B39 Development of a Method for Electrophoretic Separation of DNA for Forensic Analysis Using a UV-Transparent Microchip and a Photopolymerizable Polyacrylamide Gel Matrix

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The goal of this presentation is to demonstrate the use of photopolymerizable gel as a sieving matrix in tandem with microchips as a viable method for forensic analysis of DNA.

A method for high-resolution, CE-based separation of DNA for forensic analysis, translatable to the microchip platform, using a photopolymerizable gel in a UV-transparent capillary and microchip is described. Achieving the high-resolution DNA separations necessary for forensic analysis in a cross-linked, non-replaceable, gel-filled capillary or microchip system has previously presented a number of problems, including filling difficulties, gel placement issues, and gel degradation problems. However, photopolymerizable polyacrylamide gel differs from classic polyacrylamide gel by the presence of a UV-active initiator. The polymerization reaction will, therefore, only take place when and where the acrylamide monomer solution is exposed to UV light. The solution can, thus, be injected into the capillary or microchip as a liquid, with no polymerization taking place. This eliminates the filling difficulties previously encountered with conventional polyacrylamide gel and, in addition, simply covering regions of the capillary or microchip where gel is not desired before exposure can control gel placement. In this way, exact placement of the gel in the microchip channel or capillary is ensured, eliminating yet another obstacle encountered with conventional chemically initiated polyacrylamide. Also, the extent of polymerization and, thus, gel pore size, can be controlled by length of exposure to light. Altering the exposure (polymerization time) will change pore sizes and, by this control, resolution can be affected and the system tailored to the length of fragment being considered. Fragment mixtures of short fragment lengths can, therefore, be run through a gel with smaller pores, while longer fragments can be run through a gel with larger pores. This can also affect the length of time needed for separation, with a more tailored system allowing for higher throughput of samples. Also described is a method for the creation of a gel gradient utilizing this property by variation of the length of exposure time across a channel or capillary for high-resolution separations. In addition, the effects of other parameters (voltage, temperature, ionic strength of buffer, etc.) on separation and resolution are described. Increasing voltage can lead to better resolution, but there also exists a threshold for the gel beyond which a breakdown of the gel matrix occurs. This impacts the duration of time during which the gel is viable and effective. Gel lifetime testing is also discussed, including how the gel performs under varying conditions, how long separations can be attempted before degradation occurs, and the feasibility of this method for implementation in a crime laboratory situation. Removal and replacement of the gel in the channel and capillary is also discussed. In addition to the advantages of the photopolymerizable gel, the advantages of the microchip system are also highlighted. These advantages include smaller injection/sample volume, faster analysis time, and multi-channel, multi-sample analysis on a single chip. The microchip system is conducive to the small sample volumes often present in forensic casework and the speed with which separations can be obtained, along with the ability to analyze more than one sample at a time are of great time and cost saving benefit. This poster presents data from preliminary studies aimed at evaluating the effects of various parameters on the polymerization of the gel and the subsequent effect on DNA fragment separation and conditions on the resolution of DNA fragments in CE and microchips. Conditions necessary for attaining the high resolution separations needed for microchip analysis to be pertinent to forensic DNA analysis will be described.

DNA, Capillary Electrophoresis, Microchip

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