performed using a standard microwave and to promote the condensation reaction; standard phosphate buffer saline (PBS) buffer was used as catalyst with concentration of 1× and 10×. The characteristic Si–O–Si– moiety is observed at 1100 cm^{-1} by infrared (IR) spectroscopy; higher concentrations of TMOS ($\geq 5\%$) show higher absorbance peaks. Faster polymerization is observed with use of catalyst with 10× phosphate buffer solution (PBS; Fig. 1c; each spectrum was taken ~10 min after gelation was initiated by microwave exposure). This wide band at 1100 cm^{-1} (to $\sim 1250\text{ cm}^{-1}$) is generated by contribution of the vibrational modes of Si–O–Si– and Si–OCH₃. At 1016 cm^{-1} , the formation of CH₃OH and posterior contribution of formation of Si–O–Si– bonds are also observed at higher concentrations (see also Figure S1). A peak at 972 cm^{-1} appears as contributions of a Si–OH moiety with concentrations of TMOS $\geq 2.5\%$ and use of catalysts (Figure S1). The peak at 1100 cm^{-1} was used as an indicator for gelation and gels were prepared with DNA, where the resulting concentration was obtained by addition of DNA in PBS solution. Higher TMOS concentrations ($\geq 5\%$) and use of catalyst resulted in rapid gelation in 2 min, but less efficient recovery of DNA, showing degradation in gel electrophoresis (white arrows, Fig. 1d). Also, diluted concentration (1.25 v:v% TMOS) did not result in efficient gelation over prolonged periods of time (X days) while the quality of DNA recovered from these gels was pristine. The 2.5 v:v% TMOS concentration resulted in adequate gelation and excellent recovery of DNA. Therefore, 2.5 v:v% concentration of TMOS precursor was used further in the study. Furthermore, addition of PBS to the sol–gel introduces positively charged ions (i.e., Na⁺, schematic in Fig. 1e) and results in electrostatic interactions of DNA (–ve) with Na⁺–silica (–ve), promoting adhesion of molecular DNA to the sol–gel matrix limiting DNA recovery as observed in Fig. 1d for higher concentration of precursor. To summarize, low concentration of silica precursor is adequate to preserve DNA in contrast to prior reports. Moreover, the type of salt and ionic strength plays a critical role in adequate recovery of the DNA.

The recovery of nucleic acids is driven by salt types, pH, and ionic strength ranges, within their physicochemical formulations. The salt content in Tris EDTA (TE), phosphate buffer saline (PBS), and Tris/borate/EDTA (TBE) buffers have weakly kosmotropic monovalent (Na⁺, K⁺, NH⁺) and divalent (Mg²⁺, Ca²⁺) ions, constructing a unique microenvironment around biomacromolecules, therefore significantly altering the solute–solute and solute–solvent interactions [41, 42]. For instance, the electrostatic interaction between highly charged nucleic acids (i.e., DNA/RNA) and salts has been known to have a significant impact on structural and functional stability of nucleic acids. Both colloidal silica as well as phosphate backbone of DNA have large net negatively

a DNA encapsulation in Silica Sol-gel **b DNA Recovery and Analysis** **c** **d** **e**

Fig. 1 **a** Schematic of the silica sol-gel process for DNA encapsulation and schematic of DNA encapsulate in silica sol-gel. **b** Schematic of DNA recovery and analysis. **c** IR spectra of silica gels: 1.25, 2.5, and 5 v:v% TMOS in water without PBS catalyst at day 1. Spectra shows the faster condensation of silica sol-gel of 5% TMOS composition compared with 2.5% and 1.25% silica sol-gels (all plots taken ~10 min after gelation was initiated). **d** IR spectra of the silica gels 1.25, 2.5 and 5 v:v% TMOS in water with catalyst 10× PBS used as catalyst at day 1. Spectra shows the

faster condensation of silica sol-gel of 5% TMOS composition compared with 2.5% and 1.25% silica sol-gels and faster than samples without catalyst. **e** Gel electrophoresis of *E. coli* DNA stored in sol-gel of 1.25, 2.5, and 5 v:v% TMOS in PCR grade with catalyst (1× and 10× PBS). White arrow indicates 5 v:v% TMOS sol-gels made with 1× and 10× PBS catalysts results in the recovered DNA of the lowest quality. Sol-gels from 2.5 v:v% TMOS gives the highest quality of recovered DNA

charged, and therefore, the DNA–silica interaction is electrostatically unfavorable. However, electrostatic stability and recovery of DNA would be induced by increasing salt concentration-gradient, shielding the electrostatic interaction and compressing the electrostatic double layer surrounding the DNA and the silica surface, which facilitates DNA immobilization onto the silica networks [41, 42]. Additionally, the low-salt environment augments electrostatic adsorption of nucleases on silica precursors, resulting in an augmented DNA stability [43]. To minimize electrostatic interaction between the phosphate ions and the DNA, Tris EDTA (TE) buffer was utilized, which is a standard buffer used for nucleic acid storage at -20°C as it solubilizes DNA and prevents DNA degradation. To address the critical DNA recovery challenges faced in forensic and clinical studies, where low concentration of DNA is often observed, we studied the immobilization and recovery of two sets of sol–gels containing low DNA concentration, 4×10^2 copies/ μL , and 2×10^4 copies/ μL as compared with traditional 2×10^6 copies/ μL . Secondly, we also investigated the difference in gelation with and without DNA, as the presence of DNA may impact the gelation due to electrostatic

interactions. Time evolution of the $-\text{Si}-\text{O}-\text{Si}-$ peak at 1100 cm^{-1} is shown in Fig. 2a. The peak intensity increases over 30 days before it saturates. In contrast, gelation proceeds at a different rate in presence of DNA/TE buffer. In sol–gels with DNA (2×10^4 copies/ μL), the presence of DNA affects the gelation as the $1110\text{--}1080\text{ cm}^{-1}$ peak intensity increases up to the 5th day (Fig. 2b and Figure S2). After this point, absorbance slightly decreases and reaches plateau behavior, indicating that the sol–gel formation is limited by reactant availability. The decreased gelation serves as an advantage for recovery of DNA as compared with PBS containing gels. An eight-point *E. coli* standard curve was used to quantify the recovered DNA. Five samples of each type were analyzed in quadruplicate using qPCR at various time points (Online “Methods”), reported in Fig. 2c (sol–gel storing 4×10^2 DNA copies/ μL and 2×10^4 DNA copies/ μL , bottom and top curves, respectively). These sol–gel matrices (red data points) largely overcome the results from conventional “optimal” storage at -20°C (black data points). Although, sol–gels stored at room temperature outperform the samples stored at -20°C , we still lose about half the sample. This loss could be due to the rigid

Fig. 2 **a** IR spectra of silica sol–gel of 2.5 v:v% TMOS in TE buffer showing time dependence of gelation (days 1–27). **b** IR spectra of silica sol–gel of 2.5 v:v% TMOS in TE buffer containing DNA of 4×10^4 copies/ μL showing time dependence of gelation (days 1–12). **c** qPCR quantification results of DNA in sol–gels made with TE buffer. Sample of 5×10^2 DNA copies/ μL stored in 2.5 v:v% TMOS in TE and its control (at -20°C). Red data points (circles) present results from *E. coli* DNA stored in sol–gels at ambient temperature. The black data points (squares)

present results from the control samples (*E. coli* DNA stored in TE buffer at -20°C). Samples were analyzed at $t = 0, 3, 7, 11, 33, 90$, and 150 days. Five samples were analyzed in quadruplicate at each time point ($N = 20$). **d** DNA qPCR quantification results of sol–gels with 4×10^4 DNA copies/ μL where the red data points represent room temperature storage, and black data represents the control at -20°C . For both qPCR plots, the time points of analysis were at $t = 0, 3, 7, 11, 33, 90$, and 150 days

gel framework and possibly due to some electrostatic interactions induced by components of TE (Tris and EDTA, Fig. 2d) that results in formation of a cation bridge, bridging negatively charged silica centers. To summarize, electrostatic interactions between DNA and the silica precursor play a critical role in immobilization and recovery of DNA. Use of EDTA allows for storage of DNA at room temperature for nearly 150 days, which outperforms storage of DNA at -20°C without silica stabilization.

Therefore, to further minimize the electrostatic interactions and simplify our process even further, we prepared gels with pure PCR-grade water, without any additional reagents. Absorbance at 1100 cm^{-1} for 2.5 v:v% TMOS:PCR-grade H_2O is depicted in Fig. 3a. A similar trend is observed as in the case of TE buffer, i.e., an observed increase in peak intensity over 25 days with saturation observed after that. In the presence of DNA, the peak intensity increases up to 5 days and then decreases slightly and saturates (Fig. 3b). Higher absorbance is observed for the $-\text{Si}-\text{O}-\text{Si}-$ peak at $1080\text{--}1110\text{ cm}^{-1}$ IR, which shows a shift towards 1080 cm^{-1} (at

day 5). This shift indicates the $-\text{Si}-\text{O}-\text{Si}-$ moiety is compromised in other interactions within the matrix (days 7–49). Figure 3c shows results of qPCR and gel electrophoresis of samples with 2×10^4 DNA copies/ μl , which demonstrates that the quality of the DNA is not compromised. Electrophoresis further indicates that there is no DNA degradation or fragmentation (blue arrow, Fig. 3b) on day 1. qPCR data is shown in Fig. 3c. We have consistently observed a decrease in DNA recovery during initial days of storage (0–20 days), which also depends upon the concentration of precursor used. We attribute this phenomenon to a high concentration of methanol that might be present initially and can inhibit the qPCR cycling. Methanol is the byproduct that is released from the sol–gel matrix within 20 days—reduction of absorbance by IR (at 1012 cm^{-1}) is observed as mentioned above (see also Figure S3). After methanol residues evaporate, recovered DNA is more accurately quantified. Over a long period of storage (~ 100 days), we observe enhanced DNA recovery. For high concentration (2×10^6 DNA copies/ μl), nearly 100% recovery of initial stored DNA is observed

Fig. 3 **a** IR spectra of silica sol-gel of 2.5 v:v% TMOS in PCR-grade water silica, showing time dependence of gelation. **b** IR spectra of *E. coli* DNA of 4×10^4 copies/ μl stored in silica sol-gel of 2.5 v:v% TMOS in PCR-grade water, showing time dependence of gelation. **c** Gel electrophoresis photograph of samples stored in 2.5 v:v% TMOS- H_2O silica sol-gel and its respective controls (abbreviations: Ctrl = DNA control, Ctrl ext = DNA control vortexed, sol-gel = DNA sol-gel vortexed). Blue arrow indicates DNA recovered from silica sol-gel (2.5 v:v% TMOS in PCR-grade water) that shows outstanding quality when compared with its

respective controls. **d** Silica sol-gel sample of 2×10^4 DNA copies/ μl stored in 2.5 v:v% TMOS in PCR-grade water. qPCR data are compared with the respective control at -20°C (black data were analyzed at $t = 0, 1, 2, 7, 14, 30, 90$, and 210 days), and commercial control kit is also stored at -20°C (blue data were taken up to 250 days). The red data present results from *E. coli* DNA stored in sol-gels at ambient temperature (data were analyzed at $t = 0, 1, 2, 7, 14, 30, 90$, and 210 days). Five samples were analyzed in quadruplicate at each time point ($N = 20$)

(Figure S3). Using pure PCR-grade water for the sol–gel synthesis limits the presence of positively charged ions, promoting repulsive electrostatic interaction of silica gel surface (-ve)–DNA (-ve) that results in a more efficient DNA recovery (Fig. 3 c and d). To summarize, sol–gels of 2.5 v:v% TMOS in PCR-grade water can surpasses the traditional –20 °C storage, overcoming previous technologies (efficiency reported based on –20 °C storage, 100% efficiency).

4 Conclusions

In conclusion, we have developed a simplified rapid silica encapsulation method for molecular DNA stabilization at room temperature that allows for a 1-step DNA recovery utilizing only one chemical and a simple bench top microwave. The polymerization of the silica sol–gel is initiated by standard microwave for less than a minute, making it a technologically relevant process. This procedure avoids the use of additional chemicals and extreme conditions, promoting the rapid evaporation of methanol generated during sol–gel hydrolysis, prior to the addition of DNA or another biological component. This sol–gel could be used in field studies, forensics, and resource-challenged countries as it provides a versatile platform due to extensive control over gelation by obtaining fundamental understanding of silica DNA interactions. This method outperforms the conventional “optimal” –20 °C storage condition for encapsulation and recovery of DNA samples and can encapsulate and release low concentration of DNA, with low costs. Therefore, this sol–gel technology also overcomes the state-of-the-art and commercial technologies that report based on –20 °C conventional storage –100% efficiency. Depending upon the buffer used, the DNA structure may have an enhanced interaction with the sol–gel affecting its final recovery. Sol–gel offers an active chemical protection for ambient temperature storage, shipping, and processing of DNA and therefore architects a novel direction for the analysis of clinical specimens for disease diagnosis and monitoring. This technology could be easily adapted to encapsulate and preserve other biomolecular structures of interest, such as proteins, where the iso-electric point of the protein will dictate its preservation and recovery as the electrostatic interactions will need to be optimized. Moreover, the current technology can be utilized to stabilize RNA-based therapeutics at room temperature which has been as a critical challenge faced by COVID 19 vaccine distribution.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s42247-021-00208-3>.

Funding The work is supported by NIH R21CA251042 and Center for Integrated Nano Technologies at Los Alamos National Lab.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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