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Design, Development and Testing of Polymer-Glass Microfluidic Chips for Electrophoretic Analysis of Biological Sample

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Abstract : An important area of biological and medical research is the study of genetic mutations and polymorphisms that can alter gene function and cause inherited diseases and other diseases. The following methods to analyse DNA fragments are used: capillary electrophoresis and electrophoresis on microfluidic chip (MFC), mass spectrometry with electrophoresis on MFC, hybridization assay on microarray. Electrophoresis on MFC allows to analyse small volumes of samples with high speed and throughput. A soft lithography in polydimethylsiloxane (PDMS) was chosen for operative fabrication of MFCs. A master-form from silicon and photoresist SU-8 2025 (MicroChem Corp.) was created for the formation of micro-sized structures in PDMS. A universal topology which combines T-injector and simple cross was selected for the electrophoretic separation of the sample. Glass K8 and PDMS Sylgard® 184 (Dow Corning Corp.) were used for fabrication of MFCs. Electroosmotic flow (EOF) plays an important role in the electrophoretic separation of the sample. Therefore, the estimate of the quantity of EOF and the ways of its regulation are of interest for the development of the new methods of the electrophoretic separation of biomolecules. The following methods of surface modification were chosen to change EOF: high-frequency (13.56 MHz) plasma treatment in oxygen and argon at low pressure (1 mbar); 1% aqueous solution of polyvinyl alcohol; 3% aqueous solution of Kolliphor® P 188 (Sigma-Aldrich Corp.). The electroosmotic mobility was evaluated by the method of Huang X. et al., wherein the borate buffer was used. The influence of physical and chemical methods of treatment on the wetting properties of the PDMS surface was controlled by the sessile drop method. The most effective way of surface modification of MFCs, from the standpoint of obtaining the smallest value of the contact angle and the smallest value of the EOF, was the processing with aqueous solution of Kolliphor® P 188. This method of modification has been selected for the treatment of channels of MFCs, which are used for the separation of mixture of oligonucleotides fluorescently labeled with the length of chain with 10, 20, 30, 40 and 50 nucleotides. Electrophoresis was performed on the device MFAS-01 (IAI RAS, Russia) at the separation voltage of 1500 V. 6% solution of polydimethylacrylamide with the addition of 7M carbamide was used as the separation medium. The separation time of components of the mixture was determined from electropherograms. The time for untreated MFC was ~275 s, and for the ones treated with solution of Kolliphor® P 188 – ~ 220 s. Research of physical-chemical methods of surface modification of MFCs allowed to choose the most effective way for reducing EOF – the modification with aqueous solution of Kolliphor® P 188. In this case, the separation time of the mixture of oligonucleotides decreased about 20%. The further optimization of method of modification of channels of MFCs will allow decreasing the separation time of sample and increasing the throughput of analysis.

Keywords : electrophoresis, microfluidic chip, modification, nucleic acid, polydimethylsiloxane, soft lithography
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