PROCEEDINGS, 41st Workshop on Geothermal Reservoir Engineering
Stanford University, Stanford, California, February 22-24, 2016
SGP-TR-209

Uniquely Identifiable DNA-Embedded Silica Nanotracer for Fractured Reservoir Characterization

Yuran Zhang, Timothy Spencer Manley, Kewen Li and Roland N. Horne

Dept. of Energy Resources Engineering, Rm 050, 367 Panama St., Stanford University, Stanford, CA, USA, 94305

yuranz4@stanford.edu

Keywords: tracer, fractured reservoir characterization, DNA, silica, nanoparticles

ABSTRACT

The objective of this study was to develop and evaluate a type of uniquely identifiable nanoparticle tracer to map fracture networks without ambiguity. DNA-tagged nanotracers were synthesized by first adsorbing synthetic DNA molecules onto the surface of plain silica nanoparticles of around 140 nm diameter, and then coating the particles with a silica outer layer to protect the DNA from harsh environmental conditions. Heating and flow experiments were conducted to evaluate the durability of silica as a protective material for DNA molecules. DNA-embedded silica nanoparticles were injected through packed sand at various temperatures and analyzed in the effluent in order to test whether the DNA-silica nanotracer could flow successfully through porous medium while maintaining the integrity of the DNA. This paper summarizes the advantages and limitations of DNA-embedded silica nanoparticles as reservoir tracers, and discusses possible approaches to adjust the DNA-silica nanotracer to achieve more favorable properties for fractured reservoir analysis.

1. INTRODUCTION

Tracer tests are widely used in geothermal industry to map fracture distribution, mostly by injecting chemical tracers at the injection well and monitoring their breakthrough pattern at the production well. Because tracer transport is faster than the thermal front, tracer tests can also be used to infer reservoir temperature and hence forecast thermal breakthrough. In recent years, solid particle tracers are gaining attention because of the high degree of control of their physical and chemical properties compared with conventional solute tracers. Particle tracers also have less matrix diffusion, and hence travel more quickly through the reservoir as they tend to remain in high-velocity fluid streamlines. Alaskar et al. (2015) have investigated the properties and transport of irreversible-color-change thermochromic particles as well as fluorescent dye-attached silica nanoparticles through porous media. Li et al. (2014) studied the retention of modified carbon nanoparticles in calcium carbonate porous media in order to identify favorable physical properties for nanoparticle tracers to transport through porous media with high particle recovery. Frane et al. (2014) investigated microcapsules of polymer with dye encapsulated inside that could release dye at certain temperature threshold. In this study, we investigated a DNA-embedded nanoparticle tracer that has great capacity for information storage and good thermal stability.

DNA is well-known for its unique identifiability. DNA has infinite number of different sequences that could form an almost infinite number of unique tracers. Although reservoir conditions such as high temperature may be harsh for the DNA molecules, it is possible to protect the DNA using synthetic "fossils" by first attaching DNA onto silica nanoparticles and then adding a protective silica layer to the particles. Pioneering work of Paunescu et al. (2012) has shown that DNA protected by silica nanoparticles is able to withstand temperature as high as 200 °C, and can still be amplified and quantified through qPCR (real-time quantitative polymerase chain reaction) after being released into solution by dissolving the silica outer layer. Therefore, such DNA-embedded silica nanoparticles could conceivably be applied in geothermal fields for reservoir characterization.

The advantage of DNA-embedded silica nanoparticles lies in the unique identifiability, as DNA has an almost infinite number of sequences. The qPCR process that is used to amplify and quantify DNA is also selective, in other words, it only amplifies the DNA with a certain segment of sequence. It is therefore possible to detect and quantify each DNA sequence separately amongst a mixture of various DNA tracer samples, which, if applied in geothermal field, would allow us to understand the flow path of the tracer by identifying the DNA sequence within the recovered fluid. The large number of different DNA sequences allows every flow path to have its own unique identifier, therefore enabling the identification of well connections, as illustrated in Figure 1.

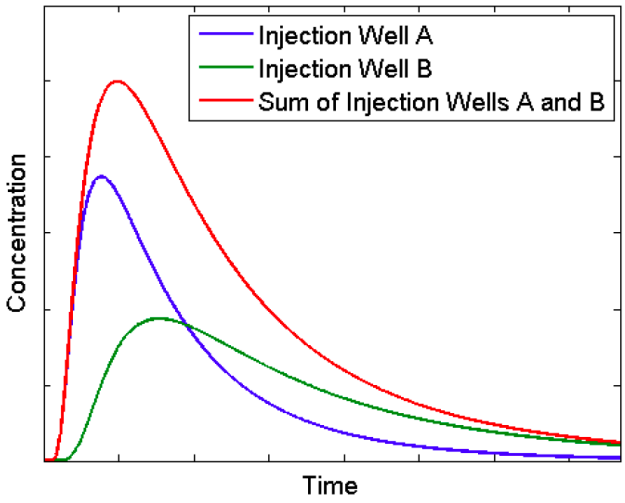


Figure 1: Schematic illustrating the well tagging application. If the same tracer were used in both wells, only the sum of the two return curves could be measured (red curve). Using unique tracers in each injection well allows us to distinguish return curves and identify the corresponding injection wells (blue and green curves). This would in turn provide more information about each interwell flowpath. Diagram by Morgan Ames.

In this study, DNA-embedded silica nanoparticles were successfully synthesized and characterized. Heating experiments on the synthesized DNA-embedded silica nanoparticles at 50 °C, 100 °C and 150 °C were conducted to confirm the durability of DNA-embedded silica nanoparticles under simulated reservoir temperatures. Injection experiments of DNA-embedded silica nanoparticles into a sand pack was also conducted both at room temperature and at 150 °C in order to investigate whether the particles are able to transport through porous medium and are still detectable after being collected in the effluent. The overall intent of this research is to establish a path toward applying uniquely identifiable DNA-based smart tracers at field scale.

2. DNA-EMBEDDED SILICA NANOPARTICLES

2.1 Synthesis of DNA-Embedded Silica Nanoparticles

The synthesis of DNA-embedded silica nanoparticles was performed according to the procedure suggested by Paunescu et al. (2013) with slight modifications. Silica seeds with a diameter of about 140 nm were first synthesized using the Stober method (Stober et al. 1968), followed by surface functionalization using trimethyl[3-(trimethoxysilyl)propyl]ammonium chloride to yield positive surface charge. Synthetic double stranded DNA (dsDNA) with 113 base pairs (Zhang, 2015) purchased from Stanford Protein and Nucleic Acid Facility were adsorbed onto the silica seeds surface, after which a seeded growth process was applied to coat the DNA-adsorbed particles with a silica layer. The DNA molecules were thereby "sandwiched" between the inner silica seed and the outer silica layer, as illustrated in Figure 2. Details of the synthesis including reagents, reaction time, etc. have been described in the paper written by Zhang et al. (2015). SEM images of the resulting DNA-embedded silica nanoparticles are shown in Figure 3. The final DNA-embedded silica nanoparticle product was diluted to yield a particle concentration of around 7mg/ml, and was used in the flow experiment described in Section 3.

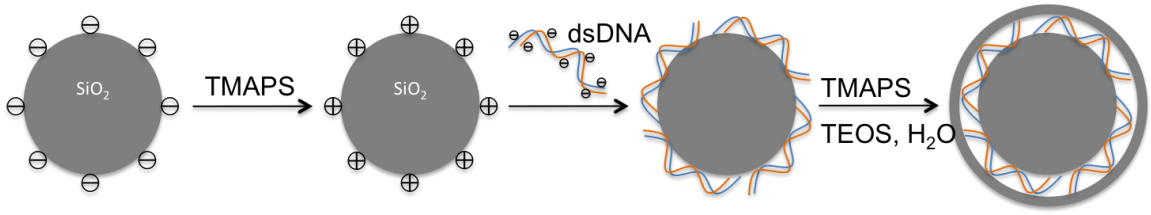


Figure 2: Schematic of DNA encapsulation into silica nanoparticles. (Derived from Paunescu et al. 2013)

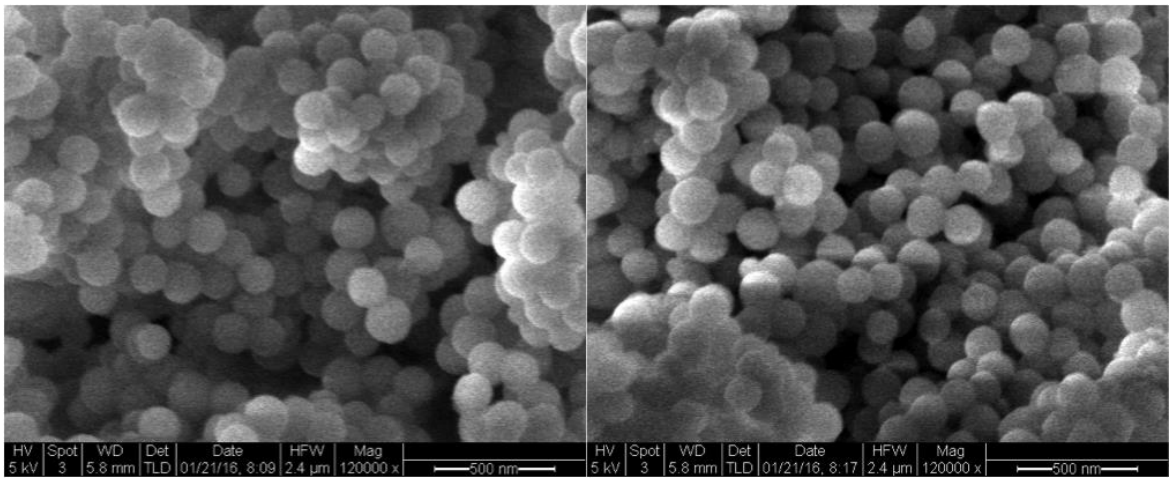
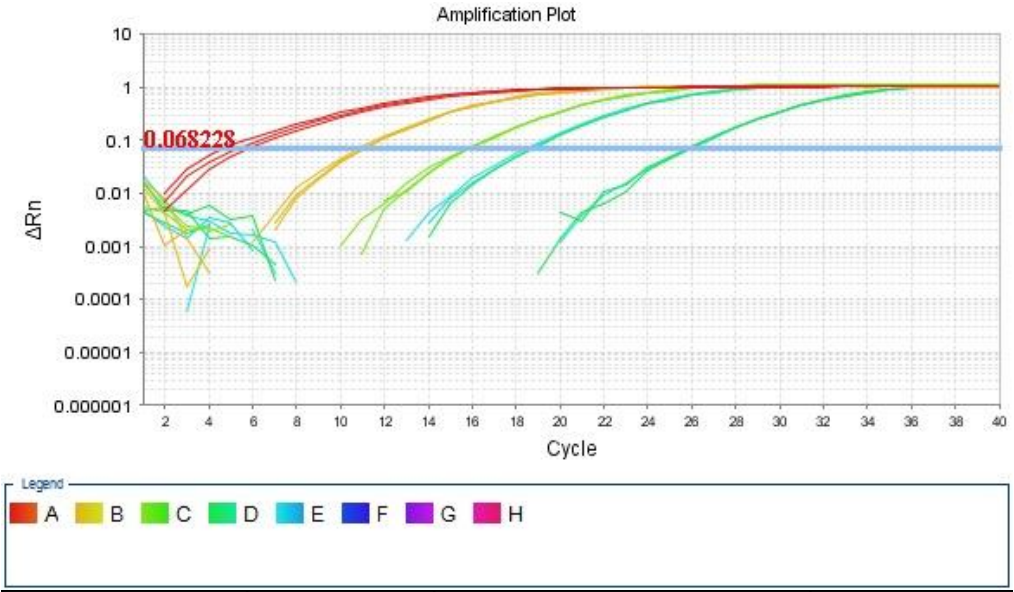


Figure 3: SEM images of DNA-encapsulated silica nanoparticles showing an average diameter of ~160 nm and particle agglomeration.

2.2 Characterization of DNA-Embedded Silica Nanoparticles

In order to quantify the amount of DNA encapsulated in the particles, DNA release experiments were conducted according to the procedure suggested by Paunescu et al. (2013) with slight modifications. Buffered oxide etch solution was made by dissolving 0.92 g of NH_4FHF in 10 ml of H_2O and 0.76 g of NH_4F in 10 ml of H_2O and mixing the two solutions together. 20 µl of the DNA-embedded silica nanoparticles solution was reacted with 12 µl of etching solution until a transparent solution was obtained (i.e. silica completely dissolved, DNA released into solution). Released DNA was then purified using GET CLEAN DNA spin columns supplied by G-Biosciences according to the manufacturer's instructions in order to remove excessive acid and salts. The DNA released from each 20 µl of DNA-silica nanofluid was finally eluted in 50 µl of TE buffer, and was diluted to a factor of 10000 before running qPCR quantification on the StepOnePlus™ Real-Time PCR System available at Stanford Protein and Nucleic Acid Facility. The result is shown in Figure 4.



Color Legend Name	DNA Type	DNA Concentration	Cycle Threshold
A	Standard	2.5 nM (~1.74×10^{-1} ng/µl)	5.23
B	Standard	0.25 nM (~1.74×10^{-2} ng/µl)	10.95
C	Standard	0.025 nM (~1.74×10^{-3} ng/µl)	15.81
D	Standard	0.00025 nM (~1.74×10^{-5} ng/µl)	25.79
E	Released DNA	~0.0094 nM (~6.54×10^{-4} ng/µl)	18.54

Figure 4: Amplification plot of the standard DNA (with known concentrations) and the released DNA from the DNA-embedded silica nanoparticles. Results summarized in the table. Note that the released DNA was diluted to a factor of 10000 before amplification, hence the original concentration of released DNA was ~94 nM (6.54 ng/µl).

As shown in the qPCR result, the DNA released from the DNA-silica nanoparticles had a concentration of around 94 nM (6.54 ng/µl). Because the cycle threshold of 18.54 was only about half of the maximum cycles allowed in qPCR (40 cycles maximum), we can infer that the DNA released from merely 20 µl of particle solution could still be amplified and quantified even after being diluted to a factor of 10^8. The abundance in encapsulated DNA leaves sufficient room for applying the DNA-silica nanoparticles in flow experiments, because the particles would go through large extent of dilution while traveling through porous media.

3. DNA-EMBEDDED SILICA NANOPARTICLES INJECTION AND HEATING EXPERIMENTS

In order to test the feasibility for the synthesized DNA-silica nanoparticles to be applied as geothermal reservoir tracers, the particles were evaluated at simulated reservoir conditions by injecting them through porous medium both at room temperature and at 150 °C. The DNA-silica nanoparticles were agglomerated due to the clustering effect caused by the sticky DNA molecules (Zhang et al. 2015). However the particle chunks were still micron sized and therefore smaller than expected fracture apertures. To test this, a sand pack with high permeability was used. In addition to the injection experiments, the DNA-silica nanoparticles were also heated in sealed tubes at 50 °C, 100 °C and 150 °C without flowing. The purpose was to investigate their heat stability separately without the porous medium or flow.

In the injection experiments, a length of stainless steel tubing packed with Ottawa sand was used as the porous medium. A differential pressure transducer (Validyne Engineering) was connected to both ends of the sand pack to monitor the pressure drop along the porous medium. The pressure transducer had a range of 0~5 psi and was calibrated according to a standard HEISE gauge. At the end of the system was a back-pressure regulator that maintained system pressure to keep water from boiling at high temperature. A standard needle gauge was connected to the downstream of the system to monitor system pressure. An Eldex Optos Series pump (Model 3) was used to push the nanoparticles through the system. A schematic of the apparatus is illustrated in Figure 5.

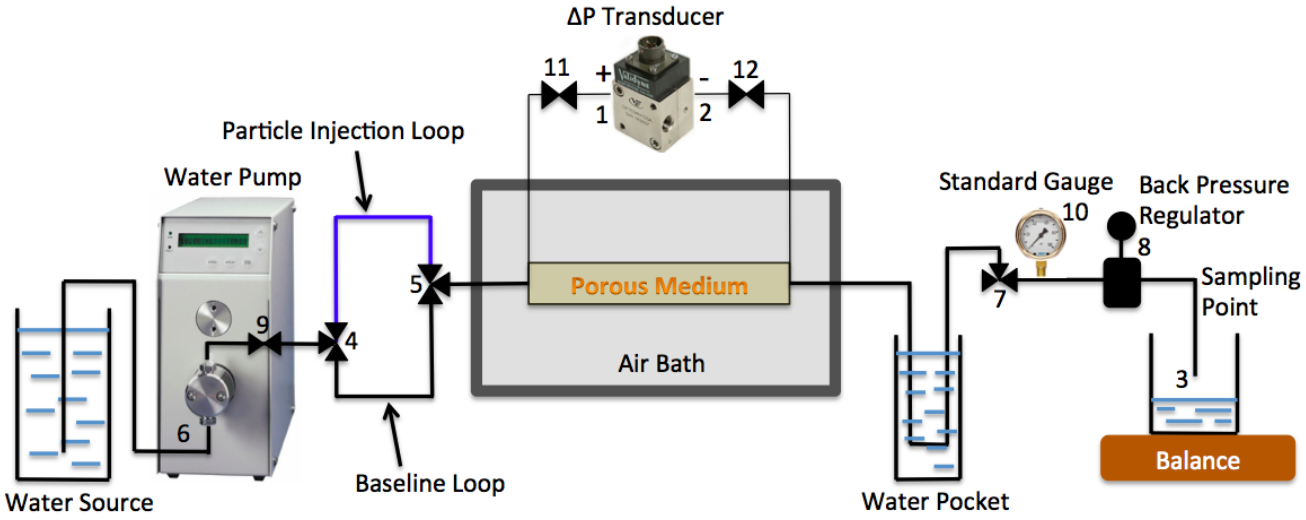


Figure 5: Schematic of flow experiment apparatus at room temperature. The porous medium was a segment of stainless steel tubing packed with Ottawa sand. Water was first flowed through the system. After achieving a steady flow, DNA-embedded nanoparticles were injected by turning valve 4 and valve 5 toward the particle injection loop. Differential pressure readings was recorded to get an estimate of the permeability of the sand pack.

In each injection experiment, a baseline flow was first established by pumping distilled water through the system until a steady flow was obtained. Then valve 4 and 5 (as shown in Figure 5) were turned toward the particle injection loop to allow the DNA-embedded silica nanoparticles to be pushed through the system. The temperature within the porous medium was controlled by the air bath. The effluent was then sampled, and subjected to SEM imaging, release experiment and qPCR quantification.

3.1 DNA-Embedded Silica Nanoparticles Injection Experiment at 150 °C

Previous studies by Zhang and Manley (2015) looked at injecting DNA-embedded silica nanoparticles into packed sand at 150 °C. The apparatus was mostly the same as the one described here. SEM images of the effluent samples confirmed that the DNA-silica nanoparticles did go through the packed sand. However, after conducting release experiments on the effluent samples, no DNA amplification was obtained by qPCR. It is possible that the exposure to high temperature (150 °C) caused the DNA to degrade to the extent that it was no longer amplifiable by qPCR. It is also possible that the particles underwent so much dilution after traveling through the porous medium that it was too difficult to recollect them and perform an effective release experiment. In the release experiment, the only way to know when the protective silica particles are fully dissolved is by observing the solution turn from milky to transparent after adding the etching solution, which is not possible if the particles were so diluted that it looked transparent even before adding the etching solution. Another possibility is that the majority of the DNA-silica nanoparticles were stuck somewhere in the system, most likely in the porous medium, and the ones that went through were too few to be detected.

In order to find out why we were not able to detect DNA in the effluent of the 150 °C injection experiment, a new injection experiment at room temperature was conducted. If it were only the high temperature that caused negative DNA detection, we should be able to achieve qPCR amplification from the room temperature injection experiment. A separate heating experiment was also conducted to look at the effect of high temperature on the DNA-silica nanoparticles without flow.

3.2 DNA-Embedded Silica Nanoparticles Injection Experiment at Room Temperature

The injection experiment of the DNA-embedded silica nanoparticles at room temperature was performed using the apparatus described in Figure 5. The system was first vacuumed for 2 hrs to eliminate air. Water was then let into the system which saturated the sand pack. Water was pumped into the system using the Eldex pump at a flow rate of 2.5 ml/min. Point 11, 12, 7, 10, 3 were checked consecutively to make sure they were dripping water. This ensured that the entire system was saturated with water. The back pressure regulator was then shut and the system was pressurized to check leakage.

The injection experiment was started by turning valves 4 and 5 toward the baseline loop and pumping in distilled water to establish a baseline. After the flow was stabilized, the pressure drop reading along the sand pack was around 2.1 psi. A sand pack permeability estimation of 102.5 darcy was obtained according to Darcy's law. The injection of DNA-silica nanoparticles was then started. The particle injection loop was initially filled with 200 µl DNA-silica nanoparticles (~7mg/ml). Valves 4 and 5 were turned toward the particle injection loop so that the DNA-embedded silica nanoparticles was pushed through the sand pack. Effluent was mostly sampled at 5 min intervals, and the sampling was continued for 2 hr. At the end of the experiment, the needle gauge 8 was back flushed to make sure there was no DNA-silica nanoparticles stuck inside. Effluent of the back flush was also collected (Figure 6).

Sampling of RT Injection Experiment	
Number of min After Starting DNA-Silica Particle Injection	Sample Number
0~8 min	1
8~13 min	2
13~18 min	3
18~23 min	4
23~28 min	5
28~33 min	6
33~38 min	7
38~43 min	8
43~48 min	9
48~53 min	10
53~58 min	11
58~63 min	12
63~120 min	13
Back Flush Needle Gauge 8	14

Figure 6: Description of sampling frequency of the room temperature injection experiment. Most samples were collected for 5 min, whereas Sample 1 and 13 were collected for longer time because the concentration of particles that broke through was expected to be very low at the beginning and the end of the experiment. Sample 14 was collected from the back flush of the needle gauge 8.

The samples were observed under SEM to gain visual confirmation on whether the particles went through. Release experiments were then performed on samples 4, 7 and 14, followed by qPCR quantification, to determine if the DNA was still detectable in the effluent. Because only a tiny amount of DNA-silica nanoparticles was used (7mg/ml × 200 µl) and because the effluent samples were heavily diluted, the samples were first centrifuged at 4816 g for 50 min in a swinging-bucket-rotor to make sure all particles were settled at the bottom of the sample tube. The supernatant was then removed before the release experiments were performed. All samples were purified using the spin column after the release experiment, and were finally eluted in 50 µl TE buffer and quantified using qPCR. In addition, the supernatant fluids from the centrifugation (before release), were also tested using qPCR.

In addition to the effluent, sand at the inlet and outlet of the sand pack was also sampled and analyzed. A release experiment was performed with this sand. The sand grains were later eliminated by separation during the DNA purification.

3.3 DNA-Embedded Silica Nanoparticles Heating Experiment

Three heating experiments (without sand or flow) and a room temperature control were conducted to investigate the heat stability of the synthesized DNA-embedded silica nanoparticles. The particles were confined in four stainless steel vessels (Figure 7). 20 µl of the DNA-silica nanoparticles was pipetted into each vessel, Milli-Q water was then added to fill the vessel. The vessel was then sealed and was ready for heating. Because the vessel was completely filled with liquid, no concern for liquid boiling during heating was necessary. The vessels were attached to metal wire and hung in the air bath to ensure accurate heated temperature. A schematic is shown in Figure 7.

Figure 7: Schematic showing the vessels that contained DNA-silica nanoparticles during heating experiment (left) and how heating was conducted (right).

The DNA-silica particles were heated in separate vessels at 50 °C, 100 °C, 150 °C, respectively, for 5 min. Five minutes is the approximate time that the particles were likely to be exposed to high temperature as they pass through the sand pack during the injection experiments. A room temperature control was also included to take into account the particle loss due to solution transfer. After heating, each vessel was placed on the bench and allowed to cool to room temperature. Solution was then removed from the vessels and centrifuged at 4816 g in a swinging-bucket-rotor for 40 min. Release experiments were conducted on the particles at the bottom and the samples analyzed using qPCR. The supernatant was also collected and analyzed separately using qPCR.

4. RESULTS AND DISCUSSION

4.1 Results for DNA-Embedded Silica Nanoparticles Injection Experiment at Room Temperature

The effluent samples of the room temperature injection experiment were first observed using SEM to confirm whether the particles went through, and then samples 4, 7 and 14 were analyzed by release experiment followed by qPCR to quantify the DNA. Supernatant of samples 4, 7 and 14 were also analyzed by qPCR.

4.1.1 SEM Visualization Results

The presence of DNA-silica nanoparticles was confirmed visually in the effluent, as shown in the SEM images displayed in Figure 8.

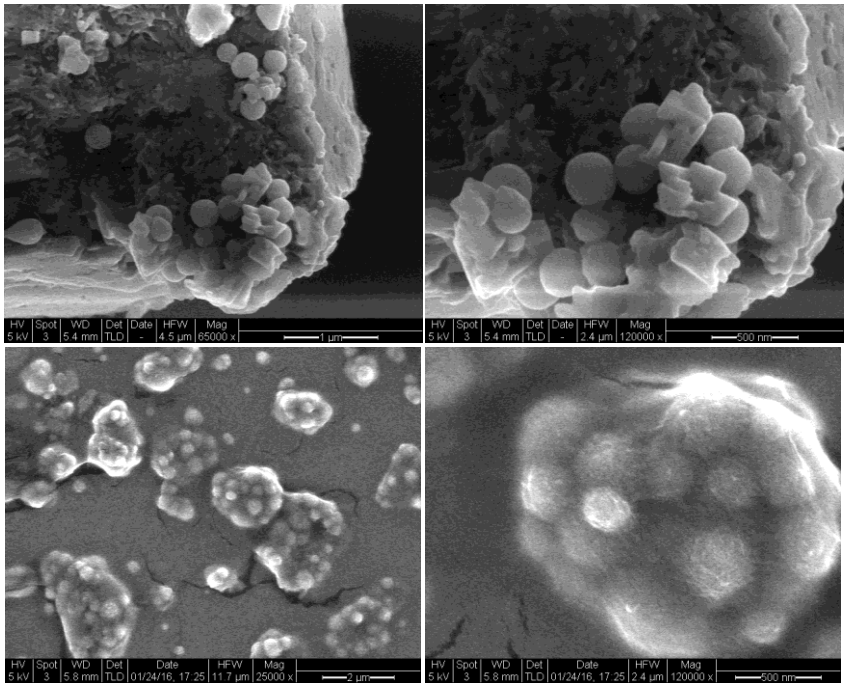
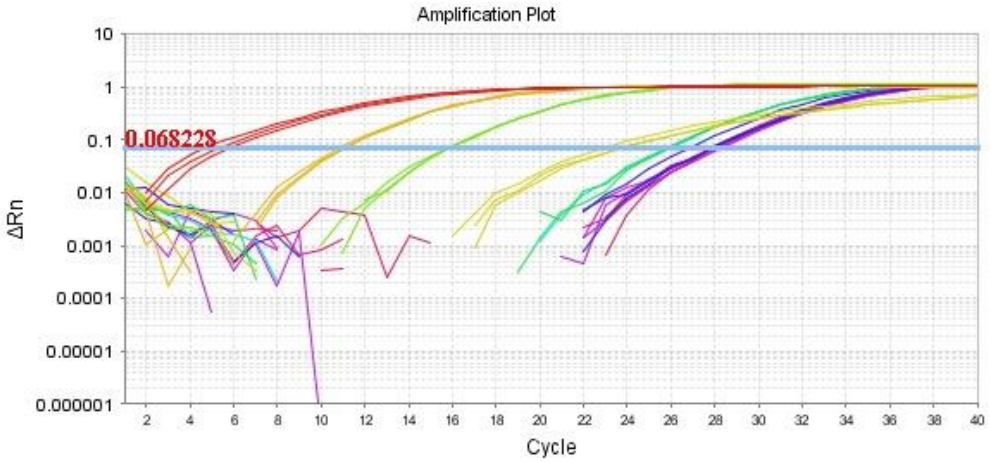


Figure 8: SEM image of effluent samples collected from the room temperature injection experiment. Figures on top show a piece of sand debris with DNA-silica nanoparticles attached to the surface. Figures at the bottom show clusters of DNA-silica nanoparticles that broke through the porous medium.

The SEM result is similar to the result of 150 °C injection experiment conducted by Zhang and Manley (2015). Despite the agglomeration of DNA-embedded silica nanoparticles, they were still able to transport through the sand pack. The critical matter now is whether we are able to gain DNA amplification out of the effluent.



Injection Experiment		
Sample Number	Source	DNA Concentration [nM]
4	Particle	--
	Supernatant (Free DNA)	2.33×10^{-4}
7	Particle	--
	Supernatant (Free DNA)	2×10^{-4}
14	Particle	--
	Supernatant (Free DNA)	2×10^{-4}
Inlet Sand	Particle	1.23×10^{-3}
Outlet Sand	Particle	--

Figure 9: qPCR results for effluent samples 4, 7 and 14 from the room temperature injection experiment. Both the particles and the supernatant of the effluent samples were analyzed. No DNA was detected in the particles whereas free DNA was detected despite the heavy dilution in the flow experiment. DNA was also detected after conducting release experiment on the inlet sand.

4.1.2 DNA Quantification Results

DNA quantification results for effluent samples 4, 7 and 14 from the room temperature injection experiment are shown in Figure 9. No DNA amplification was obtained from the particles recovered from effluent samples 4, 7 and 14. However, DNA was detected and amplified in the supernatant of all three effluent samples, although the concentration was very low (~10^{-4} nM, or ~10^{-5} ng/µl), quite close to the lower detection limit of qPCR (~10^{-6} ng/µl). DNA was also detected after performing the release experiment on the sand sampled from the inlet of the sand pack. The concentration (~10^{-3} nM, or ~10^{-4} ng/µl) was slightly higher than that of the effluent supernatant. No DNA was detected in the outlet sand.

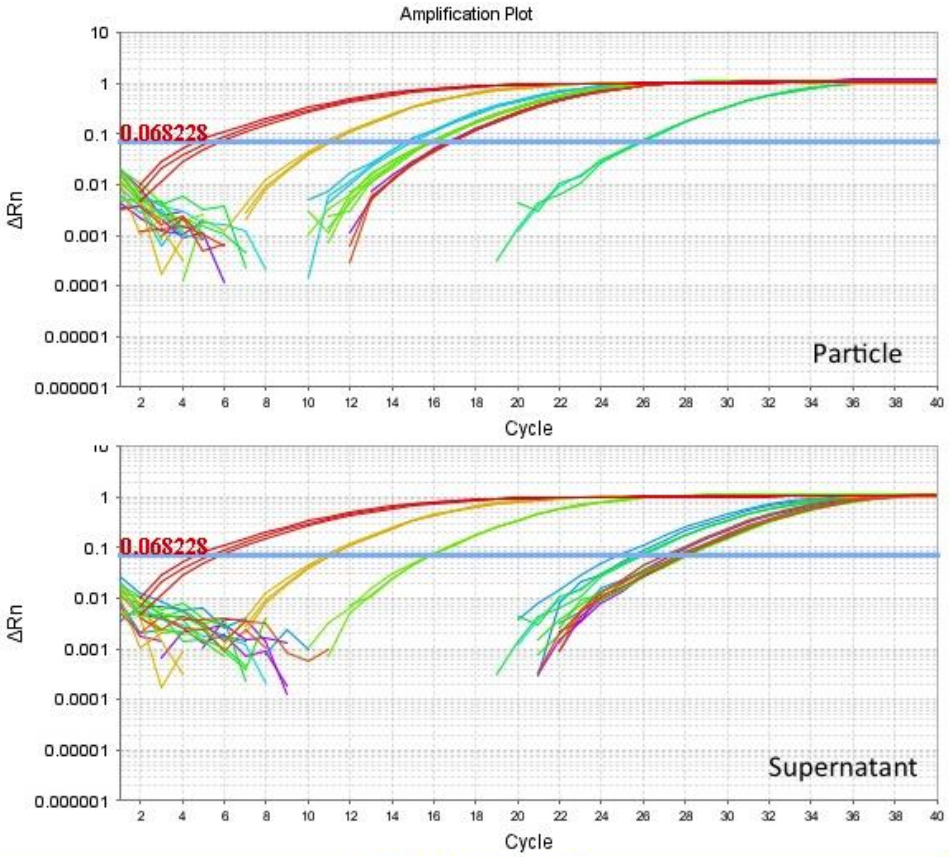
The results indicate that a number of DNA-silica nanoparticles may have been trapped in the sand pack. Although some of the particles broke through, they were so diluted after being flowed that no DNA could be detected after the release experiment. Free DNA was also present in the effluent. The free DNA was detected and amplified even after being heavily diluted in the flow experiment.

The free DNA present was very likely to be the DNA remainder that was not completely washed away at the end of the DNA-silica particle synthesis process. It is also possible that the journey through the sand pack caused some of the DNA-silica nanoparticle clusters to break down, which exposed and released some amount of DNA into the solution.

Although the DNA-silica nanoparticles in the effluent were not able to carry sufficient DNA for qPCR detection, a possible solution is simply increasing the amount of DNA-silica nanoparticles to be injected. In this experiment, only 200 µl of particle solution was used but the total amount of water that was flowing in the system was larger than 100 milliliters. Figuring out a way to mitigate particle retention could also help solve the problem.

4.2 Results for DNA-Embedded Silica Nanoparticles Heating Experiment

Similar to the sample analysis described in the previous section, the resulting particle solution of the heating experiment was also analyzed both in the particles and in the supernatant. The results are summarized in Figure 10.



Heating Experiment				
Source	DNA Concentration [nM]			
	RT	50 °C	100 °C	150 °C
Particle	28.57	47.1	20.4	20.1
Supernatant (Free DNA)	0.2	0.233	0.2	0.5

Figure 10: qPCR results of heated DNA-silica nanoparticles at room temperature (control), 50 °C, 100 °C, and 150 °C for 5 min. Although for "particle" samples there was slight concentration reduction when ambient temperature reached 100 °C, no serious degradation was observed overall. The slight fluctuations in concentration could have been caused by solution transfer loss, or operational error during qPCR. The results indicate that being heated to 100 °C and 150 °C for 5 min did not have a noticeable impact on DNA integrity.

The DNA amount in the RT (room temperature) control gives us an idea of how much free DNA there was as compared with the DNA protected within the silica nanoparticles. The results indicate that there was almost no DNA degradation when temperature was raised from below 100 °C to above 100 °C. The slight fluctuations in concentration could have been caused by loss during solution transfer, or operational error during qPCR. Therefore, it looks like the short exposure of 5 min to temperature as high as 150 °C did not have a noticeable impact on the integrity of the DNA, and the remaining DNA was still way above detection limit. It can therefore be inferred that the absence of DNA in the effluent of the 150 °C injection experiment (described in Section 3.1) was not due to the high temperature.

5. CONCLUSIONS AND FUTURE WORK

DNA has been successfully embedded within silica particles at ~160 nm diameter, and the qPCR quantification after the release experiments indicates abundant DNA within the particles. Although the DNA-embedded silica nanoparticles were agglomerated into clusters, they were transported successfully through packed sand as proven by SEM analysis of the effluent.

An injection experiment of DNA-embedded silica nanoparticles at 150 °C was performed previously by Zhang and Manley (2015), however no DNA was detected in the effluent at that time. In order to investigate the cause for null DNA detection, a room temperature injection experiment as well as several 5-min heating experiments on the DNA-embedded silica nanoparticles at 50 °C, 100 °C, and 150 °C were performed.

The results indicate that the short exposure to high temperature did not cause noticeable degradation of the embedded DNA. However retention of DNA-silica nanoparticles in the sand pack was observed, as no DNA was detected in the effluent particles even in the room temperature injection experiment. Free DNA, however, was detected, which possibly came from DNA remainder that was not

completely washed away at the end of the DNA-silica particle synthesis process. It is also possible that the journey through the sand pack caused some of the DNA-silica nanoparticle clusters to break down, which exposed and released some amount of DNA into the solution. In addition, DNA was detected after conducting release experiment on the inlet sand, again indicating retention of DNA-silica nanoparticles in the sand pack.

Because only 200 μl of DNA-silica nanoparticle solution was used in the injection experiment, it is possible that simply increasing the amount of particles injected would allow us to detect DNA in the effluent. However, future work should still focus on trying to enhance the flowability of DNA-silica nanoparticles through porous medium. It is also worth further investigating the heat stability of the DNA-silica nanoparticles, by treating them at higher temperatures and increasing the exposure time.

Given the rather low detection limit of qPCR and the observation of good heat stability of the embedded DNA, the particles show promise in being detectable after traveling through the porous or fractured media of a geothermal reservoir. Therefore, current results indicate realistic viability of DNA-embedded silica nanoparticles as tracers for geothermal flow path analysis.

ACKNOWLEDGEMENTS

The authors would like to thank the Office of the Dean of the School of Earth, Energy & Environmental Sciences at Stanford University for supporting this work, Karrie Weaver at Stanford ICP-MS/TIMS Facility for providing resources for DNA release experiments, and Alberto Lovell at Stanford PAN Facility for his time and insight.

REFERENCES

Alaskar, M., Ames, M., Liu, C., Li, K., and Horne, R.N.: Temperature Nanotracers for Fractured Reservoirs Characterization, *Journal of Petroleum Science and Engineering*, 127, (2015), 212-228.

Frane, W., Vericella, J., Duoss, E., Smith, M., Aines, R., Roberts, J.: Smart Tracers for Geothermal Reservoir Assessment, *Geothermal Resources Council Transactions*, 38, (2014), 951-957.

Li, Y., Cathles, L., Archer, L.: Nanoparticle Tracers in Calcium Carbonate Porous Media, *Journal of Nanoparticle Research*, (2014), 16:2541.

Manley, T.S.: Evaluation of DNA-Embedded Silica Nanoparticle Tracers, master thesis, Dept of Energy Resources Engineering, Stanford University, (2015).

Paunescu D., Fuhrer, R., Grass, R.N.: Protection and Deprotection of DNA—High-Temperature Stability of Nucleic Acid Barcodes for Polymer Labeling, *Angewandte Chemie*, 52, (2012), 4269-4272.

Paunescu D., Puddu, M., Soellner, J., Stoessel, P. and Grass, R.: Reversible DNA encapsulation in silica to produce ROS-resistant and heat-resistant synthetic DNA 'fossils', *Nature Protocols*, 8, (2013), 2440-2448.

Stober, W., Fink, A. and Bohn, E.: Controlled Growth of Monodisperse Silica Spheres in the Micron Size Range, *Journal of Colloid and Interface Science*, 26, (1968), 62-69.

Zhang, Y.: DNA-Encapsulated Silica Nanoparticle Tracers for Fractured Reservoir Characterization, master thesis, Dept of Energy Resources Engineering, Stanford University, (2015).

Zhang, Y., Manley, T.S., Li, K. and Horne, R.N.: DNA-Encapsulated Silica Nanoparticle Tracers for Fracture Characterization, *Geothermal Resources Council Transactions*, 39, (2015), 967-974.