Dielectrophoresis based continuous-flow nano sorter: fast quality control of gene vaccines

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We present a prototype nanofluidic device, developed for the continuous-flow dielectrophoretic (DEP) fractionation, purification, and quality control of sample suspensions for gene vaccine production. The device consists of a cross injector, two operation regions, and separate outlets where the analytes are collected. In each DEP operation region, an inhomogeneous electric field is generated at a channel spanning insulating ridge. The samples are driven by ac and dc voltages that generate a dielectrophoretic potential at the ridge as well as (linear) electrokinetics. Since the DEP potential differs at the two ridges, probes of three and more species can be iteratively fully fractionated. We demonstrate the fast and efficient separation of parental plasmid, miniplasmid, and minicircle DNA, where the latter is applicable as a gene vaccine. Since the present technique is virtually label-free, it offers a fast purification and in-process quality control with low consumption, in parallel, for the production of gene vaccines.

1 Introduction

In recent years a new class of therapy has become more prominent, the so-called gene vaccination. Gene vaccination is the injection of genes of interest into patients. Most often viral or bacterial vector systems were used as transporter systems. This generated problems concerning safety regulations if the viral or bacterial genes were also inserted and interspecies crossover appeared. In contrast, minicircle DNA consists only of the gene of interest and its regulating sequences. The established purification and quality control processes, by capillary gel electrophoresis (CGE) or affinity chromatography are time and sample consuming. Thus, the purpose of this work was to establish a new method for the purification and fast and efficient quality control of gene vaccines with low consumption.

The small dimensions of micro total analysis systems (μTAS) create several advantages, like low sample volumes, and short response times. Fast development has occurred in the field of μTAS with a high variety of devices, methods and applications, as can be seen in recent reviews. The success also shows that μTAS give access to reliable and inexpensive analytical and preparative tools complying with medical rules and regulations. Structured microfluidic devices have been used for the separation of long DNA molecules either electrophoretically or entropically. Both approaches operate in a batch process way, i.e. one fraction of the sample is processed before the next sample is injected into the operation region. When considering the process mode of purification and quality control of gene vaccines, continuous-flow processing yields several advantages like continuous harvesting, real time adaptation of separation parameters, fast response time, and easy implementation of further up- and downstream applications.

In the literature, some microfluidic devices for the continuous-flow separation of DNA species were reported. For example, Fu et al. presented a patterned anisotropic nanofluidic device for the continuous-flow separation of linear DNA molecules. The advantage of their device was that they could separate DNA over a wide range of sizes, but its separation efficiency was limited by diffusion. Since the separation mechanism was based on entropic trapping, only linear DNA molecules could be separated. Hence, gene vaccines, which are mostly in a circular conformation, could not be separated by that technique.

Huang et al. presented a DNA prism for the continuous-flow separation of long DNA molecules. They used an array of posts through which the DNA was driven by asymmetric pulsed fields, with tilting angles. DNA of more than 60 kbp could be separated within 15 s, but without baseline-separated resolution. Two disadvantages were that only linear DNA molecules could be separated, thus, excluding gene vaccines, and the diffusion-limited resolution. In 2004, Huang et al. presented a device that did not rely on linear molecules and enabled the continuous-flow separation of long DNA mole-
cules and nanobeads. The separation mechanisms either were based on the asymmetric bifurcation of a laminar flow around obstacles driven by a hydrodynamic flow (for beads), or on the bifurcation of electric fields the same way (for DNA). Depending on the particle size, different paths were followed; 0.8 μm to 1.0 μm beads could be separated within 40 s without baseline-separated resolution, whereas the separation of large DNA (61 kbp and 158 kbp) took 10 min.

One technique that allows a label-free and contactless operation of biological probes is dielectrophoresis (DEP), which is the migration of a polarizable object within an inhomogeneous electric field.

Concerning continuous-flow separation by dielectrophoresis, several approaches have been published. Church et al. used a curved channel to continuously separate 2.2 μm and 5 μm beads. The channel was arranged in serpentine shapes, over a length of 1 cm. Several other groups used insulating obstacles, e.g. blocks or oil droplets, for the dielectrophoretic continuous-flow separation of microbeads, with sizes between 5.7 μm and 15.7 μm. Gallo-Villanueva et al. used two arrays of posts that differed in post distances. Thus, two regions of varying dielectrophoretic potential were created. They could separate 1 μm and 4 μm beads into different outlets. In contrast to the two-dimensional structures other groups used, Barrett et al. fabricated three-dimensional linear ridges (slit height of 5 μm) for the separation of micrometer-sized B. subtilis and 200 nm particles. Dielectrophoresis has already been exploited for the manipulation, separation and characterization of different DNA species in batch processing. Nevertheless, the previous devices do not allow the continuous-flow dielectrophoresis of nm-sized DNA molecules.

Recently, we have shown that continuous-flow electrodeless dielectrophoresis (eDEP) at an arc-shaped nanoslit allows the fast and efficient separation of two species of small DNA fragments. Here, we extend the principle to multiple separation, i.e. the total separation of more than two species. Various approaches were considered, regarding the ridge-shape-depending ratio of the electrokinetic effects, as postulated by Hawkins et al. and 3D structuring of the ridge like a stair. Due to the ease of production and possible increased number of applications, we chose a branched channel layout with additional ridges in the branches. In section 2, a detailed discussion is given.

The chip consisted of a cross injector, two channel spanning ridges that form nanoslits of about 500 nm height, and side channels that led downstream from the ridges towards separate reservoirs. As was discussed in ref. 37 and 39, the trajectory at the ridge depends on the molecules size. We designed the layout of the chip such that the DEP potential differs at the two nanoslits. Thus, at each ridge one species could be separated from the sample. A discussion of the critical parameters and an enhanced device layout for a more efficient separation is presented.

The new method of multiple-separation is applied for the total separation of a circular parental plasmid, miniplasmid, and minicircle DNA. The separation revealed that the new technique allows a fast quality control along the production process of minicircle DNA (see Fig. 1).

2 Concept

The general concept of continuous-flow separation was based on dielectrophoresis at a bowed nanoslit that spanned the microchannel laterally. Dielectrophoresis is the migration of a polarizable object in an inhomogeneous electric field, denoted by . It is created by applying a voltage to a microfluidic channel in which at least one insulating ridge is incorporated. The resulting potential dielectrophoretic energy of a polarizable object can be written as,

\[ W_{\text{DEP}} = -\frac{1}{2} z \mathbf{E}^2, \]  

with \( z \) being the polarizability of the object. The corresponding dielectrophoretic force is,

\[ F_{\text{DEP}}^\mathbf{E} = z (\mathbf{E} \cdot \nabla)\mathbf{E}. \]  

Electrokinetic effects, namely electrophoresis and the electroosmotic flow, drive the analytes when electric fields are applied to the immersed electrodes. Additionally, a dielectrophoretic potential is generated at the nanoslit. Hence, the dynamical behavior is governed by linear and non-linear electrokinetic effects. The resulting trajectory is given by.

![Fig. 1 Working principle of continuous-flow purification and quality control of minicircle DNA production for gene vaccines. The DNA probe is injected towards the ridges and separated into its components iteratively. The nanofluidic device allows a fast operation with low sample consumption. *Sample preparation as in ref. 4.](image)
With $\mu_{eo}, \mu_{ep}$ being the electroosmotic and electrophoretic mobility, $\mu_{DEP}$ the dielectrophoretic mobility, and $E_{dc}$ and $E_{ac}$ the electric field amplitudes corresponding to the direct current (dc) voltage and the oscillating sinusoidal (ac) voltage signal. For eqn (3) it was considered that the migration of the objects in the oscillating field averages out. Additionally, the electric field strength is proportional to the electric voltage and thus in all experiments $|E_{dc}|^2 \ll |E_{ac}|^2$, so for the dielectrophoretic effects the dc component could be neglected compared to the ac component.

The quintessence of eqn (3) is that under the given experimental conditions the linear electrokinetic effects, i.e. electroosmosis and electrophoresis are controllable solely by the dc electric field. Additionally, the dielectrophoretic migration is governed by the ac electric field. Thus, the relative strength of these forces can be adapted during the experiments by tuning the applied dc and ac voltage amplitudes. Since the dielectrophoretic component scales with the gradient of the electric field, it only plays a significant role at spatial inhomogeneities, e.g. in the vicinity of the nanoslits (for more information, refer to ref. 39).

In Fig. 2 (a) the associated dielectrophoretic potential is depicted. In the vicinity of the nanoslit, the dielectrophoretic potential is significantly increased with respect to the microfluidic regime. The dielectrophoretic force is oriented essentially perpendicular to the edge of the ridge, whereas its component tangential to the ridge is very small. Thus, a particle would not migrate along the ridge, only driven by dielectrophoretic forces. In Fig. 2 (b) and (c) the electric field, generated by a dc voltage as typically used during the experiments, is shown in the vicinity of the nanoslit. Close inspection revealed that the electric field has components tangential to the edge of the ridge. In summary, the dielectrophoretic force drives the objects perpendicular to the edge of the ridge whereas the linear effects drive the analytes tangentially to the ridge. Thus, both migrations are in a separate manner and controllable.

The path of a particle is therefore governed by the ratio between the linear and the non-linear electrokinetic effects. Since these are separately tunable by the applied ac and dc voltages, for any given species with specific mobilities the migration can be fully controlled. Thus, for dominating dielectrophoretic forces the particle would be deflected at the ridge towards the other channel wall where it passes the nanoslit. The second pathway is dominated by the linear electrokinetic effects, i.e. the dielectrophoretic component is negligible. Thus, the object passes the nanoslit unaffected. So far, the system was optimized for a “binary response”, i.e. the analyte had two possible pathways at the ridge; either the analyte passed the nanoslit unhindered or was deflected towards the other channel wall. As a consequence, a mixture of two species could be separated into its components if the electrokinetic properties differed sufficiently.

Recently, we demonstrated the continuous-flow separation of nanobeads and DNA, as well as the detection of binding interactions that were performed with two different analyte species at a time. Concerning devices for the separation of three or more analytes, three different approaches were considered: (1) Hawkins et al. suggested that analytes of different polarizabilities would escape from an arc-shaped ridge at different locations, due to the angle dependent migration. Thus, separation of more complex mixtures should be possible at one single arc-shaped ridge. However, neither they nor we could observe such a behavior; (2) the ridge could be structured like a descending stair, i.e. each step would differ in the dielectrophoretic potential. So, at each step one species, that is less polarizable, would leave the ridge. The production of this device would need more than two contact lithographic steps, thus would be more sophisticated and the separation efficiency would be limited by the channel width; (3) the channel could branch downstream of the ridge into two channels. One of the branches would contain an additional
ridge. Due to the channel geometries, the dielectrophoretic potential at the second ridge could be set to a different value. Thus, a mixture of three analytes could be separated into its components. For mixtures of higher complexity, the system could easily be expanded, with more branches.

In this work, the third route was followed, because of the ease of fabrication, and since the production process for the master wafer was the same. Up to the first ridge, the device looked the same as for the binary separation. Additionally, downstream of the first ridge the channel was split into two branches, one of them with a second ridge (see Fig. 3).

When designing the device for multiple-separation, some boundary conditions have to be considered. For example, the resistance of both branches has to be (nearly) the same, otherwise the analytes would only follow the path of minimal resistance. Additionally, the channel geometries, namely the length, width and height of the micro- and nanochannels influence the strength of the electric field at the ridge and thus the dielectrophoretic potential. The geometrical factor \( \xi \) characterizes the ratio of the applied voltage and the resulting electric field at the nanoslit. \( \xi \) depends on the geometrical parameters such as channel width, height and length. From previous separation experiments of two species, the minimal electric field for a successful separation was known.\(^{37,39}\)

Hence, the device layout for a multiple-separation was designed such that the necessary field was achieved using lower voltages. Beyond these boundary conditions, the general ridge layout as well as the orientation is important for a high separation efficiency. In the present work, the ridge was arc-shaped (see Fig. 3). According to the orientation of the arc, the dielectrophoretic potential is minimal where the DNA molecules impact the ridge. Thus, an entrance into the dielectrophoretic potential well is formed.\(^{37}\) Along the ridge the dielectrophoretic force increases relative to the electrostatic forces. As a consequence, once trapped, the DNA migrates to the opposite channel wall and escapes at a well defined region as a narrow stream. Hence, a downstream separation is possible with high efficiency.

The analyte stream widened due to diffusion with respect to the migrated distance (see the ESI). For a high separation efficiency, narrow analyte streams are indispensable. Thus, it became obvious that the distance between the two ridges had to be minimized for an efficient multiple-separation. In the first device the two branches were aligned at 45° with respect to the main channel (see Fig. 3). As a consequence, the distance between the two nanoslits was too long and an efficient separation was prohibited by the wide analyte stream downstream of the first ridge. Hence, a second device was designed with the second ridge in line with the first. With that layout, the distance between the two ridges was significantly reduced, minimizing the diffusion based broadening during experiments. Thus, these were the basic parameters to consider, when designing a device for multiple-separation.

The enhanced layout is depicted in Fig. 4. The ridges were arranged in a linear channel, which revealed three advantages: (1) the “free” way in between the two ridges was minimized from 675 \( \mu \text{m} \) to 400 \( \mu \text{m} \); (2) fluorescence intensity scans of both separation steps were easily performed, since the orientation of the scan paths was the same; (3) for the separation of further species, additional branches and ridges could be inserted, with space saving. Even for several separation processes, the stream did not widen, due to the concentration of the deflected species at each step (see the ESI).

The devices were designed such that the middle-sized and largest analytes were deflected at the first ridge, hence separating the smallest one. At the second ridge, the larger analyte was deflected and the middle-sized species passed the ridge unhindered. As a consequence, the electric field at the second ridge had to be less than at the first ridge. The channel was designed such that \( \xi \) differed at the two ridges within one device. To calculate \( \xi \) we used an equivalent circuit exploiting the fact that the electric resistance is proportional to channel length/cross-section.\(^{39}\) In the first device the first ridge spanned a channel of 200 \( \mu \text{m} \) width and the second ridge a
channel of 150 µm width. As a consequence, the electric field at the first ridge was 1.5 times larger than at the second ridge. For an efficient separation of species that differ less in size, like parental plasmid and miniplasmid DNA, the ratio has to be higher. So, in the second device both ridges spanned a 200 µm wide channel. Thus, the electric field at the first ridge was twice that of the electric field at the second ridge. This was verified in the experiments (see the ESI). The heights of the nanoslits were the same at both ridges and did not influence the ratios of the electric fields.

3 Methods

3.1 Master wafer

The master wafer was fabricated by two contact lithographies. Silicon wafers (CrysTec, Germany) were cleaned in caroic acid (Merck, Germany) for 5 min twice and rinsed with water. A first layer of SU-8 photore sist (Microresist, USA, 17% solid fraction) was spin-coated (Delta 10, BLE Laboratory Equipment GmbH, Germany) at 1000 rpm for 30 s. The first layer was exposed to UV light through a chromium mask (Delta Mask, The Netherlands) in a mask aligner MJB3 (SOSS MicroTec, Germany) for 1.7 s. The height of the structure was determined, as 500 nm, with a DekTak Profilometer 3030 ST (MicroTec, Germany) for 1.7 s. The master wafer was fabricated by two contact lithographies.40 Silicon wafers (CrysTec, Germany) were cleaned in caroic acid (Merck, Germany) for 5 min twice and rinsed with water. A first layer of SU-8 photore sist (Microresist, USA, 17% solid fraction) was spin-coated (Delta 10, BLE Laboratory Equipment GmbH, Germany) at 1000 rpm for 30 s. The first layer was exposed to UV light through a chromium mask (Delta Mask, The Netherlands) in a mask aligner MJB3 (SOSS MicroTec, Germany) for 1.7 s. The height of the structure was determined, as 500 nm, with a DekTak Profilometer 3030 ST (Stanford Nanofabrication Facility, USA). A second layer of SU-8 (52% solid fraction) was spin-coated (2000 rpm for 30 s, total structure height 6.2 µm), afterwards. The wafer was again exposed to UV light for 7 s, carefully aligned to the first layer in the mask aligner. Baking and development were performed according to the supplier’s information. Finally, the wafer was silanized with tridecafluoro-1,1,2,2-tetrahydrooctyltrichlorosilane (Merck, Germany) in an exsiccator.

3.2 Chip fabrication

The chips were produced by soft lithography with poly(dimethylsiloxane) (PDMS) as published elsewhere.46 The channels were filled with working buffer, 1 mM phosphate buffer (pH 7.4, 0.2 mM NaCl) (Fluka, Germany) containing 1 mM ethylenediaminetetraacetic acid (EDTA) (Fluka, Germany), 2 mM 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) (Fluka, Germany). To reduce the unspecific adsorption of analytes to the surfaces and for the control of the electroosmotic flow, 0.25% N-dodecyl-β-D-maltoside (DDM) (Sigma Aldrich, Germany) and 0.03% methylcellulose (MC) (Sigma Aldrich, Germany) were added.41 A visual inspection of the ridge after the separation experiment revealed that only a negligible amount of DNA adhered to the nanoslit surface. A poly(methyl methacrylate) (PMMA) block with integrated reservoirs and platinum electrodes was placed on top of the chip to enlarge the reservoirs and to simplify handling.

3.3 Sample preparation

The parental plasmid (6.766 kbp), miniplasmid (4.509 kbp), and minicircle DNA (2.257 kbp) were obtained from PlasmidFactory, Germany. For the experiments, the DNA was stained with YOYO-1 (Molecular Probes, USA), ratio YOYO-1 to DNA base pairs 1 : 10. The stock solutions were diluted to several 10 pM with working buffer.

3.4 Setup

The measurements were performed on an inverted fluorescence microscope (Axiovert 200, Zeiss, Germany) with a motorized x/y-stage (995008, Ludl Electronic Products, USA). A 100 × oil-immersion objective (Plan Neofluar, NA 1.3, Zeiss, Germany) was used together with a mercury arc lamp (HBO 50) and a fluorescence filter set (BP 450-490, FT 510, BP 515-565, Zeiss, Germany). A gray filter (25% transmittance) reduced the incident light. A CCD interline-transfer camera (Imager 3LS, LaVision, Germany) with a corresponding grabber card and software (DaVis 6.2) was used for data acquisition with an 8 by 8 binning and 10 frames per second (fps). The voltage signal was created via a LabView 6i program and function generator DS345 (Stanford Systems, USA). The output signal was amplified by a high voltage amplifier (AMT-1860-L Matsusada, Japan). Three power supplies (HCL 14-12500, FUG, Germany) generated additional dc voltages.

3.5 Chip experiments

The electrophoretic migration of the DNA was adapted by dc electric voltages. The mixture was pipetted into reservoir 3 (see Fig. 3 and 4). Specific voltages used in the separation experiments were chosen as follows. First, the dc voltages at reservoirs 1 to 5 (or 6), were chosen such that a continuous molecule flow, parallel to the channel wall, from channel 3 into the separation channel was achieved with dc voltages of about 4 V. It was assumed that the inflowing DNA molecules occupy less than a quarter of the total width of the separation channel. Then, the ac voltage was switched on to relatively small amplitudes (50 V) and its effect on the particle motion was observed while varying the frequency over the interval from 50 to 1000 Hz. If no deflection was observed, the amplitude was increased, followed by varying the frequencies until two molecule streams were observed downstream of the first ridge as well as the second ridge for the first time. Hence, the frequency was optimized to the smallest voltages necessary. Each trajectory was identified afterwards at the same conditions (see the ESI).

During the separation experiment, the channel was scanned. By determining the fluorescence distribution, the separation resolution (Res) was calculated. Briefly, the fluorescence intensities were plotted and fitted with Gaussian functions. With \( \text{Res} = \frac{x_2 - x_1}{2(\sigma_1 + \sigma_2)} \) and \( x_2, x_1 \) denoting the position of the peak maximum and \( \sigma_1, \sigma_2 \) the corresponding peak width, the resolution was calculated. For more information, refer to ref. 39.

The nanoslit was inspected after the separation experiments to check for the unspecific adsorption of DNA molecules to the nanoslit surface. The inspection revealed that only a negligible amount of DNA adsorbed to the surface of the nanoslit.

3.6 Gel electrophoresis

For the gel electrophoresis, parental plasmid DNA from bacterial cells before induction and 5 min, and 30 min after induction was used. The gel electrophoresis was performed in
a 1% agarose gel with 1-fold TAE buffer (40 mM Tris (99%, Roth, Germany), 10 mM NaCl (Riedel-de Haen, Germany) and 1 mM EDTA). The samples (0.5 μg DNA) were pipetted with 1/5 of the volume of six-fold concentrated loading buffer (glycerin (30% v/v), 50 mM EDTA, bromophenol blue (0.001% w/v)) into the slots and 100 V cm⁻¹ [PowerPac 3000, BIO-RAD, USA] were applied for 30 min. The DNA was stained with 1 mg l⁻¹ ethidium bromide (Merck, Germany) for 10 min after electrophoresis and placed in water for 5 min. Finally, the gel was photographed under UV light (INTAS Science Imaging Instruments GmbH).

4 Results and discussion

4.1 Quality control of minicircle DNA

During the production of the minicircle DNA (2.257 kbp), residual miniplasmid (4.509 kbp) and parental plasmid DNA (6.766 kbp) might contaminate the probe, excluding this from any use as a vaccine. For the new chip-based quality control, the size difference between the three species was exploited. Thus, each species exhibited characteristic trajectories due to their polarizability. To demonstrate the functionality of the on-chip quality control a mixture of all three species was separated and the characteristic trajectories were observed.

The separation of the parental plasmid, miniplasmid and minicircle DNA was performed in the enhanced device, i.e. the two ridges were arranged in a linear channel (see Fig. 4). With that nanofluidic device it became possible to completely separate the three DNA species and lead them into three different outlets (see Fig. 5).

It became clearly visible that 1 min after starting the experiment downstream of the first ridge and 2 min after starting downstream of the second ridge, two molecule streams were formed. Thus, at the first ridge, two molecular species were dielectrophoretically trapped at the ridge. Within the nanoslit the electric field has a component tangential to the ridge pointing towards the opposite channel wall (cf. Fig. 2 (b)). Hence, the trapped DNA molecules are electrophoretically driven along the ridge towards the opposite channel wall. At the first ridge the parental plasmid and miniplasmid DNA, which are of higher polarizability, were deflected, whereas the smaller, less polarizable minicircle DNA passed the first ridge unaffected and was led into a separate channel. Since the

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**Fig. 5** Separation of the parental plasmid, miniplasmid and minicircle DNA. (a) Collage of the fluorescence microscopy images. A mixture of the parental plasmid, miniplasmid and minicircle DNA is injected towards the ridges from a side channel (each yellow spot depicts one single DNA molecule). At the first ridge, the minicircle DNA is separated out of the mixture and led into a separate channel. The parental plasmid and miniplasmid DNA are deflected and migrate towards the second ridge, where only the parental plasmid DNA is deflected. Thus, all three species are collected in separate channels. (b) and (c) Fluorescence intensities up- and downstream of the two ridges. The scan paths are depicted over the graphs. The black lines represent the scans upstream of the ridge; the dashed lines represent the scans downstream of the ridge. The red lines are Gaussian fits. (b) At the first ridge the resolution was $\text{Res} = 1.10$. (c) At the second ridge the resolution was $\text{Res} = 1.25$. Thus, a complete separation of the three species was performed with a very high separation efficiency.
dielectrophoretic potential was reduced by a factor of 4 (see section 2) at the second ridge, only the larger parental plasmid DNA was dielectrophoretically trapped and deflected towards the opposite channel wall. Thus, the parental plasmid and miniplasmid DNA were separated. An evaluation of the fluorescence intensity distributions revealed that at both ridges a baseline-separated resolution was achieved. Hence, a complete separation of the three species was performed with a very high separation efficiency.

For a successful separation, an ac voltage of 250 V at 350 Hz was applied to the device. Recently, we separated minicircle and parental plasmid DNA by dielectrophoresis in a 180 nm nanochannel by applying an ac voltage of 200 V at 350 Hz.\textsuperscript{37} Hence, the applied voltages were about the same. Nevertheless, here, the nanoslits were about 2.5 times higher, which results in a higher stability of the nanoslits. Thus, the channel width could be set to 200 \( \mu \text{m} \), which allows a higher separation resolution. The dielectrophoretic force was adapted via the lengths of the channel, compensating for the greater height of the nanoslits.

For applications in quality control, beyond the sensitivity, which here is on a single molecule level, the response time is one of the critical parameters. In our work, the response time for the detection of the three components was about 2 min. Thus, this new technique is considerably faster than the previous on-chip techniques and significantly faster than the established affinity chromatography used in today’s laboratories.

4.2 Comparison to gel electrophoresis

Throughout the minicircle DNA production process, several analytical samples were taken to ensure the quality, e.g. by gel electrophoresis.\textsuperscript{4} In Fig. 6 a photograph of an electrophoretic agarose gel of various samples along one production process is depicted. In each slot, 500 ng DNA was applied and the runtime of the gel was 30 min. Five minutes after the induction of recombination, the characteristic bands of the minicircle and miniplasmid DNA are visible.

The comparison of both techniques reveals the significant superiority of the new chip-based quality control compared to the gel electrophoresis. The chip-based technique needs only 0.3 ng DNA, hence 1666 times less sample, and allows the detection of single molecules. Particularly, with a response time of 2 min, it is significantly faster than the gel electrophoresis assay.

5 Conclusion

We have shown a new approach for the continuous-flow separation of three DNA species with a potential application in the production of gene vaccines, like minicircle DNA. The non-invasive and virtually label-free technique of dielectrophoresis at ridges that span the channel laterally was used. The multiple-separation was realized by an iterative separation in a branched channel system with two ridges. Thus, at each ridge one species was removed and collected in a separated channel. By an enhanced channel layout, due to the distance between the two ridges, the separation efficiency could be significantly increased. Hence, even for the multiple-separation of three species, a baseline-separated resolution was achieved.

The continuous-flow separation presented in this work exhibits several advantages: fast separation time, in less than 2 min; accessibility to linear as well as circular DNA;\textsuperscript{37} baseline-separated resolution, for multiple-separation and the on-line adaptability of selectivity parameters via applied voltages. Hence, it combines the advantages of the three continuously operating microfluidic devices published,\textsuperscript{13,20,21} in one single device. Furthermore it allows an in-process-control with versatile applications in the optimization of plasmid production.

As fluorescent staining is not mandatory for the presented method, beyond its use in the quality control of gene vaccines, demonstrated above, the implementation of the method in the purification of gene vaccines, by the parallel operation of several devices is possible. As a label-free variant, the method could be applied to e.g. spatially resolved optical adsorption. In future developments, for the purification of gene vaccines, additional analysis of the separated samples investigating damage and degradation of DNA and the separation efficiency will be undertaken by capillary gel electrophoresis.

Since this novel technique does not rely on classical filtration steps, shear force associated analyte deterioration is reduced to a minimum. Together with the fact that this approach is inherently independent of toxic or mutagenic reagents, this new method could be implemented in a much easier way regarding the rigorous concerns of safety regulation in drug administration.\textsuperscript{41}

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References
