Biomed Microdevices (2009) 11:685–692
DOI 10.1007/s10544-008-9280-6

Application of an asymmetric helical tube reactor for fast identification of gene transcripts of pathogenic viruses by micro flow-through PCR

R. Hartung · A. Brösing · G. Sczcepankiewicz · U. Liebert · N. Häfner · M. Dürst · J. Felbel · D. Lassner · J. M. Köhler

Published online: 24 January 2009
© Springer Science + Business Media, LLC 2009

Abstract We have established a fast PCR-based micro flow-through process consisting of a helical constructed tube reactor. By this approach we can detect transcripts of measles and human papilloma virus (HPV) by continuous flow allowing for reverse transcription (RT) and amplification of cDNA. The micro reaction system consisted of two columnar reactors for thermostating the different reaction zones of the RT process and the amplification. The PCR reactor was built by asymmetric heating sections thus realizing different residence times and optimal conditions for denaturation, annealing and elongation. The system concept is based on low electrical power consumption (50–120 W) and is suited for portable diagnostic applications. The samples were applied in form of micro fluidic segments with single volumes between 65 and 130 nL injected into an inert carrier liquid inside a Teflon FEP tube with an inner diameter of 0.5 mm. Optimal amplification for template lengths of 292 bp (lambda-DNA), 127 bp (measles virus) and 95 bp (HPV) was achieved by maximal cycle times of 75 s.

Keywords Virus diagnostic · Reverse transcription · Rapid PCR · Micro reactor · Flow-through process · Fluid segment technique

1 Introduction

Viral infections are responsible for different human and animal diseases. The molecular diagnostics of such infec-

R. Hartung · A. Brösing · J. M. Köhler (✉)
Institut für Physik, Physikalische Chemie/Mikroreaktionstechnik,
Technische Universität Ilmenau,
Weimarer Str. 32,
98693 Ilmenau, Germany
e-mail: michael.koehler@tu-ilmenau.de

R. Hartung
Institut für Biochemie II,
Klinikum der Friedrich-Schiller-Universität Jena,
Nonnenplan 2,
07743 Jena, Germany

G. Sczcepankiewicz · U. Liebert
Institut für Virologie, Universität Leipzig,
Johannisallee 30,
04103 Leipzig, Germany

N. Häfner · M. Dürst
Frauenklinik, Klinikum der Friedrich-Schiller-Universität Jena,
Bachstrasse 18,
07743 Jena, Germany

J. Felbel
Institut für Photonische Technologien Jena,
Albert-Einstein-Str. 9,
07745 Jena, Germany

D. Lassner
Institut Kardiale Diagnostik und Therapie GmbH Berlin,
Moltkestr. 31,
12203 Berlin, Germany

Present address:
A. Brösing
Senckenberg Forschungsinstitut und Naturmuseum,
Senckenberganlage 25,
60325 Frankfurt, Germany

Present address:
J. Felbel
Analytik Jena AG,
Konrad-Zuse-Str. 1,
07745 Jena, Germany

tions depend largely on detection of viral genomes (DNA and RNA) or its transcripts (mRNA). Polymerase chain reaction (PCR) has emerged as the prime tool for rapid and reliable detection of viral gene sequences in clinical settings. In particular reverse transcription (RT)-PCR is used to distinguish between replicating and latent viral infection stages of DNA-viruses. Generally, RT-PCR depends on a well equipped molecular biological laboratory and this approach takes several hours when standard thermocyclers are used.

A reduced process time can be achieved by high-speed capillary cyclers but only at the expense of a more difficult sample handling and high power consumption. There is an urgent need of easy-to-use devices for diagnostics based on thermocycling techniques. This problem is addressed by the development of miniaturized PCR devices since the beginning of the nineties. An extreme reduction of sample volumes and device size was realized by the introduction of silicon chip based micro chamber thermocyclers (Woolley et al. 1996). These devices were marked by low parasitic heat capacitance and fast heating and cooling rates despite of low power consumption (Poser et al. 1997). Nevertheless, the routine use of the devices was hampered by difficult sample application and regeneration of these devices. An improved sample handling and the application of sample series was achieved in micro flow-through chip thermocyclers (Wilding et al. 1994; Kopp et al. 1998; Schneegass and Köhler 2001; Fukuba et al. 2004; Auroux et al. 2004; Felbel et al. 2008). High surface to volume ratios of these devices demanded sophisticated techniques for surface treatment in order to avoid an inhibition of the PCR by interactions of biomolecules—in particular of the Taq DNA Polymerase—with the channel walls (Felbel et al. 2004). A significant improvement of the chemical compatibility was achieved by the substitution of silicon/glass chips by polymer-based micro devices. Furthermore the manipulation of oil covered droplets by surface acoustic waves led to an improved handling of miniaturized samples in on-chip PCR (Guttenberg et al. 2005).

Micro flow-through thermocyclers based on Teflon FEP (fluor-ethylen-propylen) tubes are a promising way for using the advantages of miniaturized continuous flow PCR reducing problems of surface compatibility. A planar system was presented by the group of J. Roerrade in 2003 (Curcio et al. 2003). Such planar systems are simple in construction, but the spiral-like tube arrangement causes a changing residence time from cycle to cycle. Constant residence times were achieved by a helical arrangement of the reaction tube in a columnar reactor (Park et al. 2003; Zhang et al. 2007). This PCR reactor type of cylindrical shape included three thermostatic sectors. The adaptation of sector sizes on the requirements of PCR lead to an asymmetric columnar reactor with helical arrangement of the reaction tube.

Tube reactors are of particular importance due to the straightforward generation and handling of micro fluid segments (Thorsen et al. 2001; Burns and Ramshaw 2001; Nisisako et al. 2002; Song et al. 2003). Micro fluid segment techniques exhibit following advantages: (1) fast aliquotation of samples, (2) realization of stepwise varied concentrations of samples and additives (Köhler et al. 2004) and (3) support of convenient manipulations of small sample volumes (Henkel et al. 2004). Therefore micro fluid segment techniques had applied for cell incubation, cultivation (Martin et al. 2003, Grodrian et al. 2004) and for toxicological screenings (Funfak et al. 2007).

Results concerning the application of a columnar reactor and the micro fluid segment technique for the identification of isolated virus DNA and the detection of viral transcripts (mRNA) are reported here.

2 Materials and methods

2.1 Construction of reactor, micro fluidic arrangement and characterization of arrangement

The concept of PCR amplification in aqueous segments embedded in an immiscible inert carrier liquid was applied. This was realized by using a Teflon FEP tube for all process steps. A columnar reactor consisting of three thermostatic segments was used for DNA amplification. The subdivision of the thermostatic block into three asymmetric parts was chosen in order to match the demands of the temperature program in an optimal manner. The construction of the columnar amplification reactor is shown in Fig. 1(a). The three copper segments are equipped with electrical heaters and with resistive sensors for controlling the temperature precisely. The three parts are mounted together with a slit between them in order to avoid a direct thermal contact among the three thermostatic zones. The thermostatic column has a diameter of 7 cm and a height of 8 cm. It can be used for the realization of up to 42 temperature cycles. The length of tube amounts 924 cm in case of 42 cycles. In addition, a tube length of 15 cm is applied for the first denaturation step, resulting in a maximal total tube length of 939 cm. PC-controlled syringe pumps for fluid actuation and the flow-through fluorimeter (Fig. 1(c)) were also integrated in the complete setup (Fig. 2). The flow-through fluorimeter is custom made; the UV-LED (Hero Electronics Limited, England) and the spectrometer (Avantes, RB Eerbeek, The Netherlands) are commercially available.

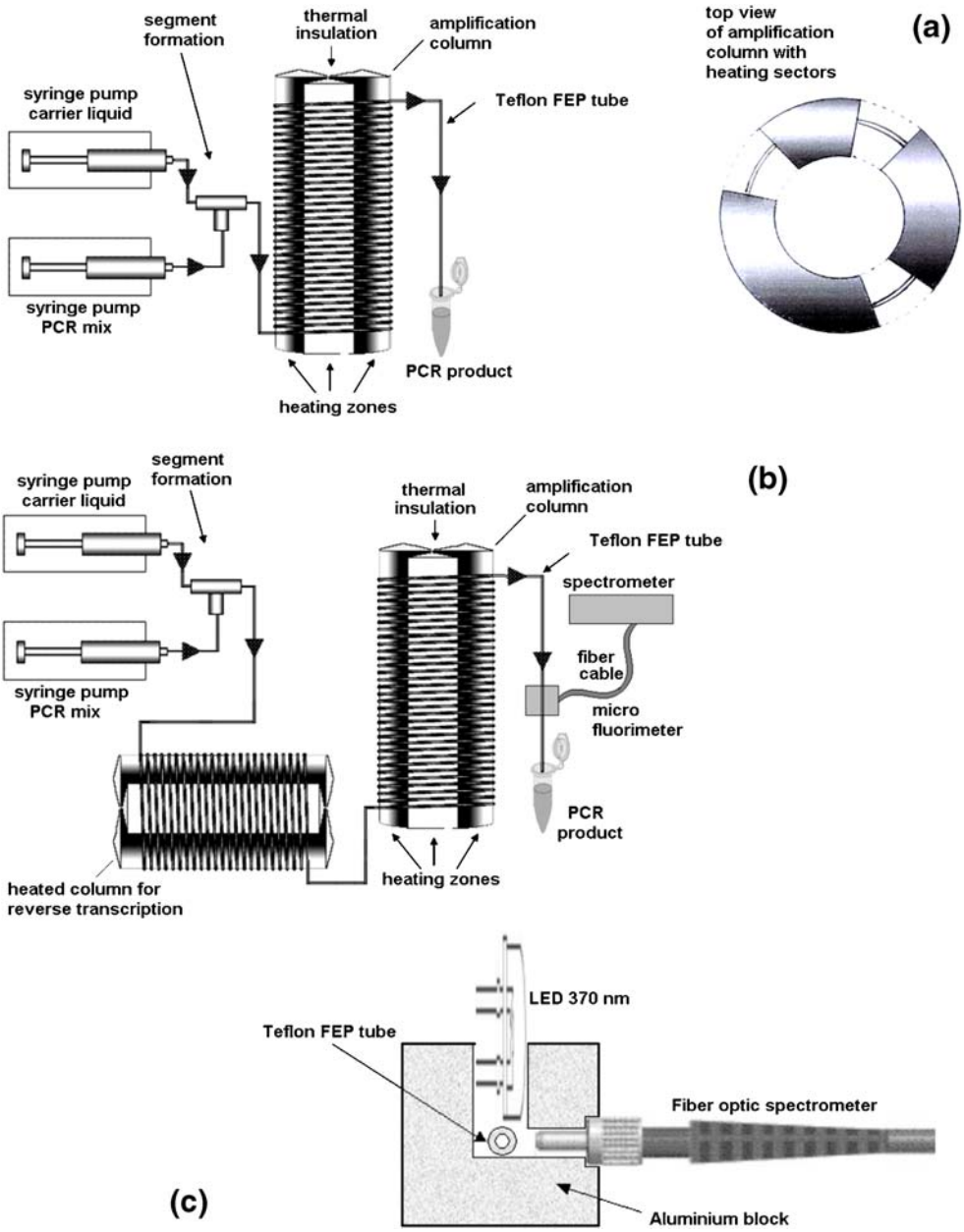

Fig. 1 Experimental arrangement: (**a**) construction principle of the columnar amplification reactor with asymmetric heating zones, (**b**) complete micro fluidic setup for RT-PCR (schematically), (**c**) construction principle of micro flow-through fluorimeter

The whole experimental arrangement for the micro-flow-through RT process included an additional columnar reactor for the reverse transcription beside the amplification reactor (Fig. 1(b)). The two columnar reactors were connected by a common Teflon FEP tube. The reverse transcription was realized by a temperature of 50°C in case of HPV and of 60°C in case of measles viruses. A residence time of 30 min was realized in the reverse transcription zone. Therefore a tube length of 42.5 cm was needed for the RT process if a flow rate of 12 μL/min was used. After the reverse transcription reactor a hot start region was implemented enabling a residence time of 5 min at a temperature of 94°C for activation of hot start polymerases. Subsequent amplification of the cDNA occurred in the second columnar reactor. The length of the whole tube for all processes (linear connected tubes in both reactor columns) added up to 981.5 cm.

2.2 Samples and templates

Isolated lambda DNA (Fermentas, St. Leon-Roth, Germany, original concentration=0.3 g/L) was chosen as a simple and well known example for genotyping. It was used for testing the function of the amplification reactor. Samples containing

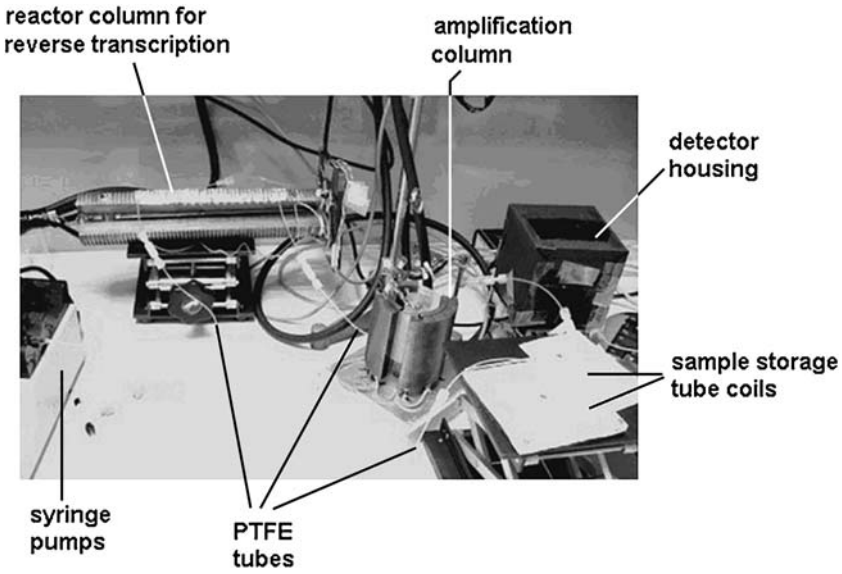

Fig. 2 Columnar amplification reactor with asymmetric heating zones integrated in the experimental setup

human SiHa cells were used for the identification of virus derived transcripts. SiHa cells are an established cervical carcinoma cell line (ATCC cell line HTB-35). Each cell contains two copies of the HPV16 genome integrated into the host chromatin. Detection of messenger RNA specific for measles viruses was performed with persistently infected human glial cells (C6/SSPE) (Halbach and Koschel 1979). Prior to amplification, cells were fixed with paraformaldehyde (2%) for 1 h at 4°C, spinned down and then resuspended in *pure* bidistilled water.

2.3 Chemicals and protocols for micro flow-through PCR and RT-PCR processes

For our purposes the conventional PCR protocols established for micro reaction cups or micro titer plates had to be adapted for micro fluidic experiments. Modifications became necessary due to the application of carrier liquid, due to changed conditions of heat transfer resulting in deviations in temperature profiles and because of reduced sample sizes and enhanced surface-to-volume ratios. The amplification of lambda DNA was carried out in 25 μL under following conditions: 15 ng lambda-DNA, 1 μL dNTPs (each 10 mM), Genaxxon PCR buffer, SYBR Green (1:10,000 diluted), 1 mM $MgCl_2$, 0.1 μg BSA, 1 μL of each primer (3SE 5′-TTC CCT GTA TTG CTG AAA TGT G-3′, 4AS 5′-GGA AGG TTT TAC CAA TGG CTC-3′ 10 pmol/μL), and 0.5 μL Taq polymerase (5 U/μL, Genaxxon Biberach, Germany).

The composition of reaction mixture for the experiments with HPV containing samples was adapted from a protocol developed for the miniaturized rapid PCR (Felbel et al. 2008). Therefore, a one step RT PCR kit (Invitrogen, Karlsruhe, Germany) was used. 25 μL of the mixture were composed of 12.5 μL reaction buffer (containing dNTPs), 0.2 μL RNAse inhibitor (Invitrogen, 40 U/μL), 1 μL of each primer (F151 5′-CAC AGA GCT GCA AAC AAC TAT ACA-3′, R-E6*I 5′-CTT TTG ACA GTT AAT ACA CCT CAG G-3′, 10 pmol/μL), 1 μL mix of enzymes and 4.75 μL template solution. The solution was completed by 1.25 μL SYBR Green solution (diluted 1:500) for fluorescence labelling of generated PCR amplicons, additives of 1.8 μL BSA solution (1 mg/mL), 0.5 μL magnesium chloride solution (25 mM) and 1 μL of Hot Start Taq polymerase (5 U/μL, Genaxxon).

The composition of the reaction mixture for the measles virus identification was adapted from a protocol developed earlier for the rapid PCR with the Light Cycler. Therefore, a One-Step-RT PCR kit (Qiagen, Hilden Germany) was used. 20 μL of the initial mixture were composed by 0.2 μL RNAse inhibitor (RNAsin), 0.8 μL dNTP (each 10 mM), 1 μL of each primer (MV-FLA 5′-GGT TTA TCG AGC ACT AGC AT-3′ and MV-FLA rev 5′-GAC ATA CCA ACT TGT TCT CC-3′, 10 pmol/μL), 0.8 μL mix of enzymes and 3 μL template. The solution was completed by 4 μL SYBR Green solution (diluted 1:10,000) for labelling and additives of 1.5 μL BSA solution (2 mg/mL), 0.4 μL magnesium chloride solution (50 mM), 4 μL reaction buffer (5x) and 3.3 μL water. A half micro liter of Super Hot Start Taq polymerase (5 U/μL, Genaxxon) was added between the reverse transcription and the segmentation for the amplification; and then initial denaturation step was prior to PCR.

All primers were selected from us according to GC content, lack of hybridization with each other and ordered from a commercial producer.

2.4 Characterization of successful PCR amplification

Characterization of the PCR products was realized by gel electrophoresis. Therefore, about 150–250 segments (about 100 nL-segments from 25 μL PCR mix) were collected and analyzed by gel electrophoresis. In addition, nucleic acid sequencing, Southern hybridization and specific fluorochromated hybridization probes (FRET technology) were used to prove the specificity of amplicons. For Southern hybridization of lambda, HPV and measles PCR products we used the oligonucleotide probes GTGAGACGTTGTGACGTTTTAGTT, CTCACGTCGCAGTAACTGTTG and CTTGGAGGGTTGATAGGGATC, respectively and a standard protocol was applied (Ausubel et al. 1999).

An optical micro flow through device was applied for monitoring the amplification product in single moving fluid segments. The construction principle of this fluorimeter is shown in Fig. 1(c). The device allowed the direct measurement of fluorescence in the Teflon FEP tube and in individual segments with an UV-LED (370 nm). The angle between excitation and emission was 90 degrees. A compact spectrometer and a photodiode were used as detector.

3 Results and discussion

3.1 Reactor characterization and application of micro segmented flows

The reactor arrangement was characterized at first with respect to generation, transport and monitoring of fluid segments. The reproducibility of segment generation in Teflon FEP tubes and T-connectors was tested by the application of dyed aqueous segments embedded in PP9 (perfluoromethyldecaline) as carrier liquid (Fig. 3). The reproducibility of distance and size of fluid segments was measured by a micro flow-through photometer. Photometric measurements were carried out with a measurement frequency of 1 kHz. The flow rates were adapted to the conditions of PCR covering the range between 10 and

Fig. 3 Micro channel (inner diameter of 0.5 mm) with dyed aqueous segments (0.1 μL) embedded in PP9 as carrier liquid (device: IPHT Jena)

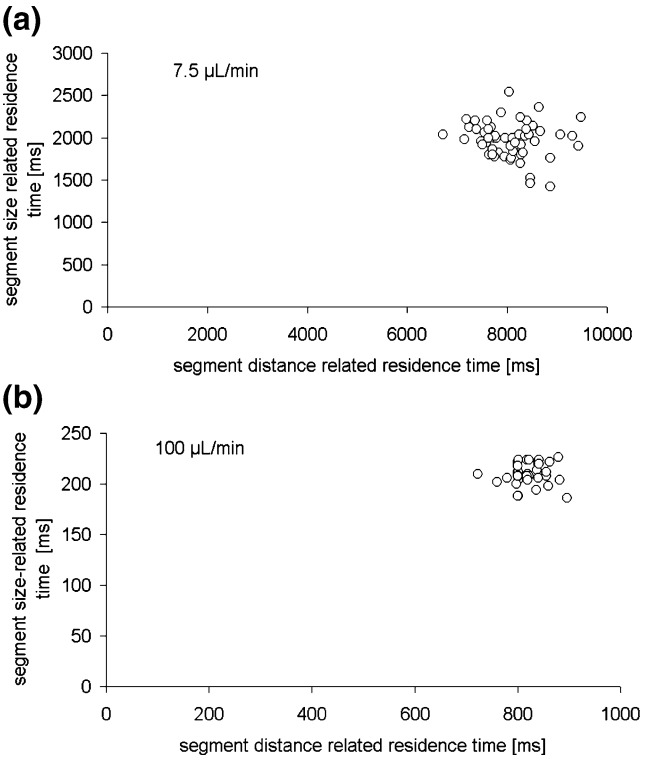

Fig. 4 Segment size/segment frequency plot for segment formation in Teflon FEP connectors and Teflon FEP tubes at different flow rates: (**a**) carrier liquid=7.5 μL/min, aqueous solution=2.5 μL/min, (**b**) carrier liquid=100 μL/min, aqueous solution=100 μL/min

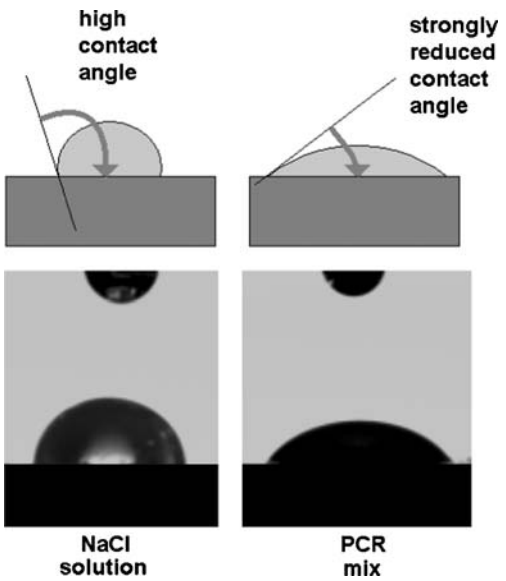

Fig. 5 Influence of PCR components on interface tensions: strong enhancement of wetting/decrease of contact angle by application of PCR solution in comparison to 0.9% NaCl solution on a glass surface

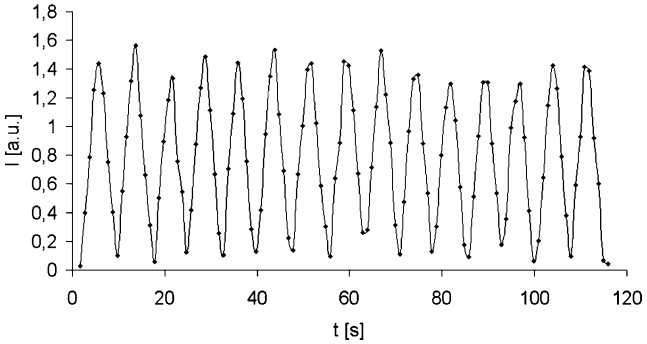

Fig. 6 Example of segmented micro flow-through fluorimetry for *in-situ* detection of double strand DNA (total flow rate 20 μL/min, flow rate ratio 9:1) The changing fluorescence intensity (*I*, arbitrary units) reflects alternating phases of aqueous segments (SybrGreen labelled dsDNA) and non fluorescent carrier liquid

50 μL/min (total flow rate). The change of flow rate results in increase or decrease of the residence time in the different temperature zones.

After an initial phase with a larger scattering in segment size and distance, the reproducibility in size is better than 20%, in segment distance better than 12% at low flow rates and becomes better if the flow rate is enhanced. The distribution of segment parameters is well reflected by the size-distance plots (Fig. 4).

The segment stability is strongly influenced by the wetting behavior of the capillary walls and by the liquid/liquid interface. The amphiphilic components of the PCR solution cause a significant decrease of surface as well as of interface tensions. Thus, the change of contact angle and interface energy was measured using PCR solution in comparison to 0.9% NaCl solution (Fig. 5). The reduction of interface energy results in decreased robustness of segment stability. This problem is of major importance in the case of changes in channel diameter and in the case of deviations from a circle-shaped cross section. In the present concept of tube PCR such problems can be avoided due to the nearly ideal cross section of tubes. So, micro fluid segments are much less sensitive against reduced interface energies inside the tube reactor than in case of other micro channels, as normally applied in chip PCR devices.

The characterization of PCR products can be performed either by a gel electrophoresis after collecting a sufficient number of fluid segments or by *in-situ* measurement of enhanced fluorescence. It was possible to realize a single segment monitoring by application of the micro flow-through fluorimeter. Figure 6 gives an example of fluorescence measurement in a series of micro fluid segments by micro fluorimetry.

3.2 Amplification of lambda-DNA in micro fluid segments

The amplification reactor was tested by an amplification procedure with lambda phage DNA. The amplification within 42 temperature cycles corresponding to a total tube length on the columnar reactor of 939 cm was realized. We tested the dependence of the efficiency of PCR to residence time, what realized with different flow rates.

We could successfully amplify a 292 bp product from lambda DNA. The specificity of the amplification process was approved by Southern hybridization (Fig. 7(a)). Only the sample containing lambda DNA revealed a specific hybridization signal, whereas the non template control do not show an amplification product. PCR products of expected molecular size (292 bp) could be found by gel electrophoresis up to flow rates of 50 μL /min (Fig. 7(b)). This flow rate corresponds to residence times of about 6 s in the denaturation zone, 9 s in the annealing zone and 15 s in the primer extension zone.

The product yield was improved by enhancing residence time. An amplification yield of about 500 ng from 80 pg template DNA was obtained at a flow rate of 20 μL/min,

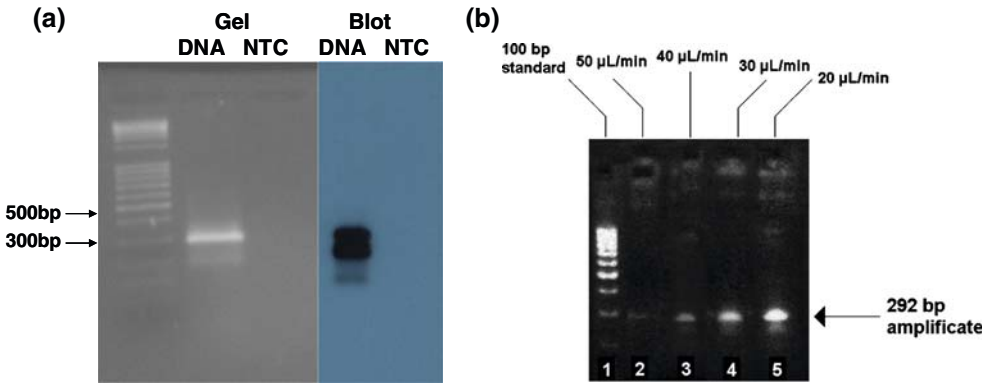

Fig. 7 Analysis of products from lambda-DNA amplified by flow-through PCR in micro-fluidic segments. (**a**) Validation of specific amplification by Southern hybridization (*DNA* 100 ng lambda DNA, *NTC* non template control). The second hybridization signal likely originates from underrepresented, internally false primed PCR product. (**b**) Amplification at different flow rates in the columnar tube reactor shows PCR products up to 50 μL/min

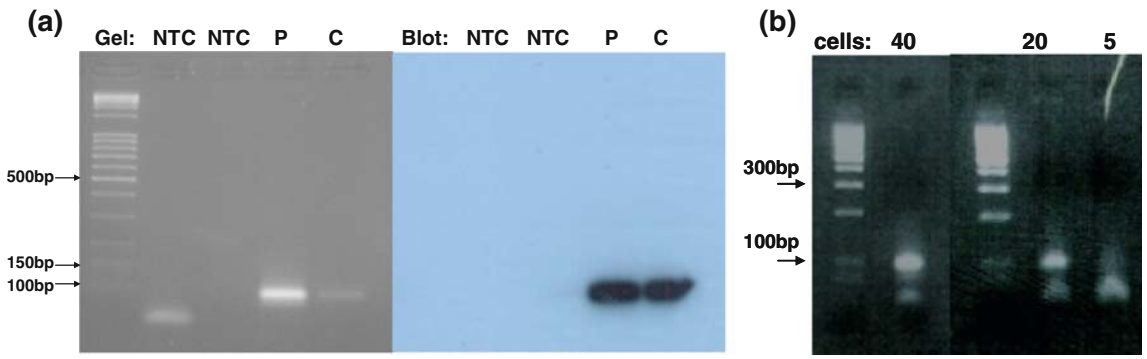

Fig. 8 Results of micro flow-through RT-PCR using HPV-infected human SiHa cells. (**a**) Gel and Southern Blot of PCR products from 100 pg plasmid (*P*) and 10 SiHa cells per microliter (*C*) confirms a specific amplification of the 95 bp product. Non template controls (*NTC*) amplify occasionally primer dimers which do not gave Southern blot signals. (**b**) Amplification of HPV16 sequences could detect down to five SiHa cells per microliter. Primer dimer amplification is contrary correlated to the amount of SiHa cells

corresponding to residence times of 15 s in the denaturizing zone, 22 s in the annealing zone and 38 s in the primer extension zone. The electrophoresis gels show the flow rate dependence of amplification yield (Fig. 7(b)).

3.3 Micro flow-through RT-PCR of HPV 16 positive SiHa cells

Reverse transcription and amplification of samples were carried out by use of an integrated flow-through process with the experimental arrangement shown in Figs. 1(b) and 2. The goal of the experiments was to investigate the possibility to detect HPV transcriptional activity in human cells at the level of a few cells. Therefore, samples with different concentration of infected human SiHa cells were introduced to the system after a pre-treatment with paraformaldehyde for supporting both cell permeabilisation and fixation. We could not observe PCR inhibition by residual paraformaldehyde in resuspended cells. Additionally cell suspensions of fixed single cells do not cause clogging of the tubes because of sufficient difference between cell- and tube diameter (10 and 500 μm, respectively).

After reverse transcription (30 min at 50°C) and hot start (5 min at 94°C), the amplification by PCR was started with the first passage of the denaturation zone. 42 temperature cycles were realized by 42 loops of reaction tubes on the amplification reactor.

Optimal amplification was found at an annealing temperature of 58°C and an elongation temperature of 68°C. For the PCR-product of 95 bp, a cycle time of 120 s was found to be suitable. This cycle time corresponded to a residence time in the denaturation zone of about 15 s, in the annealing zone of about 22 s and in the elongation zone of about 38 s. Additional 45 s per cycle are consumed by the residence time in tube sections between the reaction areas.

The quality of the obtained products was analyzed by gel electrophoresis (Fig. 8). Used HPV primers were carefully validated to selectively amplify cDNA of spliced transcripts avoiding the co-amplification of genomic DNA (data not shown). The specific amplification of HPV16 sequences was guaranteed by Southern hybridization of exemplary samples (Fig. 8(a)). The expected viral mRNA could be detected at cell concentrations down to five cells per microliter (Fig. 8(b)). At an average segment volume of about 0.1 μL, the average probability of finding one cell in one segment was about 50%. About 25 μL of product solution from fluid segments was collected for gel electrophoresis and Southern hybridization.

3.4 Micro flow-through RT-PCR of transcripts from the measles virus

For the molecular characterization of measles-infected cells, a template length of 127 bp was chosen. The whole experiments were carried out in analogy to the experiments with HPV-infected cells. Samples with different concentration of infected human C6/SSPE cells were introduced to

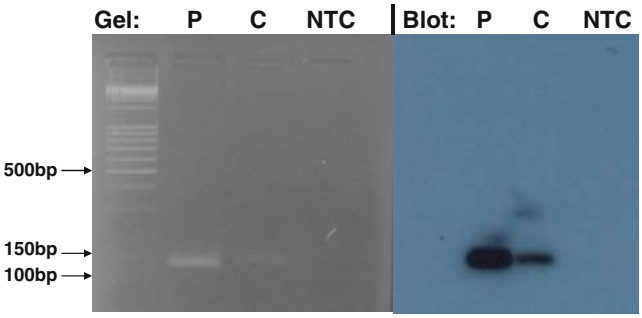

Fig. 9 Results of micro flow-through measles RT-PCR showing successful amplification of the 127 bp PCR product. The faint PCR product from infected cells (*C*) is confirmed by the more sensitive Southern Blot. (*P* 10 ng plasmid DNA containing the measles genome, *C* 10,000 measles-infected human C6SSPE cells, *NTC* non template control)

the system after a pre-treatment with paraformaldehyde for supporting the cell permeabilisation and fixation.

The RT process was carried out at elevated temperature. After the reverse transcription (30 min at 60°C) and hot start (5 min at 94°C), the amplification by PCR was started with the first passage of the denaturation zone. 32 temperature cycles were realized by 32 loops of reaction tubes on the amplification reactor. Good conditions for amplification were found at an annealing temperature of 60°C and an elongation temperature of 72°C. For a template length of 127 bp, a cycle time of 120 s was found to be suitable as in case of HPV cells.

The quality of the obtained products was checked by gel electrophoresis and the specificity of the amplification was controlled by Southern hybridization and a control reaction using a plasmid containing the measles genome (Fig. 9). The expected viral mRNA could be found at cell concentrations of 10,000 cells per microliter.

4 Conclusions

The investigations show the suitability of an asymmetric constructed columnar reactor system for the performance of PCR and RT-PCR in micro fluid segments. Templates of lambda-DNA could be amplified very quickly. The tube reactor using Teflon FEP tube with an internal diameter of 0.5 mm allows a whole amplification process of a 292 bp amplicon within 32 temperature cycles in about 24 min. The products can be either characterized by gel electrophoresis after collecting about 25 μL of product solution or by direct monitoring using a miniaturized fluorescence detector for measurements of fluorescence intensity of the single fluid segments inside the reaction tube. Fluorescence artifacts caused by primer dimers or unspecific amplifications could be generally excluded by the application of probe based detection systems rather than SybrGreen based assays in future experiments.

In case of HPV-infected human SiHa cells, the tube reactor concept was modified to include an integrated continuous reverse-transcription process. By this approach it was possible to detect viral transcripts in cell concentration as low as five cells per microliter. Measles virus mRNA could also be detected by the flow-through RT-PCR process. However, the minimal cell concentrations required were significantly higher than in the case of the HPV infected cells. The expected products were visualized by gel electrophoresis and confirmed by Southern blotting. The difference in sensitivity between HPV and measles virus detection is conceivably caused by the lower transcriptional activity of measles viruses and is certainly biased by the higher number of PCR cycles for the detection of HPV transcripts.

Acknowledgement We thank J. Weber (Analytik Jena) and C. Gärtner (microfluidic ChipShop) for the cooperation and helpful discussions. The technical support by S. Schneider (development of tube reactors and optical detectors, software development) is gratefully acknowledged. We are gratefully for the financial support by the Ministry of Culture and Education Thuringia in the frame of the project "Zellex".

References

P.A. Auroux, Y. Koc, A. deMello, A. Manz, P.J. Day, Lab Chip **4**, 534 (2004). doi:10.1039/b408850f
F.A. Ausubel, R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, J. A. Smith, K. Struhl (eds.), "Current Protocols in Molecular Biology", Wiley, New York (1998)
J.R. Burns, C. Ramshaw, Lab Chip **1**, 10 (2001)
M. Curcio, J. Roeraade, Anal. Chem. **75**, 1 (2003)
J. Felbel, I. Bieber, J. Pieper, J.M. Köhler, Chem. Eng. J. **101**, 333 (2004)
J. Felbel, A. Reichert, M. Kielpinski, M. Urban, T. Henkel, N. Häfner, M. Dürst, J. Weber, Chem. Eng. J. **135S**, S298 (2008)
T. Fukuba, T. Yamamoto, T. Naganuma, T. Fujii, Chem. Eng. J. **101**, 151 (2004)
A. Funfak, A. Brösing, M. Brand, J.M. Köhler, Lab. Chip. **7**, 1132 (2007)
A. Grodrian, J. Metze, T. Henkel, K. Martin, M. Roth, J.M. Köhler, Biosens. Bioelectron. **19**, 1421 (2004)
Z. Guttenberg, H. Müller, H. Habermüller, A. Geisbauer, J. Pipper, J. Felbel, M. Kielpinski, J. Scriba, A. Wixforth, Lab Chip **5**, 308 (2005)
M. Halbach, K. Koschel, J. Gen. Virol. **42**, 615 (1979)
T. Henkel, T. Bermig, M. Kielpinski, A. Grodrian, J. Metze, J.M. Köhler, Chem. Eng. J. **101**, 439 (2004)
J.M. Köhler, T. Henkel, A. Grodrian, T. Kirner, M. Roth, K. Martin, J. Metze, Chem. Eng. J. **101**, 201 (2004)
M.U. Kopp, A.J. Mello, A. Manz, Science **280**, 1046 (1998)
K. Martin, T. Henkel, V. Baier, A. Grodrian, T. Schon, M. Roth, J. Michael Köhler, J. Metze, Lab Chip **3**, 202 (2003)
T. Nisisako, T. Torii, T. Higuchi, Lab. Chip. **2**, 24 (2002)
N. Park, S. Kim, J.H. Hahn, Anal. Chem. **75**, 6029 (2003)
S. Poser, T. Schulz, U. Dillner, V. Baier, J.M. Köhler, D. Schimkat, G. Mayer, A. Siebert, Sens. Actuators **62**, 672 (1997)
I. Schneegass, J.M. Köhler, Rev. Mol. Biotech. **82**, 101 (2001)
H. Song, J.D. Tice, R.F. Ismagilov, Angew. Chem. Int. Ed. Engl. **42**, 768 (2003)
T. Thorsen, R.W. Roberts, F.H. Arnold, S.R. Quake, Phys. Rev. Lett. **86**, 4163 (2001)
P. Wilding, M.A. Shoffner, L.J. Kricka, Clin. Chem. **40**, 1815 (1994)
A.T. Woolley, D. Hadley, P. Landre, A.J. deMello, R.A. Mathies, M. A. Northrup, Anal. Chem. **68**, 4081 (1996)
C. Zhang, J. Xu, J. Wang, H. Wang, Anal. Lett. **40**, 497 (2007)