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Rate limiting factors for DNA transduction induced by weak electromagnetic field

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ABSTRACT

DNA transduction across aqueous solutions has been reported previously. In this study, we examined a few key factors affecting DNA transduction rate in an extremely low frequency electromagnetic field. These include: the chemical composition of the aqueous solutions, the type of experimental vessel, the dilution step, and the origin of the DNA fragments. The results indicate that partially introducing essential ingredients for DNA amplification (i.e. dNTPs and PCR buffer) to the aqueous solution enhanced the transduction rate greatly, and transduction vessels made of hydrophilic quartz yielded more favorable results than vessels made of hydrophobic plastic. In addition, performing a serial dilution to the transduction solution more than doubled the transduction rate compared to that without the dilution step. For the DNA fragments used in this study, there was one with a pathogenic origin and two with non-pathogenic origins. However, all three fragments achieved DNA transduction regardless of the difference in their origins. The experimental setup for eliminating the false positives caused by both biological and potentially physical contamination is also described.

Introduction

A number of reports indicate that chemical molecules have an intrinsic electromagnetic (EM) signature which carries the information and function of the molecule. Furthermore, such molecular information can be separated from the physical molecule and transferred to a separate aqueous solution using an exogenous EM field in the ELF range (Foletti et al., 2012a, 2012b; Kim, 2013; Montagnier et al., 2010, 2015).

Numerous publications from Montagnier’s lab have measured low-frequency EM signals from DNA in serially diluted solutions after activation by exogenous EM fields. These EM signals can be measured and stored using conventional EM detectors (Montagnier et al., 2010). In addition, these EM signals can be transferred via a resonance phenomenon to separate aqueous solutions and can induce the formation of water nanostructures which serve as a DNA template to recreate the intact original DNA (Montagnier et al., 2009, 2015). A mechanism for the recreation of DNA using water nanostructures has been proposed where the Taq-DNA polymerase enzyme can apparently “see” the EM signature of the DNA by exchanging wave fields. Such a mechanism is consistent with the gauge theory paradigm of quantum field theory (Kurian et al., 2018; Montagnier et al., 2017).

The EM signals from pathogenic bacteria and viral DNA can further be recorded as analog files. When bacterial EMS files were played back to immobilized human cell lines, all cells with a tumor origin synthesized the bacterial DNA and resulted in cell death, while normal cells were not affected (Montagnier et al., 2015). These findings are of great importance in the field of medicine as DNA spectral information can be used not only for diagnostic purposes (Giuliani et al., 2011), but also for treatment (Montagnier et al., 2015). Similar results were obtained using P53, a potent tumor suppressor. By transferring molecular information of P53 to aqueous solutions, the EM imprinted solutions were shown to inhibit cancer cells proliferation, have anti-metastasis activity and increased apoptosis (Kim, 2013). Other reports demonstrated the suppression of the metabolic activity of neuroblastoma cells by EM signals coming from retinoic acid (Foletti et al., 2012b; Grimaldi and Grieco, 2013) and the inhibition of bacterial growth by the molecular signals generated from antibiotic agents (Heredia-Rojas et al., 2015; Rad and Jalali, 2018). It is clear that the development of electromagnetic information imprinting in aqueous solutions as an inexpensive and non-toxic medical treatment holds great promise (Norman et al., 2016). Commercial devices now exist which can
transfer molecular information into the water and are currently being used by practitioners of Electronic Medicine to make “remedies”. Thus real-life applications of this new technology exist despite a shortage of rigorous scientific research on the subject of water memory. However, since the macroscopic quantum properties of water have been recently measured, a mechanism of action using quantum field theory has been proposed and a recent conference was held at the Royal Society of Medicine in London, it is reasonable to consider water imprinting a new emerging field of science. We believe our article contributes to the scientific understanding of this new field.

Given the significance of Montagnier’s work (Montagnier, 2010; Montagnier et al., 2017, 2010, 2009, 2015), we set out to examine the factors affecting DNA transduction, with the goal to increase DNA transduction rate and gain a better understanding of the conditions required for successful transduction. The transduction experiments spanned over 1 year, during which the experimental design continued evolving with the accumulative knowledge gained by the group. The experiments reported in this article were carried out sequentially instead of simultaneously. Here, we report the results of the variables examined over time which showed a significant impact on the transduction rate. The factors include: (1) the composition of the aqueous solutions, (2) the material of the vessel, and (3) the dilution steps. In addition, the transduction rate for DNA with different origins is also presented.

As the experiments progressed, we realized that “false positives” are an issue that needs to be addressed. Here, we report our experiment design with the goal to reduce “false positives” incurred both biologically and physically.

Materials and methods

General setup

DNA and the aqueous solutions were placed next to each other in a copper coil for an extended period of time (16–18 h) where information transduction was expected to take place. At the end of the transduction, we examined the success of the transduction by performing a PCR and gel electrophoresis on the transduction solutions or the serially diluted transduction solutions. We conducted experiments with three distinctive stages.

During the first stage, the aqueous solution used for transduction was purified water (Milli-Q water, with a resistivity of 18.2 MΩ•cm at 25°C) treated with microwave radiation and is here referred to as activated water. After transduction, the aqueous solution, referred to as the transduction solution, went through PCR and gel electrophoresis directly without going through a serial dilution. The DNA used during this stage was from non-pathogenic Streptomyces donated by a collaborator, M145-285 (DNA285). The vessels used included Eppendorf tubes (hydrophobic material) and a quartz cuvette (hydrophilic material) (Figure 1. Upper left panel). The negative control used a different batch of activated water and was placed outside of the coil under similar environmental conditions till the end of the transduction experiment. We’d like to stress that it is critical that the aqueous solution used for transduction and negative control are NOT from the same batch of water, and they need to go through the same procedure separately to avoid “false positives”.

During the second stage, the aqueous solution was activated water plus dNTPs and PCR buffer, with the goal to increase the transduction rate by introducing the ingredients for DNA replication. The DNA fragment was slightly shorter, P4TS-183 (DNA183), which is a hybrid DNA synthesized commercially. Based on the results from the first stage, we used quartz cuvette only and quit using plastic Eppendorf tubes from this point forward. At the end of the transduction experiment, the transduction solution went through a PCR and gel electrophoresis procedure directly without going through a dilution step. Negative control during this stage contained the same ingredient as the aqueous solution used for transduction, but prepared separately as stated above (Figure 1. Upper right panel).

During the third stage, we expanded on the second stage by carrying out an additional serial dilution step on the transduction solution, with the goal to further increase the transduction rate. In addition to DNA183, we also synthesized HIV-LAV-105 (DNA105), which is the same DNA used in Montagnier’s lab that has a pathogenic origin (Montagnier, 2010). For this stage, there were two different negative controls, with the first one (NC1) prepared separately as the aqueous solution used for transduction, and the second one (NC2) prepared separately as the diluent used for the dilution step. The diluent and negative control two contained physically treated water plus dNTPs and PCR buffer. The physical treatment for the Milli-Q water used to make the diluent and the negative control 2 included a step of autoclaving first and a second step for freezing at −20°C (Figure 1 Lower panel).

After transduction, the transduction solution was removed for decimal serial dilution for 10 times. To verify the result of the DNA transduction, PCR and gel electrophoresis analyses were performed on all serially diluted solutions, the positive control, and both negative controls.

One type of DNA cross contamination could be caused by the equipment, the shared space, the experimenters, as
well as from the air circulation. This type of contamination is referred to as “biological contamination” in this paper. To avoid biological DNA cross contamination, we separated the whole procedure into different modules, and each module was carried out by an independent experimenter in a lab located in a different building. For the transduction step, the DNA solution was capped, rinsed thoroughly and then wrapped with layers of Parafilm before putting into the coil next to the aqueous solution. In addition, the aqueous solution was also capped and wrapped with layers of Parafilm. At the end of the transduction, the aqueous solution was taken out of the coil and rinsed thoroughly before removing the Parafilm. Finally, the vial was thoroughly rinsed again to completely remove any potential trace of DNA stuck to the outside of the vessel, although the likelihood of this happening is very slim given the extremely strict experimental protocol used. In this setup, the biological contamination of DNA is nearly impossible to occur.

Another type of contamination we came across could be caused by the EM “field” or its “information”. We speculate that water with the same source, especially water treated by physically perturbation, e.g. microwave, could exchange information and lead to “false positives”. To avoid this kind of contamination, we avoided using water from the same container for both transduction and control. Instead, water for control was collected and handled separately as indicated in Figure 1.

Figure 1. Key variables for three stages of transduction experiments. First stage: (a) no dilution step, (b) DNA285, (c) plastic and quartz transduction vessel, and (d) without dNTPs and buffer in aqueous solution; Second stage: (a) no dilution step, (b) DNA183, (c) quartz transduction vessel only, and (d) with dNTPs and buffer in aqueous solution; Third stage: (a) with dilution step, (b) DNA183 and DNA105, (c) quartz transduction vessel only, and (d) with dNTPs and buffer in aqueous solution.

DNA fragments

Three DNA fragments were used for this study, HIV-LAV-105 (DNA105), P4TS-183 (DNA183) and M145-285 (DNA285), with the numerical numbers indicating the length of each DNA fragment in base pairs (bp). The DNA105 fragment is part of the sequence of the HIV1/HTLV-III/LAV virus as described in (Montagnier, 2010); the DNA183 fragment is a hybrid of the Arabidopsis TAG gene and the Cyanobacterial D1 protein gene; and the DNA285 fragment was donated by a collaborator with a non-pathogenic origin. The relevant DNA templates and primers were synthesized at Beijing Rui Bo Xing Ke Biotechnology Co., Ltd. (www.ruibiotech.com). DNA was amplified using PCR with the PCR product further purified by electrophoresis using Qiagen Gel Recovery Kit. Purified DNA was then stored at −20°C. See Table 1 for DNA sequence information.

Transduction vessel

Two types of containers were used to hold the aqueous solutions during the DNA transduction process, one was made of quartz and the other of polypropylene. The rectangular quartz cuvette (3.5 mm x 10 mm x 30 mm) had a 1 mm light path, a final volume of 350 ul and was purchased from a company specialized...
in optical equipment (http://yxhggx.com/index.php/List/index/cid/31.html). In addition, a 0.5 ml thin wall polypropylene tube with a diameter of 0.75 mm was purchased from Xygen Inc. (www.axygen.com).

**Preparation of the aqueous solution**

For stage 1, aqueous solution was Milli-Q water treated with microwave radiation. 30 ml of Milli-Q water (resistivity of 18.2 MΩ•cm at 25°C) was placed in a 100 ml unsealed flask and heated to boiling for 2 min in a microwave oven (300 W at 2.45 GHz); for stage 2 and 3, aqueous solution contained microwaved Milli-Q water, dNTPs and PCR buffer solution which was prepared by mixing 770 μl microwave treated water with 100 μl PCR buffer and 80 μl dNTPs and then mixed thoroughly using a vortex mixer for 15 s. Finally 350 μl of the mixed aqueous solution was added into the transduction vessel for the transduction procedure.

**Preparation of the DNA solution**

A DNA stock solution at the concentration of 25 nM was diluted 10 fold in an Eppendorf tube using Milli-Q water and vortexed for 15 s to obtain the DNA working solution (D1). Four hundred microliter of D1 was transferred into the transduction vessel. All the steps were carried out in a laminar flow hood designated only for DNA preparation in a separated building.

**The electromagnetic coil**

The coil had a width of 25 mm and a height of 100 mm. It has a resistance (R) of 8.6Ω and an impedance (L) of 1.8 mH. The coil was placed in between two magnets (230mT each) and was connected to a commercially available signal generator (Figure 2). The output from the signal generator was a 10 V sine wave scanning the ELF frequency range from 8 to 500 Hz. The repetition rate was 500 s.

**DNA transduction procedure**

DNA and an aqueous solution were placed side by side in the copper coil for DNA transduction. All experiments were carried out in a metal incubator (Beijing Yashilin testing equipment Co., LTD, Cat#, GDJS-800A) for 16–18 hours. The two containers were separated by multiple layers of Parafilm wrapped around
both containers so the outer surface of the two chambers did not touch. The temperature was set at 22°C during the transduction process.

**Preparation of the diluent**

The diluent contained pretreated Milli-Q water and dNTPs and PCR buffer. The pretreatment procedure for the water is as follows: a 300 ml of Milli-Q water in a glass container was autoclaved at 120°C for 15 min. Once cooled down to room temperature, it was dispensed into 1.5 ml Eppendorf tubes and stored at −20°C. To make the diluent, we took two tubes of pretreated water out of the freezer to thaw, and then mixed dNTPs and buffer with it. For example, 0.95 ml diluent consisted of the following constituents: 770 μl activated water+ 80 μl dNTPs +100 μl PCR buffer AND vortexed for 15 s before using.

**Serial dilution for the transduction solution**

After transduction, the transduction solution (the informed aqueous solution) underwent a decimal serial dilution using the diluent. After removal from the coil, the transduction solution was extensively rinsed under running water for a minimum of 3 min to remove any potential DNA molecules outside of the cuvette. The solution was transferred into Eppendorf tubes using a designated pipette and vortexed for 15 s to obtain D0 of the transduction solution. A decimal serial dilution was then conducted for 10 times. For example, to make D1, we took 10 μl of the D0 and added into a 90 μl of the diluent and then vortexed for 15 s. The successive dilution was carried out for 10 times to obtain D1-D10.

**Preparation of PCR premix**

The PCR premix was prepared in a laminar flow hood designated for DNA handling. For a 50 μl PCR reaction, 5ul buffer, 4ul dNTP mixture (containing dATP, dTTP, dCTP, dGTP at 2.5 mM each), 0.5 μl Taq DNA polymerase, 1μl forward primer and 1 μl reverse primer were added into a clean vial and vortexed for 15 s. The PCR premix does not contain DNA template. For samples with buffer and dNTPs added in previous steps, these constituents were left out at this step. All chemical reagents were purchased from Takara Clontech (www.takara-bio.com).

For the serially diluted samples and the negative controls, 2.5 μl of the premix was added to a 200 μl PCR tube, followed by 47.5 μl of the negative control and 47.5ul D1-D10 from each sample, respectively. Therefore the serially diluted samples and the negative controls did not contain the DNA template. For the positive control, 38.5 μl of the DNA template (25 pM) was introduced to the premix as shown in Table 2, which then went through PCR procedure as all the other samples. This step was ONLY initiated after all the above steps were fully completed.

**PCR and gel electrophoresis**

The PCR mix for all samples was centrifuged briefly, and then placed into a thermal cycle for PCR following a program as indicated by Figure 3. For most experiments, a standard 40 cycle program was used although in some cases an additional 10 cycles were performed when the band was too weak. A standard gel electrophoresis protocol was used using 2% agarose gels. For better resolution, the standard buffer was replaced by a TBE buffer and the voltage was kept below 5V/cm.

**Results**

In these experiments, three conditions were required for DNA transduction to be successful: (1) the positive control must generate a band with the correct size of the target DNA, (2) there must be no band for all negative controls, and (3) there must be at least one band at any of the serially diluted samples (D0-D10). Electrophoresis graphs below are representative of successful DNA transductions. The qualitative results indicate that, depending on the experiment, one or more of the serially dilute samples showed a band corresponding to the target DNA. The particular dilution showing a successful DNA transduction varied from experiment to experiment. There appears to be no logical preference for one dilution over another despite the fact that all experimental conditions were kept the same. Nonetheless, the results indicate that information from the intact DNA is carried on the low frequency EM field into the aqueous solution inside the transduction chamber. This transduction phenomena appears to be independent of the origin of the intact DNA as all three fragments produce successful information transfer.

<table>
<thead>
<tr>
<th>Components</th>
<th>Volume (μl)</th>
<th>End concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Buffer</td>
<td>5</td>
<td>1X</td>
</tr>
<tr>
<td>2 dNTP mix</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>3 Taq DNA polymerase</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>4 Forward primer</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>5 Reverse primer</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>6 DNA Template</td>
<td>38.5</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>50</strong></td>
<td></td>
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</tbody>
</table>
Figure 4 shows a representative GE for DNA105 with no positive bands identified for both negative controls but several distinct positive bands with the correct size as the target DNA for the serially diluted samples D2, D7 and D10.

Figure 5 shows another typical experiment using the exact same experimental conditions with the same DNA105 as the previous experiment. In this experiment, no positive bands are identified for the negative controls, but positive bands are identified for D0, D2, D3, D8, and D9.

The results in Figure 6 were also done under the exact same experimental conditions except that a larger DNA fragment (183bp) was used. Again the negative controls show no peaks, but there are distinct positive bands (albeit weak) at serial dilutions of D7 and D8. The bands in this experiment are less intense than in the previous experiment.

**Quantitative data on transduction rates under different conditions**

**Composition of the aqueous solutions**

Using DNA285, we observed that the composition of the aqueous solution affected the transduction rate (see Figure 7). As stated in the methods section, two types of aqueous solutions were tested, activated water (treated by microwave), and the activated water mixed with PCR buffer and dNTPs. The aqueous solution with partial PCR ingredients is more effective than activated water alone, with a 38.5% transduction rate for the aqueous solution \((n = 13)\) and only an 11.1% for the activated water \((n = 18)\).

**Transduction vessel**

The material of the transduction vessel is also a rate-limiting factor for DNA transduction as demonstrated in Figure 8 using DNA285. The quartz cuvette yielded much better results than the polypropylene Eppendorf tube, with a success rate of 38.5% \((n = 13)\) for the cuvette and 8.7% \((n = 23)\) for the plastic tube. Therefore all future experiments were done in a quartz cuvette.

**The dilution step**

The data indicates that the serial dilution of the aqueous solutions after DNA transduction increases the likelihood of a successful information transfer. For Both DNA105 and DNA183, the success rate of transduction is more than doubled by conducting the serial dilution step as demonstrated in Figure 9.

**The origin of DNA fragments**

We have achieved successful DNA transduction for all three DNA fragments. However, the DNA fragment with a pathogenic origin (DNA105) was appeared to be more active compared to the non-pathogenic DNA (DNA183 and DNA 285), demonstrated by a slightly higher transduction rate for DNA 105 than the other two fragments (Figure 10).
Discussion

These experiments replicate and confirm previous studies by Luc Montagnier (Montagnier et al., 2009, 2015) demonstrating that molecular information from intact DNA molecules can be transferred into an aqueous solution in a separate container. For this transduction to be successful, one of the essential requirements is a low-frequency EM field which can be produced from...
Figure 7. Average transduction rate for aqueous solutions with and without dNTP and buffer for DNA285.

Figure 8. Average transduction rate for two types of vessels using DNA285.

Figure 9. Average transduction rates with and without the dilution step for two DNA fragments.
either natural sources (the Schumann resonances) or artificial sources. In this study, we used a combination of natural Schumann waves and artificial EM waves generated by scanning from 8–500 Hz as described in the method section.

Two of the DNA fragments used in this study, DNA183 and DNA285, are not of pathogenic origin and yet are still capable of transferring information. In contrast, under somewhat different experimental conditions, Montagnier reported that only pathogenic DNA is capable of emitting EM signals (Montagnier et al., 2009, 2015). DNA transduction was successfully achieved with all three DNA fragments regardless of their origins under the experiment conditions used in this study. This is an important conclusion, because using the final experimental protocol, transduction of other chemical compounds can be investigated without the limitation of a pathogenic origin. Indeed, other biomolecules like thyroxin (Weber et al., 2008) are reported to be capable of transduction.

Compared to polypropylene chambers, the hydrophilic vessel yielded significantly higher transduction rates. The hydrophilic property of the quartz vessel used in this study facilitates the formation of an ‘exclusion zone’ near its surface (De Ninno, 2017; Giudice et al., 2013; Hwang et al., 2018). The exclusion zone itself is believed to contain numerous Coherence Domains (CD) (Ho, 2012) which appear to increase the transduction rate substantially. The formation of CDs is likely to be critical for DNA transduction as they are ensembles of nearly free electrons functioning as a disruptive structure and ready to accept externally supplied energy (Bono et al., 2012; Del Giudice et al., 2011; Marchettini et al., 2010). This could lead to the formation of nanostructures in water and serve as the template for the DNA replication during PCR amplification (Montagnier et al., 2009).

Compared to experiments with undiluted transduction solution, performing an additional serial dilution to the transduction solution increased DNA transduction rates. This is a critical factor in the preparation of homeopathic remedies (Weingartner, 2007; Wolf et al., 2011) and is assumedly responsible for the altered physical and chemical properties of homeopathic remedies. These altered properties in turn are believed to offer a feasible mechanism for water to have “memory” (Chaplin, 2007). Although the concentration of the solute decreases upon serial dilution, the number of coherent domains increases, thereby increasing its ability to imprint water across all frequencies associated with large biomolecules like DNA (Henry, 2017). According to Del Giudice (Del Giudice et al., 2011), molecules undergoing serial dilution will resonate with the CD and become “guest molecules” on its surface thereby initiating a chemical reaction (i.e. amplification process) once the enzyme and primers are introduced into the system.

Such a mechanism is feasible with respect to the present data and could explain why a hydrophilic vessel and serial dilutions were able to promote the transduction rate so significantly. The same mechanism may also explain why the addition of PCR buffer and dNTP enhanced the transduction rate so dramatically in the present study. We speculate that the addition of these ingredients will initiate and facilitate a chemical and energetic interaction with coherent domains formed in the transduction solution.

Figure 10. Average transduction rates for DNA fragments with different origins.
Based on the year-long experiments conducted by the group, we conclude that there are two types of false positives, one being “biological” and the other “physical”. The biological contamination is due to the handling of DNA solutions in the lab, which could cause cross contamination through various routes including, but not limited to, experimental vessels, bench top space, pipette and tips, air circulation, experimenter’s clothes/hair etc. Physical contamination could be caused by the low frequency electromagnetic field or by macroscopic entanglement of water from the same source or treated by the same physical perturbation, e.g. microwave.

Negative controls do not contain DNA polymerase or DNA template and therefore should not produce positive bands on PCR analysis. Nonetheless, false positives did occasionally occur. At the early stage of the work being carried out in the lab, false positive seemed to be a repeated issue despite the extreme precautions taken to avoid DNA contamination. Thus we physically isolated the DNA, the aqueous solution, the transduction solution, and the EM device to different buildings and the solutions were handled by designated operators at each step. This set up leaves no room for biological cross contamination between DNA and the aqueous solutions. Nonetheless, false positives still occurred occasionally and are most likely due to some unknown physical perturbation.

Since water has macroscopic quantum properties that give rise to long-range effects and entanglement between separated systems (Smith, 2004), we speculate that the false positives may be associated with macroscopic quantum entanglement in water. By using water treated with different physical methods (i.e. microwave vs. autoclave+ freezing) at different times as negative controls, we should have been able to eliminate false positives. Indeed, the final setup described in this paper allowed us to achieve clean results without the occurrence of false positives. However, the transduction protocol is still not optimized and many unknown factors still need to be identified.

Conclusions

We have achieved DNA transduction into aqueous solutions under the influence of an EM field, regardless the origin of the DNA. Many factors played crucial roles in the transduction rate, including the type of vessel used, the dilution step, and the composition of the aqueous solution. This line of work must be considered from the perspective of quantum electrodynamics (Del Giudice et al., 2011) in order to shed light on the issues like false positives and operator-dependent effects.

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Declaration of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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