Enhancement of DNA Microarray Hybridization using Shear-Driven microfluidics

J. Vanderhoeven[1], K. Pappaert[1], B. Dutta[2], P. Vanhummelen[2], G.V. Baron[1] and G. Desmet[1].
[1]Vrije Universiteit Brussel, Department of Chemical Engineering
Pleinlaan 2, 1050 Brussels, Belgium
tel.: (+).32.2.629.37.81, fax.: (+).32.2.629.32.48, e-mail: kris.pappaert@vub.ac.be
[2]MicroArrayFacility Lab, Flemish Institute for Biotechnology (VIB), Belgium

ABSTRACT

Hybridization on microarray slides is a very slow process. The transport of target molecules towards the immobilized probe spots occurs solely by molecular diffusion and as a result, microarray analyses require overnight incubation and are highly inefficient in terms of capture rate and detection limit. To overcome this diffusion limitation, several solutions have already been reported: electrokinetic flows, ultra-sound mixing, pump-around systems, etc. The present study demonstrates how DNA microarray analysis is enhanced by miniaturization. Only a small sample volume (<5µL) is applied on the microarray and is continuously convected across the microarray surface using a shear-driven flow system. We performed hybridization experiments in a rotating micro-chamber for different hybridization times and with different chamber depths. We demonstrated the benefits of the shear-driven rotating flow system in a biological assay, with complex RNA mixtures (mouse lung and testis total RNA). The shear-driven system is compared with two commonly used hybridization techniques: overnight coverslip diffusion and a commercial hybridization-station (Automated Slide Processor or ASP). The shear-driven system enhanced the hybridization, both in terms of analysis speed and in final spot intensity. We observed that the shear-driven system leads to a tenfold decrease in analysis time and a sixfold increase in final spot intensity.

1. INTRODUCTION

In the past years, many research groups have focused on the enhancement of DNA microarray analysis, and a large number of different solutions have been proposed and commercialized. The proposed methods include surface acoustic wave (SAW)-based micro-agitation[(1)], pressure- and electrically-driven flow generation[(2)], the use of pump-around systems[(3)], shear-driven flow systems[(4)], etc. Central to all these approaches is the use of some form of mechanical or electrical forces to increase the transport rate of the sample DNA strands beyond their normal (extremely slow) diffusion transport rate. Trying to compare the different proposed systems on the basis of the published hybridization experiment results is very difficult because the available data relate to widely differing experimental conditions (probe spot density, length and complexity of the sample target strands, size and concentration of the applied samples,…). In addition, the reported final spot intensity data are very diffuse and disperse. In some cases[(1)] a gain of up to a factor of five is claimed, whereas in other cases the diffusion control and the transport enhanced experiment clearly converge to the same limit. Sometimes, the gain with respect to the traditional diffusion-driven coverslip experiments is not even mentioned.

When approaching the problem of enhancing DNA microarray analysis it seems that the sample concentration can be premised as the key factor determining both the initial hybridization rates and the final spot intensities. Both the initial binding rates and the maximally achievable final spot intensities are proportional to the sample concentration. Therefore, a maximal sample concentration is beneficial during the entire course of the hybridization process. The following very simple design rule can hence be put forward: if a given mass of DNA sample is available, the fastest hybridization rates and final spot intensities will be obtained if the available amount of DNA dry mass is dissolved in the smallest possible buffer volume. To handle these small volumes, minimal volume hybridization chambers are needed, and the use of pump-around sample reservoirs and tubing should be avoided, or at least minimized. If at the same time the total microarray surface has to remain fully covered by the entire sample, the minimization of the hybridization chamber volume can only be achieved by decreasing the thickness of the fluid layer covering the microarray spots. Since in this case the volume of fluid which is in direct contact with each individual target spot becomes very small, a strong convective transport enhancement is needed to continuously renew the sample at the spot surface. Similar conclusions have recently also been made from the study of a planetary centrifugal DNA hybridization enhancement system[(5)].

In previous publications the enhancement of the microarray hybridization process using the principle of shear driven flows was already proven. Here we describe hybridization experiments performed in a rotating micro-chamber (Figure 1) for different hybridization times and with different chamber depths. These results however, were obtained on a setup with a limited number of spots. In order to demonstrate the benefits of our shear-driven rotating flow system on biological assays, we also used a microarray setup where the Mouse-microarray-chip (MicroArray Facility) was hybridized with mouse lung and testis total RNA samples. We compared our shear-driven

system with two commonly used hybridization techniques: overnight diffusion under coverslip and a commercial hybridization station (ASP).

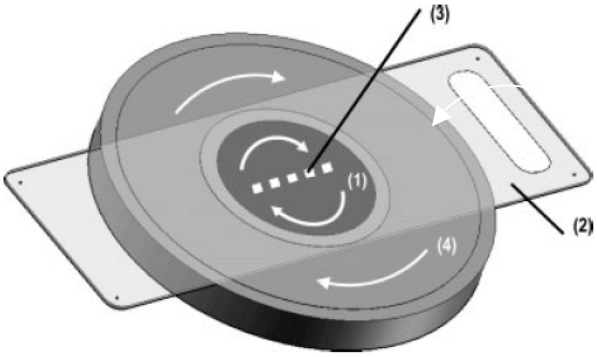

Figure 1. *Schematic representation of the hybridization set-up, showing the rotatable sample chamber (1) and the DNA microarray slide (2) with the spotted regions (3) covering the sample chamber during the operation of the device. The outer etched ring (4) is only there to limit the contact area between the rotating bottom substrate and the microarray slide.*

2. METHODICAL SECTION

The concept of a shear-driven flow circumvents pressure-drop and double-layer overlap limitations of respectively pressure-driven and electrically driven flows. This flow system is an excellent way to generate ultra-high velocity liquid flows through micro- and nanometric deep channels. The flows are generated by mechanically moving the bottom half of a flat-rectangular channel past the top part (or vice versa). The fluid present in the channel region between the two channel halves is displaced by the viscous dragging effect caused by the moving wall. The flow is sustained along each point of the channel and a linear velocity profile is established in the channel (Figure 2). The mean flow velocity is equal to half the velocity of the moving wall and is independent of the fluid viscosity and channel depth and length.

The circular micro-chambers that were used (diameter = 2 cm and with depths varying between 1.6 and 70 µm), were etched in the center of a round, flatly polished (flatness = $\lambda/20$ at λ= 512 nm) borosilicate glass wafer (Photox Optical Systems, UK) with a thickness of 6 mm and a diameter of 5 cm. The etching was carried out using a 50 % HF-solution for different time intervals, depending on the desired micro-chamber depth. The fluid in the micro-chambers could be flown in two different modes. Both continuous and discontinuous rotating experiments were performed.

All microarray experiments were performed using conventional microarray procedures that are used at the Microarray Facility Lab (www.microarrays.be). Two types of microarray systems were used for these experiments. The first system used for the primary tests consisted of an array of 6x12 probe spots spotted eight times over the entire slide. The spots were positioned in a regular way across the micro-chamber (Figure 1) A limited number of target molecules were used to simplify the analysis.

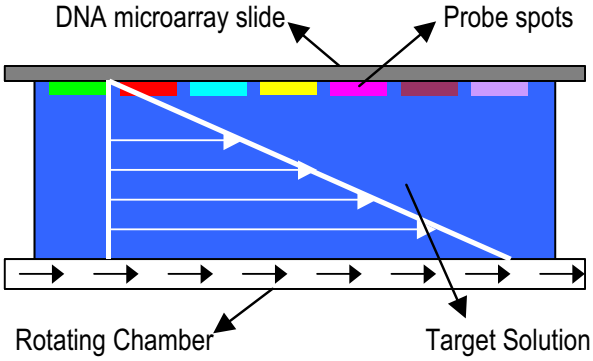

Figure 2. *Basic principle of a shear-driven flow. The flow is generated by moving one flat surface relative to the other one. A linear flow field will be created and the fluid layer between the 2 surfaces will move at an average velocity, which is half the velocity of the moving wall.*

In a second more complex microarray system the VIB_Mouse_5K_II chip (MicroArray Facility, Leuven, Belgium) was used. This more complex system contains more than 9000 different probe spots. Mouse testis and lung total RNA was used as target.

3. RESULTS

In earlier publications we have already proven the benefits of using a shear-driven system for enhancing the hybridization rate on microarrays[4]. However, in this system only a small number of spots could benefit from the shear-driven flow. To alleviate this low surface coverage a rotating system was developed (Figure 1). Numerous experiments showed that continuously rotating the fluid was not as beneficial as a discontinuous rotating experiment. A comparison of 30' hybridization experiments with stop periods of 1, 2, 3, 5 and 10 minutes showed that an optimal stop period can be determined for each micro-chamber depth and this optimal stop period becomes shorter with smaller micro-chambers. These findings are in agreement with one's physical expectation since local target depletion will occur sooner in smaller micro-chambers and therefore, the required frequency of sample refreshment will be higher than in larger micro-chambers. Figure 3 clearly shows that whereas the continuous rotation experiments yielded intensities which were sometimes not significantly larger than the purely diffusion driven conditions, the hybridization intensities obtained during the discontinuous rotation experiments are, without any exception, at least twofold larger. The introduction of stop-periods, during which the disturbing effect of the velocity field is switched off, hence clearly has a beneficial effect.

The differences between the results obtained in the different micro-chamber depth cases are shown in Figure 3. The advantage of using the thinnest possible micro-chamber is obvious, as was already theoretically predicted[6]. The 1.6 µm deep micro-chamber for example yields a 5 to 7 times larger final hybridization intensity than the 10 µm deep

micrco-chamber. This roughly compares with the concentration factor obtained when putting the same amount of DNA into a 10/1.6 ≅ 6 times thinner fluid layer. A similar conclusion can be made when comparing the 1.6 µm micro-chamber with the 3.7 µm micro-chamber. The concentration factor is approximately 2.5. Comparison with the 70 µm micro-chamber is more difficult because in this case the obtained intensities flirt with the background signal.

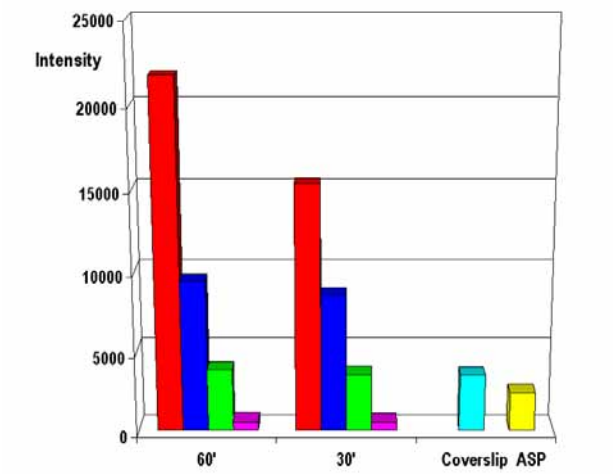

Figure 3. *Comparison of the shear driven rotating flow system (30 & 60 minutes) for four different micro-chamber sizes (red:1.6 µm, blue:3.7 µm, green:10 µm and pink:70 µm). The hybridization experiments were compared with 16 hour hybridizations under coverslip (light blue) and 12 hour hybridization using the ASP-hybridization system (yellow)*

As can be noted from Figure 3, the gain in hybridization intensity between the 30' discontinuous rotation in the thinnest micro-chamber and the overnight diffusion-driven hybridization under coverslip is already a factor of about four. Waiting for 30 more minutes in the discontinuously rotating micro-chamber system, to obtain a total hybridization time of 1 hour, the gain with respect to the diffusion-driven overnight coverslip experiment amounts up to almost a factor of six. This is quite impressive taking into account that the hybridization time in the discontinuous rotating system is sixteen times shorter. For the ASP system the dilution factor is obviously so large that it can not be compensated by the increased transport rates of the pump-around operation mode.

Obviously, the gains obtained from the concentration factor (spanning a range of nearly a factor of 40) are much larger than the gains originating from the rotation itself, as can be noted from the fact that the difference between the purely diffusion-driven experiments and the best possible discontinuous rotation experiments is only maximally a factor of four (Figure 4). Figure 4 also shows that the optimal displacement timing depends on the micro-chamber depth. The thinner the micro-chamber, the shorter the optimal stop-periods. This is in agreement with one's intuitive expectations based on the shorter radial diffusion times which are needed if the micro-chamber depth decreases.

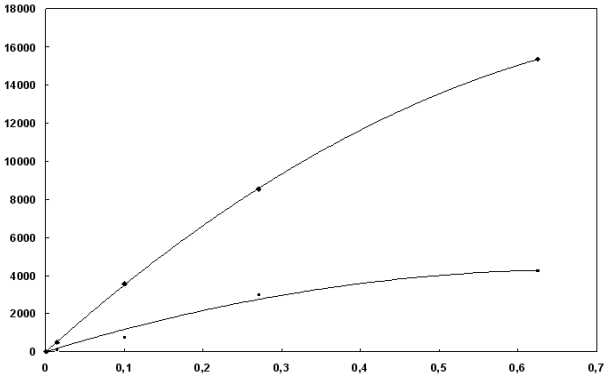

Figure 4. *Maximal spot intensity versus the inverse of the micro-chamber depth for 30' discontinuous rotation (♦) and 30' diffusion-driven (□) experiments.*

In order to test the shear driven rotating hybridization system in a more complex hybridization system, experiments were performed on high density microarray chips. As target molecules Mouse testis and lung total RNA was used. The shear-driven system was compared to normal diffusion under coverslip and to a commercial system (ASP) from Amersham. Figure 5 shows the obtained microarray images.

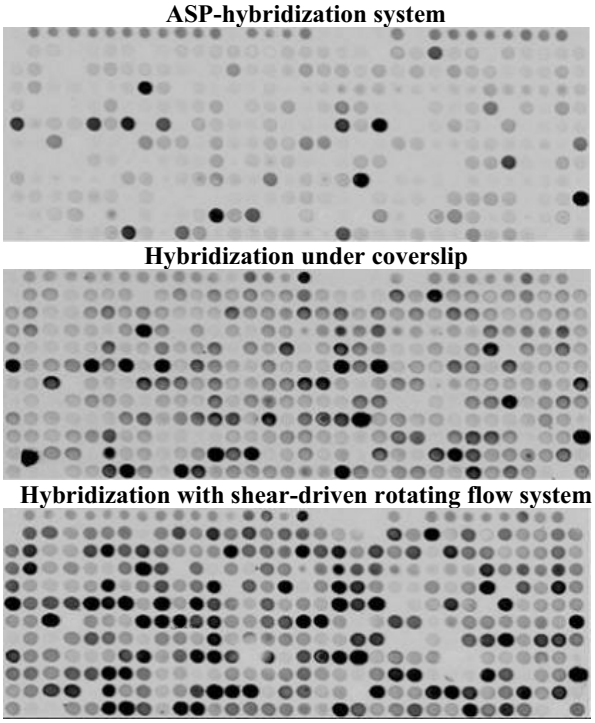

Figure 5. *Overview of the obtained results from three different sets of experiments: hybridization with the ASP (720 minutes), hybridization under coverslip (960 minutes) and hybridization with the shear-driven rotating flow system (60 minutes). It is clearly visible that the obtained signal is much higher for the shear-driven system.*

The obtained results are remarkable. The shear-driven system enhanced the hybridization and the gain was large, both in terms of analysis speed and in final spot intensity. We observed that, starting from the same amount of DNA,

only a small volume, relative to the other two techniques, is required for our application and that the combination of miniaturization and the introduction of the shear-driven principle leads to a more than tenfold decrease in analysis time while the final spot intensity increases significantly (Table 1). We noticed that, in comparison with the two other hybridization systems, the use of the shear-driven flow-system led to a higher amount of hybridized spots. Within one hour, 89% of the spots were hybridized using the shear-driven flow system. For the diffusion-driven hybridization under coverslip, we obtained 80% hybridized spots after overnight (16 h) incubation, while only 67% of the spots were hybridized after 12 h hybridization using the ASP system.

Table 1. *Comparison of different hybridization systems for Mouse lung and testis total RNA samples on the VIB_Mouse_5K_II microarray chip.*

	SDF-System	Coverslip Diffusion	ASP
Cy3 Incorporation (pmol)	40	40	40
Sample Volume (µl)	5	30	210
Concentration (pmolCy3/µl)	8.00	1.33	0.19
Hybridization Time (min)	60	960	720
Hybridized Spots (max.1760)	1559 (89%)	1415 (80%)	1173 (67%)
Relative Intensity	369%	189%	100%

4. CONCLUSIONS

The present study has demonstrated that if a given amount (expressed in mass or number of moles) of DNA is available, each attempt to increase the DNA microarray hybridization intensity or the hybriddization speed should preferentially involve a reduction of the total volume of the hybridization system, because this allows to increase the initial sample concentration, which is the key factor controlling both the initial hybridization rate and the equilibrium spot intensity. Due to the induced dilution, hybridization systems requiring an enlarged sample volume to generate an enhanced convective transport cannot be expected to yield a large final equilibrium spot intensity, in spite of the enhanced transport rates. The dilution also acts counterproductive in the initial phase of the hybridization.

The currently proposed rotating micro-chamber system allows to combine a miniaturization of the sample chamber with the creation of a strong lateral convective transport. In only 30 minutes, the system can yield hybridization intensities which are about four times larger than a traditional overnight experiment under a coverslip and more than five times larger than in an experiment using the same amount of DNA conducted in a fully-automated pump-around system. One of the essential keys to this gain seems to be that the convective displacement is performed in a discontinuous mode.

Application of the shear-driven flow system resulted not only in a reduction of the hybridization time and higher spot intensities, it was also possible to detect a larger amount of hybridized spots, emphasizing its strength for potential use in assays focusing on the presence of low abundant genes.

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