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Introducing a New and Simple Protocol for Capillary Electrophoresis of Cell Free Fetal Double Stranded DNA

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Abstract

Isolation of cell free fetal DNA (cffDNA) from maternal serum usually leads to very low concentrations of DNA impeding further resolving through conventional methods of electrophoresis. Although several protocols have been described for capillary electrophoresis (CE) of double stranded DNA, they usually need using special polymers or coated capillaries which degrade over time. Herein, we proposed a new and very simple protocol using uncoated fused silica capillary for CE of cffDNA purified from serum of pregnant women. The proposed protocol was able to determine the presence of small fetal DNA fragments and it also confirmed the optimization of sonication performed on purified cffDNA samples.

Key words: Capillary electrophoresis, Free fetal DNA, Maternal serum.

Introduction

Recognizing cell free fetal DNA (cffDNA) in the maternal serum have opened a new hopeful window toward non-invasive prenatal diagnosis (NIPD) in recent decade [1]. However, due to the very low percent of fetal DNA in the maternal circulation (2-6%), getting access to cffDNA especially in the early weeks of pregnancy seems to be so hard [1]. Confirmation of the presence of fetal DNA in total isolated DNA from maternal serum can ensure that the subsequent steps of NIPD would be performed fruitfully. Although, some strategies as well as enrichment of fetal specific methylated sequences have been described, they necessitate all the steps of procedure to be completed. It was demonstrated that the fetal DNA are almost less than 150 bps and therefore, the electrophoresis of purified DNA can roughly show the existence of desired DNA fragments [2]. Given the low concentration of serum cffDNA especially in early pregnancy, fragments usually have to be resolved on Polyacrylamide gel electrophoresis (PAGE) which would be laborious, time consuming and toxic. Moreover, the initial step of NIPD tests is sonication of purified DNA to shear it in to 300-500 bps DNA fragmens that it also requires verification. Capillary electrophoresis (CE) provides a rapid, 10 fold sensitive and convenient alternatives to the traditional methods of electrophoresis to determine the size of double strand DNA and PCR products. Some new formulations of polymers have been introduced to increase the resolution of DNA and RNA fragments beside using polyacrylamide gel filled capillaries [3, 4]. However, these polymers are usually expensive making the need for cheaper and more user friendly protocol more prominent. In addition, using polyacrylamide gel filled capillaries not only is toxic but also they usually degrade so earlier than uncoated capillaries that cause

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the CE procedure to be so expensive.

Given the progressive advances in using NIPD tests in prenatal screening and diagnosis of fetal genetic abnormalities, introducing the safer and more rapid methods in each steps of NIPD tests can enhance the strength of these tests. Herein, we were aimed to show a novel optimization of CE of free fetal double stranded DNA purified from serum of pregnant women before and after sonication. We employed surface modified capillaries to absolutely suppress the electroosmotic flow.

Materials and Methods

Free fetal DNA was isolated from maternal serum of 10 pregnant women and two non-pregnant women using our previously proposed method (unpublished data). The quality and quantity of obtained DNAs were examined by NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). CE was implicated to determine the quality of obtained DNA through our proposed method and its capability in trapping cffDNA fragments. An ultrasonic processor UP50H (Hielscher, Germany) was then implicated to shear isolated DNA samples in to 300-500 base pairs fragments. DNA samples were maintained in an ice container to avoid of extra DNA degradation. Sonication process was optimized according to following protocol: three pulses of 90 seconds at 30 kHz with 30 seconds interval between each two pulses, amplitude of 40 and cycling 1. All the CE experiments was optimized and carried out on an Agilent Technology HP 3DCE Capillary Electrophoresis system (Berlin, Germany). Running of DNA samples were fulfilled using a fused silica capillary with an inner diameter of 75 µ m, 57 cm total length and 49 cm to the detector which was purchased from Agilent Technology (Berlin, Germany). The capillary was undergone preconditioning before initiating each run according to the following suggested protocol: 15 min flushing with NaOH (1M) followed by 5 min flushing with ddH2O and then running buffer, respectively. The capillary was washed using ddH2O for 10 min between each run. TAE 1X (Tris, Acetic acid, EDTA, PH: 8) and TBE 1X (Tris, Boric acid, EDTA, PH: 8) were compared as running buffer. The used running buffer was degassed, filtered and adjusted for both anion and cation poles vials to be in the same volume and reduce the chance of arising background signals. The 10 µl of each DNA samples (20 ng/ µl) was mixed with the same volumes of running buffers and SYBR Green dye (0.03µg/µl) to achieve better differentiation between various signals displayed by different DNA fragments. Pressure was adjusted to be 30 kPa to inject each sample in to the capillary in 3 second. Running the samples was tested at 25°C and only the signal peaks regarding to UV absorbance at 260 nm were selected to be drawn. The required voltage for running was examined in 7, 15 and 20 and samples were run in each voltage for 15, 20, 30 and 45 minutes. Initial optimization was performed by running the Ladder 100 bps (Fermentase) as control. The DNA sample purified from serum of a non-pregnant woman was also included to be run as another control in comparison with DNA samples isolated from pregnant women. The DNA samples were sonicated in three 90 seconds pulse accompanying two 30 seconds intervals on ice between every two pulse. After that the sonicated DNA was also subjected to CE with the same protocol as optimized for non-sonicated DNA.

Results

Implicating the voltage 7 and 15 Kv could not show the different sizes of DNA present in our samples and Ladder in durations of 15 and 20 min. However, one peak may representing the DNA fragments altogether was detected when the samples were run for 30 and 45 min in 15 Kv. By increasing the voltage to 20 Kv within the duration of 20 min, running Ladder and then samples was optimized to show the expected peaks (Fig 1a). None of running with TAE buffer has yield peak arising and therefore the TAE was excluded from the experiment as running buffer. CE demonstrated the DNA fragments less than 100 bps which have been saved using only our proposed method of DNA isolation (Fig 1b). Very low quantity of these small fragments in the CE of DNA derived from a non-pregnant woman was a confirmation of our results (Fig 1d). Capillary electrophoresis of sonicated DNA has revealed that the peaks of larger DNA fragments shifted to right to show the increase in the quantity of smaller fragments around 300-500 bps (Fig 1c).

Discussion

CE is frequently used for short tandem repeat (STR) analysis especially when the separating 1-2 nucleotides is difficult through even PAGE. In the present study, CE was employed to electrophoresis of double stranded cffDNA isolated from the serum of non-pregnant and pregnant women before and after sonication. It revealed that our previously proposed method of DNA isolation from maternal serum (unpublished data) was able to enrich high quantity of DNA fragments less than 150 bps. We could also determine that our DNA sonication

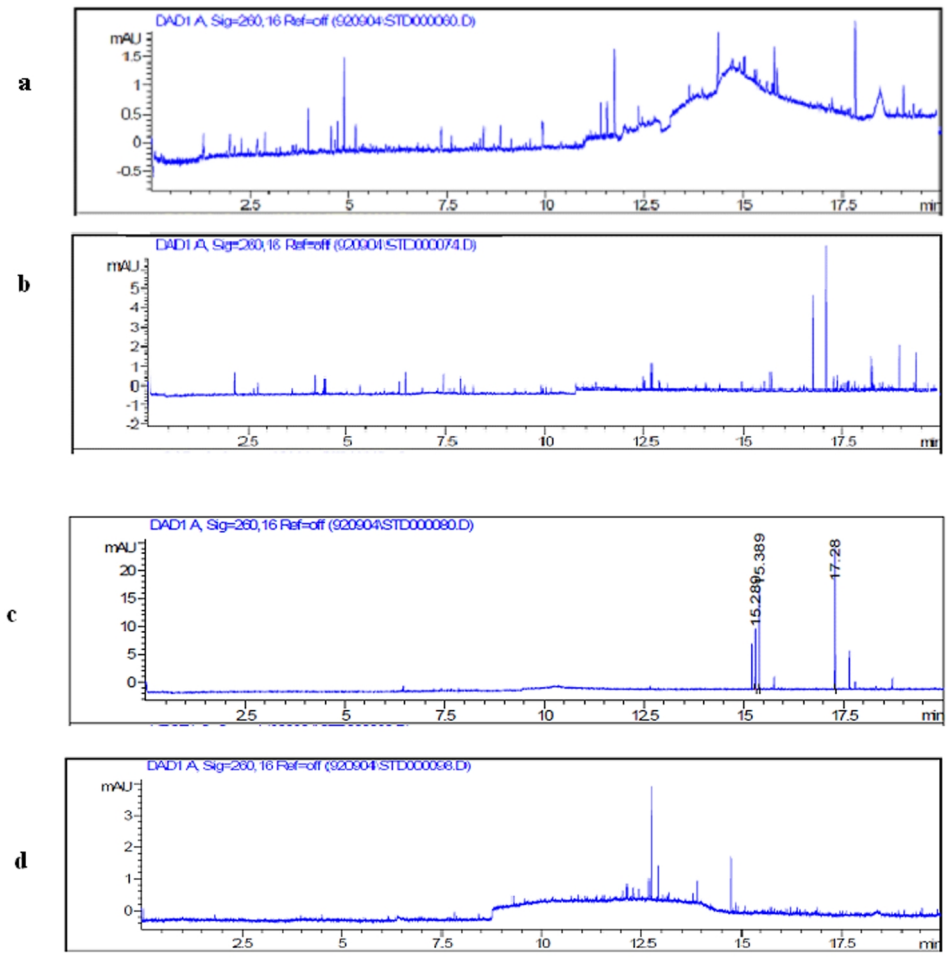

Figure 1. a: CE of Ladder 100 bps**; b:** CE of the cffDNA isolated based on our proposed method; **c**: CE of sonicated DNA isolated through our proposed method; **d**: cell free serum DNA isolated from non-pregnant woman.

optimization was appropriate in producing 300-500 bps DNA fragments ideal for downstream processes through CE of sonicated DNA. Although it may be also possible through PAGE, the priority of CE is that it not only determines the exact boundary of the sizes of obtained DNA fragments but also it can show the quantities of each DNA fragment. It is worth to note that owing to using bare silica, the large DNA fragments migrated faster than smaller fragments toward cationic pole and therefore the smaller fragments would be appeared at the end of run [3]. To our knowledge, the literature of DNA capillary electrophoresis is too scant and is restricted to a few studies. The capability of CE separation of double strand DNA fragments created by restriction enzyme digestion was initially examined using monomeric intercalating dyes in comparison with dimeric dyes. The quality of separation was shown to be increased when 9-aminoacridine (9AA) was included in both column and running buffer [5]. In the following study it was revealed that either the polyacrylamide coated or uncoated capillaries were able to separate large DNA fragments in dilute cellulosic polymer solutions [6]. However, the potential of CE in resolving the small DNA fragments less than 2 kbps was not explored in the mentioned assay. The optimized CE protocol described in the present work would be useful in resolving either large or small DNA fragments. Although, gel coated capillaries are still frequently used in separating DNA especially PCR products, they have two major disadvantages including their high expense and column degradation. These main drawbacks of coated capillaries engaged the conduction of two investigations to focus more on polymer solutions with uncoated capillaries. In the first one, a non cross-linked interpenetrating polymer network (IPN) included poly N, N-dimethylacrylamide (PDMA) and polyvinylpyrrolidone (PVP) was considered to be used as a medium in double strand DNA analysis without any

purification. Upon the conductive effect of TBE 1X buffer which has shown to be the preferred running buffer in the present work, PVP and PDMA were able to separate the pBR322/HaeIII DNA fragments by the resolution of 1 bps [4]. The second assay studied the capability of hydroxypropylmethyl cellulose (HPMC) to resolve the DNA fragments and plasmids purified from the Escherichia coli strain RRI [7]. It was revealed that electrophoresis of DNA in HPMC polymer and uncoated CE would be a safe, reproducible and stable alternative to more toxic coated CEs. However, the potential capability of another cellulose polymer ,hydroxyethylcellulose (HEC) was determined to be low in separation of double strand DNA compared to single strand DNA and RNA sequences [8]. Separation of double strand DNA using HEC polymer was described through pulse field gel electrophoresis in a recent study [9]. In the present work, we optimized a new method of CE in which the need for using coated capillaries and polymer has been absolutely removed. In addition, it was demonstrated that double strand binding SYBR Green dye had significant role in increasing the resolution of the present fragments.

Taken together, we could primarily demonstrate that CE using our suggested protocol would be an easy, cheap and rapid method of cffDNA running, in particular, when the DNA concentration is too low to be resolved by even PAGE. Although CE instrument is relatively expensive, our proposed protocol can increases its applications whenever it is present. Implicating of CE not only could help us to determine the size of cffDNA fragments in our samples for NIPD assays, but also it is so constructive in optimizing sonication of cffDNA samples.

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