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Notes & Tips

Electrophoresis of periodontal pathogens in poly(ethyleneoxide) solutions with uncoated capillary

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ABSTRACT

Periodontitis is a prevalent inflammatory disease caused by different species of anaerobic bacteria such as *Porphyromonas gingivalis* (*P.g*), *Treponema denticola* (*T.d*), and *Tannerella forsythia* (*T.f*). We compared the separation result of DNA ladders in hydroxyethyl cellulose, poly(ethyleneoxide) (PEO), and polyethylene glycol and analyzed the effect of polymer concentration, electric field, and temperature of the background electrolyte on the separation performance. Results demonstrated that there was a linear relationship ($R = 0.942$) for 100 to 700 bp of DNA and its migration time. Finally, the polymerase chain reaction products of *P.g*, *T.d*, and *T.f* were successfully identified within 8.5 min in 0.5% PEO with uncoated capillary.

Periodontitis, which is a prevalent human disease, is an inflammatory disease caused by different species of anaerobic bacteria. In 1996, the World Workshop in Periodontics described *Porphyromonas gingivalis* (*P.g*),[1] *Treponema denticola* (*T.d*), and *Tannerella forsythia* (*T.f*) as microorganisms that play a key role in periodontal destruction [1]. The multi-factoriality of this infection is due to a complex bacteria population with high dynamicity and adaptability, and the elimination of these pathogenic bacteria may be impossible once they are established in periodontal pockets [2]; thus, timely diagnosis of periodontal pathogens is important for the control of periodontitis.

The conserved sequences in 16S ribosomal RNA or DNA (16SrRNA or 16SrDNA) has been frequently used as a target of the polymerase chain reaction (PCR) examination and gene probe test, and the tests are noninvasive and extremely accurate in sensitivity and specificity [3]. Periodontal pathogens, as a kind of the bacteria, can also be identified by DNA or RNA tests. Cosgarea and coworkers compared the outcome of the two different commercially available microbiological tests: gene probe and real-time PCR for *P.g*, *T.f*, and *T.d* analysis [4]. However, the methods mentioned above not only had complicated operations but also had high costs.

Capillary electrophoresis (CE) is a powerful analytical tool in post-PCR analysis because of its numerous advantages [5]. The polymers employed in CE can be classified into cross-linked polymers and non-cross-linked polymers. Compared with the former, non-cross-linked polymers are more easily prepared and with high repeatability. So far, the non-cross-linked polymers mainly include polyethylene glycol (PEG) [6], poly(ethyleneoxide) (PEO) [7], polyvinylpyrrolidone (PVP) [8], and hydroxyethyl cellulose (HEC) [9]. Barron and coworkers carried out CE of DNA restriction fragments (2.0–23.1 kbp) in dilute cellulosic polymer solutions [10]. Our lab has also studied the φ×174-Hirc II and λ-EcoT14 I DNA analysis in mixed HEC polymer with different molecular weight (M_r), and results show that the separation performance was greatly improved [11]. Compared with the other polymers, it is easy to prepare homogeneous PEO solution for DNA separation, and Yeung's group finished single-base resolution of double-stranded DNA between 123 and 124 bp in 2.5% PEO ($M_r = 8{,}000{,}000$) [12]. Chang's lab performed separation of φ×-174/Hae III DNA restriction fragments in PEO solutions containing ethidium bromide [13]. In this work, we have analyzed the periodontal pathogens by CE in PEO solutions coupled with PCR technology. Such a method is rapid and inexpensive and has high selectivity; thus, it may provide an effective method for clinical diagnosis of periodontal pathogens in patients.

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[1] *Abbreviations used*: *P.g*, *Porphyromonas gingivalis*; *T.d*, *Treponema denticola*; *T.f*, *Tannerella forsythia*; 16SrRNA, 16S ribosomal RNA; 16SrDNA, 16S ribosomal DNA; PCR, polymerase chain reaction; CE, capillary electrophoresis; PEO, poly(ethyleneoxide); HEC, hydroxyethyl cellulose; M_r, molecular weight.

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First, we resolved the standard 100-bp DNA ladders (Takara, Japan) with the total concentration at 20 ng/μl in HEC (1300k) and PEO (1000–4000k). All electrophoresis experiments were carried out on a self-built CE system [14,15]. The polymer solution containing 1× SYBR Green I (Invitrogen, USA) was prepared by dissolving in 0.5× TBE buffer (Bio-Rad, USA). Then, they were separated in the polymer with the same concentration (0.5%) at 100 V/cm electric field. The resolution (R) was evaluated by the following equation [11]:

$$R = \Delta t/1/2(W_1 + W_2),$$

where Δt is the difference between two adjacent peaks and W_1 and W_2 are the peak widths measured at the baseline. Results showed that 11 DNA fragments could be completely resolved in the three polymers. The resolution for the adjacent DNA fragments was improved with the increase of PEO molecular weight (Fig. 1A), but the separation time was increased, which is possibly attributed to the self-coating characteristic of PEO. Furthermore, it took 7.5 and 10.0 min for DNA fragments in 0.5% PEO (4000k) and 0.5% HEC (1300k), respectively, but the resolution for DNA fragments in the former solution was better than that in the latter solution. Therefore, PEO (4000k) was selected as the sieving medium for further experiments.

Next, we investigated the effect of PEO concentration on the separation performance. Here, 100-bp DNA ladders were resolved in PEO solutions from 0.1 to 1.0% at 100 V/cm electric field. Results showed that the resolution (Fig. 1B) of DNA fragment in PEO solutions from 0.1 to 0.5% was improved with the growth of the polymer concentration due to the increase of ionic strength, and the migration time also was prolonged with the increase of the polymer concentration. However, when the polymer concentration grew from 0.5 to 1.0%, the resolution of DNA fragment between 800 and 1000 bp became low. This is possibly because there are highly entangled networks when the polymer solution is above 0.5%, and the average distance ξ between the polymer chains will be smaller with the increase of PEO concentration; thus, large DNA fragments can hardly go through the sieve pore.

Next, the influence of the electric field on the separation performance was investigated. Results demonstrated that when the electric field was increased from 80 to 200 V/cm, the migration time of the 1000-bp DNA fragment was reduced from 10.5 to 3.0 min. This is because the electric field force enforced on the DNA molecule was increased with the increase of the electric field. However, the resolution of the DNA fragments was deteriorated if the electric field force was increased (Fig. 1C) because the additional heat due to the electric field may affect the resolution [12]. Heat generated during electrophoresis is proportional to the DC voltages. For 200 V/cm, the total power is 1.5 times larger than that of 80 V/cm. The additional heating may deteriorate the resolution because of the enhancement of DNA fragment diffusion during the run. Thus, we selected 100 V/cm for further experiments.

Next, we studied the effect of temperature of background electrolyte on the separation performance. Experiments demonstrated that by increasing the temperature of the polymer, the migration time of DNA will be decreased, and this is partly because the viscosity of the polymer was reduced with the increase of temperature [16]. However, the resolution for the adjacent DNA fragments will be degraded at the same time (Fig. 1D). Based on the experiments

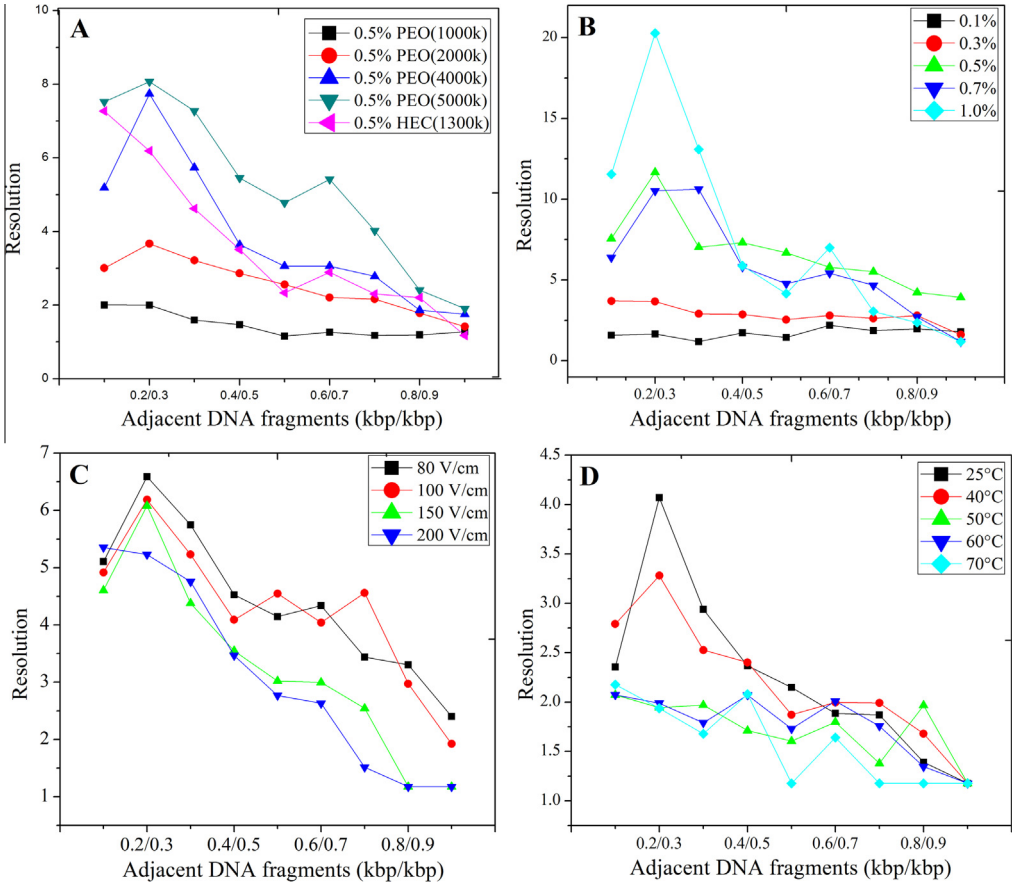

Fig.1. Resolution for 0.1-kbp DNA ladders at various electrophoretic conditions. (A) Polymers: HEC (1300k) and PEO (1000k, 2000k, 4000k, and 5000k); electric field strength: 100 V/cm. (B) PEO concentrations: 0.1 to 1.0%; electric field strength: 100 V/cm. (C) Electric field strength: 80 to 200 V/cm; PEO concentration: 0.5%. (D) Temperature of background electrolyte: 25 to 70 °C; electric field strength: 100 V/cm; PEO concentration: 0.5%. The total length and effective length of the capillary were 12 and 8.0 cm, respectively. Sample loadings: 100 V/cm (1.0 s).

above, we selected 0.5% PEO (4000k), 100 V/cm electric field, and 25 °C temperature to accomplish a compromise for CE.

Then, we carried out the PCR of the periodontal pathogens. Bacterial strains of *P.g* (ATCC 33277), *T.f* (ATCC 43037), and *T.d* (ATCC 35405) were bought from Microbiologics (USA). The reagents for PCR included a 1.0-μl DNA template and 49-μl reaction volumes containing 5.0 μl of 10× Fast Buffer I, 4.0 μl of deoxynucleoside triphosphate (dNTP) mixture (2.5 μM), 200 nM primers, and 0.25 μl of SpeedSTAR HS DNA polymerase. The primers for *P.g*, *T.d*, and *T.f* were as follows [17]:

P.g: forward, 5′-TGTAGATGACTGATGGTGAAAACC-3′; reverse, 5′-ACGTCATCCCCACCTTCCTC-3′
T.d: forward, 5′-AAGGCGGTAGAGCCGCTCA-3′; reverse, 5′-AGCC GCTGTCGAAAAGCCCA-3′
T.f: forward, 5′-GCGTATGTAACCTGCCCGCA-3′; reverse, 5′-TGCT TCAGTGTCAGTTATACCT-3′.

Those primers were synthesized by FASMAC (Kanagawa, Japan). PCR was performed in a T-100 thermal cycler (Bio-Rad) and programmed with an initial step of denaturation at 95 °C for 2 min. The cycling conditions were as follows: 95 °C for 10 s (denaturation) and 64 °C for 30 s (annealing and extension). In total, 40 cycles of the above program were performed. The sizes of the corresponding PCR products for *P.g*, *T.d*, and *T.f* were 197, 311, and 641 bp, respectively.

Based on the optimal electrophoretic conditions obtained above, we equally mixed the PCR products of *P.g*, *T.d*, and *T.f* and then separated the mixture coupled with 100-bp DNA ladders in 0.5% PEO (4000k) at an electric field of 100 V/cm (Fig. 2C). Results showed that they were baseline resolved within 8.5 min. Data in Fig. 2C show that the PCR product of *P.g* cannot be identified because the resolution between the PCR product of *P.g* (197 bp) and 200 bp was too low. Therefore, we carried out electrophoresis of 100-bp DNA ladders (Fig. 2D) and PCR products of *P.g*, *T.d*, and *T.f* (Fig. 2B) in the same electrophoretic conditions. It demonstrates that they were resolved with high resolution. A typical plot of migration time versus molecular weight of DNA ladders demonstrated that there was a linear relationship between the migration time and the molecular weight from 100 to 700 bp. The correlation coefficient *R* was approximately 0.942; thus, the size of the PCR products of periodontal pathogens was determined. We also extracted the periodontal pathogens from 40- to 50-year-old volunteers according the method proposed in Ref. [17] and then carried out PCR of *P.g* with the procedure above. Results demonstrated that *P.g* was well recognized (Fig. 2A) but that *T.d* and *T.f* were not found by CE. This was possibly because they existed only in patients with severe periodontitis.

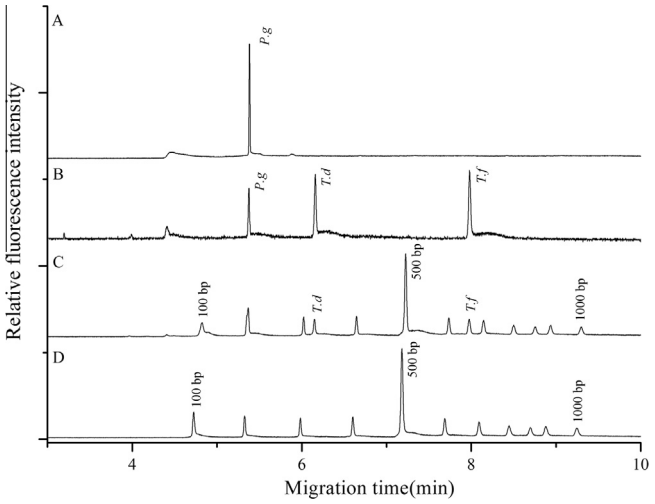

Fig.2. Capillary electrophoresis of PCR products of *P.g* from real sample (A); PCR products of *P.g*, *T.d*, and *T.f* (B); PCR products of *P.g*, *T.d*, and *T.f* and 0.1-kbp DNA ladders (C); and 0.1-kbp DNA ladders (D). Capillary electrophoresis was performed in 0.5% PEO (4000k) polymers with 100 V/cm electric field strength. Other electrophoretic conditions were the same as those in Fig. 1.

In summary, we compared the separation results of 100-bp DNA ladders in HEC (1300k) and PEO (1000–4000k) by CE, and the PEO (4000k) polymer was selected in this work. Then, we optimized the electrophoretic conditions by varying electrophoretic parameters. Finally, the PCR products of periodontal pathogens were resolved within 8.5 min in the optimal electrophoretic conditions.

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