PROTOTYPING AND TESTING BASIC DESIGNS OF CENTRIFUGAL MIRCOFLUIDIC PLATFORMS FOR BIOMEDICAL DIAGNOSTICS

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Abstract

This paper presents a design approach, based on the idea to develop a product that allows a considerably faster diagnose of bacterial infections in the physicians office and thus, supports avoiding the unnecessary use of antibiotics. Nowadays, physicians send probes to central labs for diagnoses. These Labs usually use either manual work or microfluidic platforms. Most microfluidic platforms require expensive machinery for realizing complex motion sequences. However, there also is a cost-efficient alternative: centrifugal microfluidic platforms, which are also known as 'lab-on-a-disk'. In order to control flows, these disks take advantage of centrifugal forces, which can be easily regulated via rotation speed. In a first step, the technical feasibility was investigated with focus on basic designs of centrifugal microfluidic platforms for reliable controlling flows, only by regulation of rotation speed. The channel and reservoir designs were evaluated based on the results gained by physical testing of a lab-on-a-disk prototype in operation. The results and implications support the further development of a fully automated diagnostic device to be used as point-of-care.

Keywords: Biomedical design, prototyping, testing, lab-on-a-disk

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1 INTRODUCTION

Modern technologies and designs strongly facilitate the substantial progresses in clinical diagnostic and medical treatment. However, medical science still meets huge challenges, and some of these challenges are even emerged from its most important achievements. Nowadays, a major public-health problem is the resistance to antibiotics. Several national and international studies revealed that antibiotic use is being increasingly recognized as the main selective pressure driving this resistance (Goossens et al. 2005, Hicks et al. 2011). Indeed, broad-spectrum antibiotics are often prescribed prophylactically without an assured diagnose confirming a bacterial infection. This common practice originates from the fact that attending physicians usually have to wait up to three days, before they receive final test results from the lab. During this time the patient's status can change for the worse or even reach a critical level.

The research presented in this paper is based on the idea to develop a product that allows a considerably faster diagnose of bacterial infection and thus, supports avoiding the unnecessary use of antibiotics. In order to save transfer time from and to the lab, the product should be used directly by the attending physician on the spot. Due to the fact, that at this point in time it is especially important to know, whether or not the patient is infected by a specific bacterium, a binary classification test seems to be sufficient. Consequently, the product design can be simplified to that level where this question can still be reliably answered by a clear yes or no. To sum up, the overall aim is to develop a user-friendly sample-to-answer disposable for rapid point-of-care applications.

In labs, the diagnosis of bacteria is usually carried out by means of polymerase chain reaction (PCR), which is a biomedical method to amplify DNA sequences. Here, first a fluid sample (e.g. saliva) is taken. If this sample contains DNA of one specific bacterium (e.g. streptococcus), then PCR allows amplifying the DNA to a quantity where it can be detected as soon as a corresponding fluorescent marker is added. A basic PCR setup requires several fluidic components (primer, polymerase, nucleotide, etc.) that have to be managed correctly during the procedure. Therefore, labs usually use microfluidic platforms providing a system of channels and reservoirs to control the single component's flow. Most microfluidic platforms require expensive machinery for realizing complex motion sequences. However, there also is a cost-efficient alternative: centrifugal microfluidic platforms, which are also known as 'lab-on-a-disk'. In order to control flows, these disks take advantage of centrifugal forces, which can be easily regulated via rotation speed and thus, can be operated by a single drive unit. But, in return for the simplified drive technology, the design of a lab-on-a-disk's channels and reservoirs becomes considerably more complex.

This paper addresses the overall research question of to what extent centrifugal microfluidic platforms are applicable to be used as sample-to-answer disposable for rapid point-of-care applications. To answer this question, in a first step the technical feasibility was investigated with focus on basic designs of centrifugal microfluidic platforms for reliable controlling flows only by regulation of rotation speed. The channel and reservoir designs were evaluated based on the results gained by physical testing of a lab-on-a-disk prototype in operation.

The lab-on-a-disk prototype presented in this paper consists of three layers made of acrylic glass. Channels and reservoirs have been engraved into the layers by a computer controlled laser cutter machine. This technology was chosen, because it allows a cost-efficient and rapid realization of simple as well as complex channel designs. Due to laser cutter prototyping, new insights gained from testing a design can be directly utilized to quickly realize adapted designs and thus, it allows to systematically investigate the interrelations between design and performance (cf. Meboldt et al. 2014).

The paper proceeds as follows. Section 2 gives a brief overview of the basic reservoir and channel design of centrifugal microfluidic platforms. In this context, especially designs for valving fluids are illustrated and compared to each other. In section 3, the design of a lab-on-a-disk prototype (LabDisk) is presented. It is explained how the single layers of the prototype are manufactured and assembled. Furthermore, the setup for testing the LabDisk is described and the single sequences of the corresponding spinning profile are listed. Section 4 presents the test results. On basis of photographic pictures taken during operation, it is reported, how the filled fluids were moved inside the LabDisk depending on the rotation speed. In section 5 the results are discussed in regard to reliability and reproducibility of flow control. Based on the findings, weak points of the presented design are identified and approaches for improvements are outlined. Section 6 concludes.

2 CENTRIFUGAL MICROFLUIDIC PLATFORMS

2.1 Basic Reservoir and Channel Design

The design of reservoirs and channels on centrifugal microfluidic platforms are based on taking advantage of two physical effects. As the name implies, the first one is the centrifugal effect. If spinning a disk, then a fluid inside the disk is pressed outwards. Centrifugal forces acting on that fluid increase depending on rotation speed n and distance to the center of rotation r. The second effect used is capillary action. In a narrow hydrophilic channel a fluid flows through the channel until it is filled or a balance of forces is reached. The capillary force depends on the fluid's material properties (surface tension, contact angle and viscosity) and the channel's cross-section area, but not on the rotation speed (cf. Ducrée et al. 2007).

Figure 1 illustrates, how the interaction of these effects allows controlling the flow of a fluid by rotation speed. On the left of Figure 1 the disk has stopped and consequently, there is no centrifugal force (passive state). A fluid filled into the outer reservoir is only affected by capillary action and thus, the fluid flows through the narrow channel towards the center of the disk until the channel is filled. If the rotation speed is increased to a certain amount, the centrifugal forces become larger than the capillary forces and the fluid is pushed back into the channel. Now, the distance between the fluid front and the center of rotation can first be adjusted and then hold by balancing the forces (balance state). In case the rotation speed is further increased, the fluid is fully repressed outwards until there is no further space left for the fluid to flow to (high speed state).

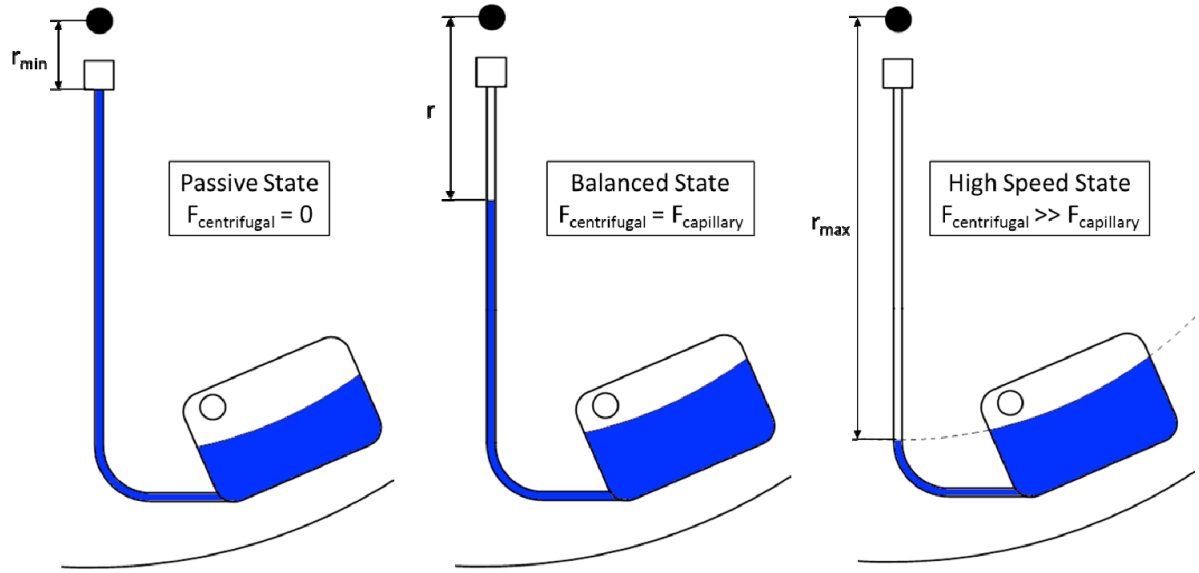

Figure 1. Basic design of reservoirs and channels on a centrifugal microfluidic platform and fluid states at low (left), medium (middle) and high (right) rotation speeds

2.2 Basic Valve Design

In order to precisely control the fluid movement, valving is most important functionality to realize. Gorkin et al. (2010) distinguish between four basic methods of valving a fluid on centrifugal microfluidic platforms: (1) capillary valves, (2) hydrophobic valves, (3) siphon valves and (4) sacrificial valves.

Capillary valves are simply realized by a sudden expansion in channel diameter, such as when a channel meets a reservoir (Madou et al. 2006). Due to the fact that capillary action only takes effect in narrow channels, the fluid stops flowing at the point where the channel ends respectively, when the channel is filled completely (see Figure 2, middle). The fluid passes the capillary valve as soon as rotation speed is increased to a level where the resulting centrifugal forces causes a burst (see Figure 2, right). Experiments conducted by Cho et al. (2007) showed that the burst rotation speed (also referred to as burst frequency) can be predicted based on the valve dimensions and the wetting properties of the liquid/solid combination.

Hydrophobic valves rely on either a sudden narrowing in a hydrophobic channel or functionalized hydrophobic regions in channels to impede fluidic movement (Gorkin et al. 2010). Similar to capillary valves, hydrophobic valves are overcome by centrifugal forces. However, additional geometrical and material options in designing the hydrophobic regions allow a refined adjustment of the burst rotation speed. Due to this, hydrophobic valves have been successfully applied to even control fluid flows in the nanoliter range (Andersson et al. 2007).

Siphon valves consist of a liquid-filled reservoir with a connected siphon channel first extending upwards above the radial position of the reservoir and then down to a position radially below the reservoir (Siegrist et al. 2010). Figure 2 depicts this basic siphon valve design and illustrates its operation in three sequential steps. Immediately after the fluid is filled in, the rotation speed of the disk is increased (n_1=high) so that the fluid is pressed outwards and thus cannot flow through the siphon. As soon as the rotation speed is lowered, the centrifugal forces decreases and the capillary forces make the fluid climb the left channel of the siphon until a balanced state is reached. If the rotation speed is low enough (n_2=low), the fluid overcomes the siphon's crest and enters the right channel. In this part of the siphon centrifugal and capillary forces are now oriented in the same direction, i.e. the siphon valve is passed and the fluid is driven through the channel till the flow is stopped again by the capillary valve. By increasing the rotation speed above burst rotation speed (n_3=high), this capillary valve can be passed as well and all fluid enters the receiving reservoir.

Siphon valves are especially used to simultaneously control the movements of several fluids. Siegrist et al. (2010), for instance, have designed a disk, at which the first fluid have to pass only one siphon valve, whereas the second fluid have to overcome two serial siphon valves. This design allows to precisely control after how many speed changes each fluid is entering the receiving reservoir. Another example is given by Steigert et al. (2007), who presented a siphon-based disk design that allows to separate blood plasma from a sample of whole blood.

Sacrificial valves are valves that can only be used once. Sacrificial valves are usually realized by embedding plugs of fusible materials into the disk to block fluid passages. The single valves are activated by local energy injection temporally melting the material. Gorkin et al. (2010) describe that in contrast to the rotation-controlled valves, sacrificial valves are vapor-tight, which is most important, if reagents are to be stored on the disk or high temperature heating steps are required. Park et al. (2007) investigated the operation of both normally-closed and normally-opened sacrificial valves. In their experiment they used paraffin wax with embedded iron oxide nanoparticles, which can be easily melted by laser irradiation. As a central result they were able to show that their valves were working reliable and that the response time to operate was less than 0.5 s.

The use of siphon valves in combination with capillary valves allows very robust designs while keeping the costs low, since no additional actors need to be introduced into the system. It allows initial filling of the reservoir without the fluid crossing several siphons because of the capillary force.

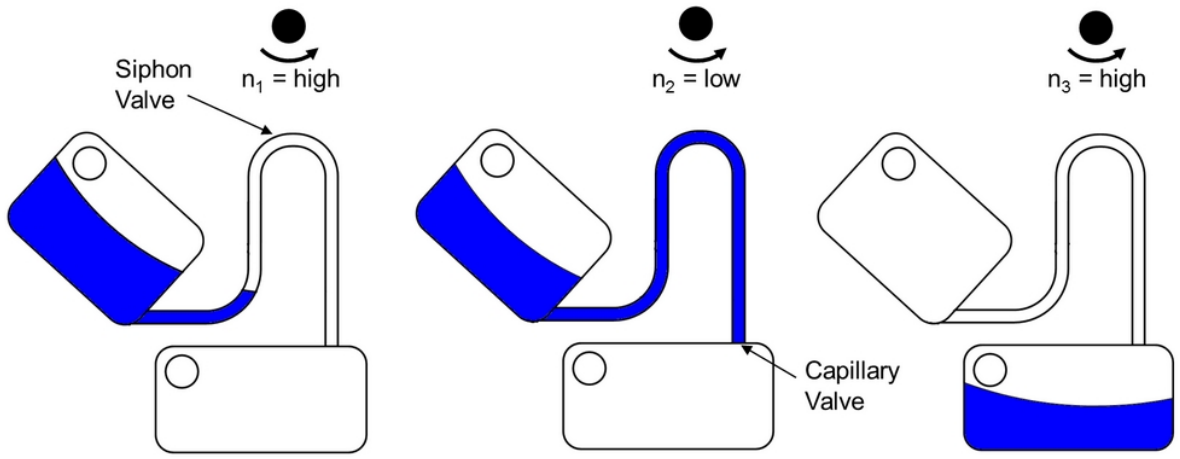

Figure 2. Basic design of a siphon valve (including a capillary valve) for rotation speed controlled fluid movement on a centrifugal microfluidic platform

3 MATERIALS AND METHODS

3.1 Design of the LabDisk

The layout of the LabDisk prototype can be seen in Figure 3. The design used to mix two fluids is marked 1 and shown enlarged in Figure 3. The other three designs that are visible in Figure 3, which are marked 2, 3 and 4, are there to evaluate the operating performance of the channels and their hydrophilic force.

The fluid mixing is realized with a combination of capillary and siphon valves (Figure 3, right). The design is composed of two loading reservoirs, initially containing the fluids to be mixed (blue), and a third bigger reservoir in between, further from the rotation center, to receive both fluids in the end (blue). All reservoirs have an opening hole on the upper layer for inlet and ventilation. The small reservoirs are linked by channels, which merge above and lead into the big reservoir. The channels are set small enough to induce capillary forces. Both channels have several valves along the way. The first valve on the channel coming from the small reservoir is a capillary valve (red dot on channel), which needs high rotation speed to surpass. It is followed by a siphon valve crest, which needs low rotation speeds to overcome. To make the mixing commence sequentially, the left channel is equipped with a second combination of capillary and siphon valves. This enables the right fluid to enter the mixing reservoir first and the left fluid to enter afterwards.

The operating performance of the channel design, respectively their capillary force is investigated by the measure design explained in section 2.1 and can be seen in Figure 3 (left). This concept is implemented three times on different radii (design 2,3,4). A fluid reservoir with an inlet hole is connected with a riser channel, which is directed to the center of rotation and ending with a vent. A small capillary valve is inserted for handling purposes to stop the fluid from wetting the channel at the initial filling. Additionally, a measuring scale with millimeter steps is implemented in the design to simplify the read out of the forefront position.

Different to the usual diameter of 12 cm of a regular compact disk, the diameter of the presented disk is set to be 18 cm to create a larger area for testing. The design consists of 1.1 mm deep reservoirs and capillary valves, connected by channels of 270 µm depth and 1 mm width. The reservoirs of design 2, 3 and 4 are capable of holding 220, 330, and 440 µl with a start-radius of 45, 65 and 85 mm, respectively. The small mixing reservoirs hold 110 µl fluid flowing into the mixing chamber with an inner volume of 330 µl. All vents have a diameter of 3 mm. The capillary valves base diameter is 1.5 mm and their distance radius from the center is 40 mm. The siphon valves crests are 30 mm away from the disk center and their overall height is 15 mm. The radial distance between the loading and the mixing reservoir is 25 mm.

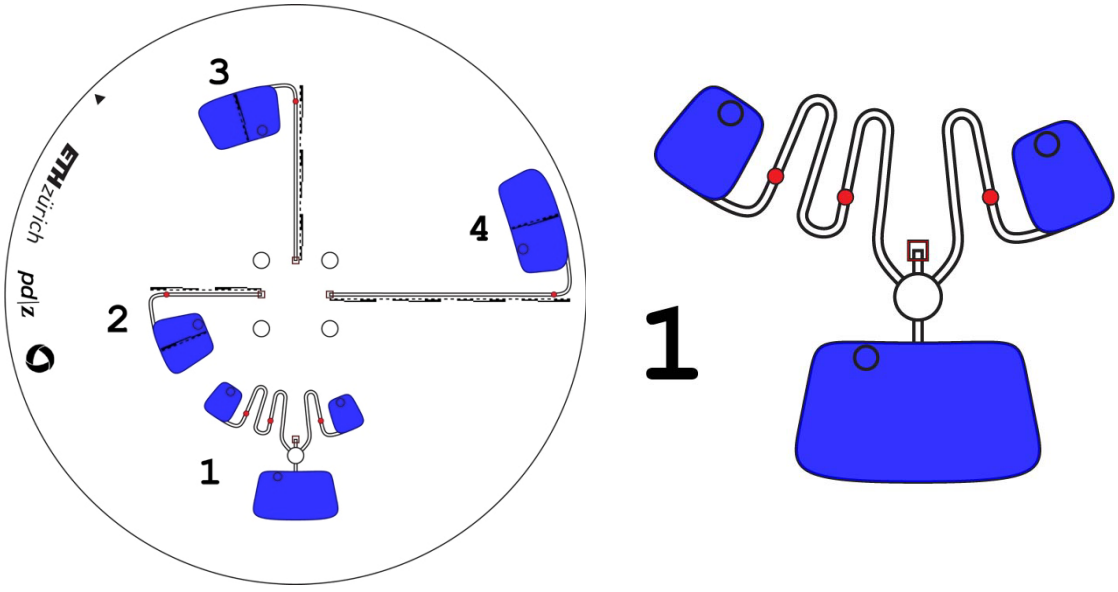

Figure 3. Prototype Disk containing basic reservoir and channel designs (left) including a serial siphon valve design presented by Siegrist et al. [2010] (right)

3.2 Prototyping of the LabDisk

The reservoirs and channels need to be sealed leak-proof and the inner surfaces of the channels require high surface tension to engage hydrophilic effects moving the liquid. This is solved with layer architecture. The composition is shown in Figure 4. The prototype is manufactured from three different disks, consisting of three layers each. Acrylic Glass (PMMA), double sided adhesive film and hydrophilic film are used. The bottom disk is made from 3.0 mm thick acrylic glass to provide stability in the system and joined with hydrophilic film by a double-sided adhesive film. The hydrophilic side is facing the inside. The top disk is manufactured identically, but since no additional stability is needed, the acrylic glass thickness is reduced to 1.5 mm for less inertia. Two layers of double-sided film enclose a thin 0.2 mm acrylic glass to create the middle disk. The three disks are machined separately with a Trotec Speedy 300, a 60W CO_2 Lasercutter. With four consecutive engraving steps, the reservoirs are processed into the bottom disk. The channels are cut directly into the middle disk and the inlets and ventilation holes into the top disk. In a last step, the three disks are pressed together at room temperature with a regular bench vise.

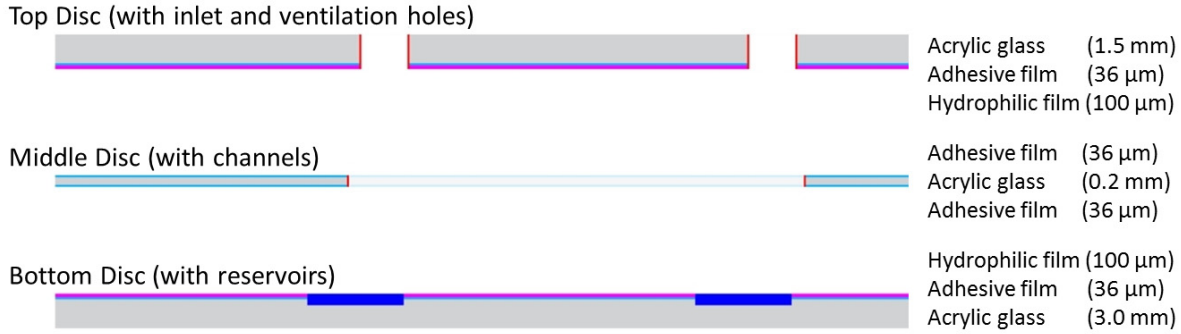

Figure 4. Layers of the prototype disk: reservoirs, channels and holes machined by laser cutter, layer material and layer thickness listed right hand side

3.3 Testing of the LabDisk

In order to test the functionality of the valve design, the prototype disk was connected to a 250 W mechanically commutated DC motor (Maxon RE65), which provided the required rotary motion. After placing 100 µl of distilled water containing food color dye Brilliant Blue FCF (E133) inside each loading reservoir, the rotation speed was increased from 0 rpm to 500 rpm.

The setup was designed to allow vision of the fluidic forefront in movement. After data acquisition the images were analyzed regarding the location of the liquid's forefront in the channels. Using this setup, the speed was manipulated in steps of 20 rpm. After each increase and a five second run-in, pictures were taken. Depending on the position of the forefront, the speed was either increased or decreased. The decision is made on the following logic: If the forefront passed the coming valve, the speed modification is changed into the other direction. This means, from increase to decrease or vice versa. If the forefront did not yet pass the coming valve, the speed is increased or decreased further, respectively. This procedure took less than 20 seconds for each step and resulted in the following sequences, starting from an initial speed of 0 rpm:

- Sequence 1: rotation speed was increased from 0 to 500 rpm and then decreased to 80 rpm
- Sequence 2: rotation speed was increased from 80 to 500 rpm and then decreased to 60 rpm
- Sequence 3: rotation speed was increased from 60 to 200 rpm and then decreased to 80 rpm
- Sequence 4: rotation speed was increased from 80 to 400 rpm and then decreased to 0 rpm

Images of the microfluidic states were recorded during operation by a 12.3-megapixel digital reflex camera (Nikon D90) and a photographic flash unit with a 100 Hz strobe light function (Nikon Speedlight SB-900), which was used to decrease exposure time to reduce blurry effects appearing at high speeds.

4 RESULTS

The results generated in the experiment are presented below. The channels, the reservoirs, and the fluid captured in operation are shown in Figures 5-7. The pictures taken are chronically ordered from left to right with initials from a to i in the upper left corner, the actual rotational speed is show in rpm (rounds per minute) in the lower left corner.

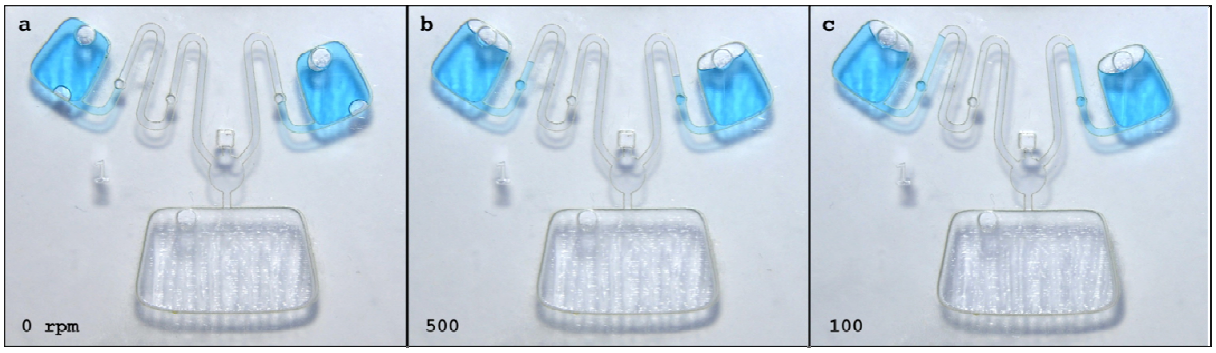

Figure 5. Capture of the liquids at initial position, at surpassing the capillary valve during the first speed cycle and at the passage from fast to low speed

In the first step the LabDisk is standing still in its initial position. The reservoirs are filled with fluid. In the course of filling, the liquid ran through the channels up to the first capillary valve and stopped Figure 5(a). Next, the first high speed is reached in Figure 5(b). It was captured at 500 rpm rotation speed and shows that both liquid forefronts surpassed the capillary valve. The fluid height level adjusted visibly radial to the center throughout the complete system. The passage from high to low speed, and the behavior of the liquids can be observed in the third picture (c). At 100 rpm, both liquid forefronts ascended further to the center. The fluid height level of the reservoirs decreased slightly. The crest is not surpassed.

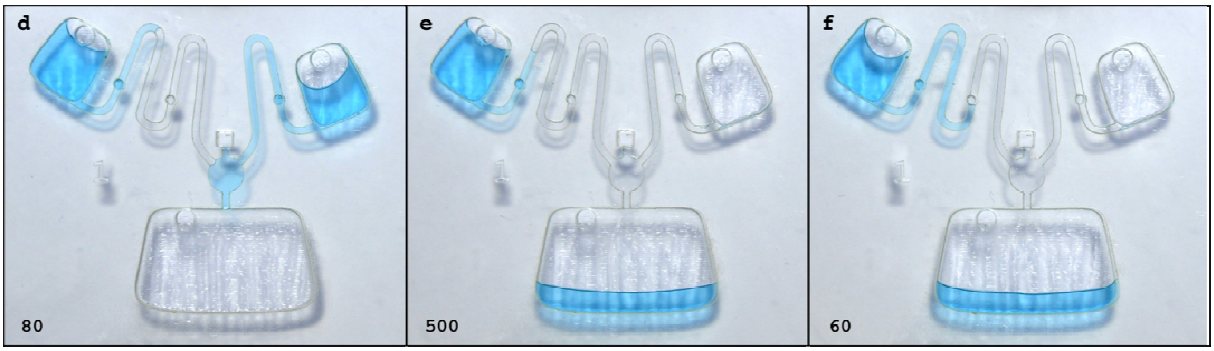

Figure 6. Capture of the right liquid passing the siphon valve and flowing into the big reservoir, while the right fluid retreats before passing the first crest

Shown in Figure 6(d) is the first low speed setting at 80 rpm. The right liquid forefront overcame the crest and ran through the channel to the outlet. It stopped on the orifice of the left channel and on the outlet to the mixing chamber. The right fluid height of the reservoir is significantly lower than the left. The second high speed setting with the LabDisk at 500 rpm is reached in the second picture (e). The right fluid ran through the outlet into the mixing chamber. The left liquid returned to the same state as in Figure 5(b), the first high speed setting, and did not overcome the first crest. As shown in Figure 6(f) the liquid forefront ran through the first crest and stopped at the second capillary valve at the second low speed setting with 60 rpm.

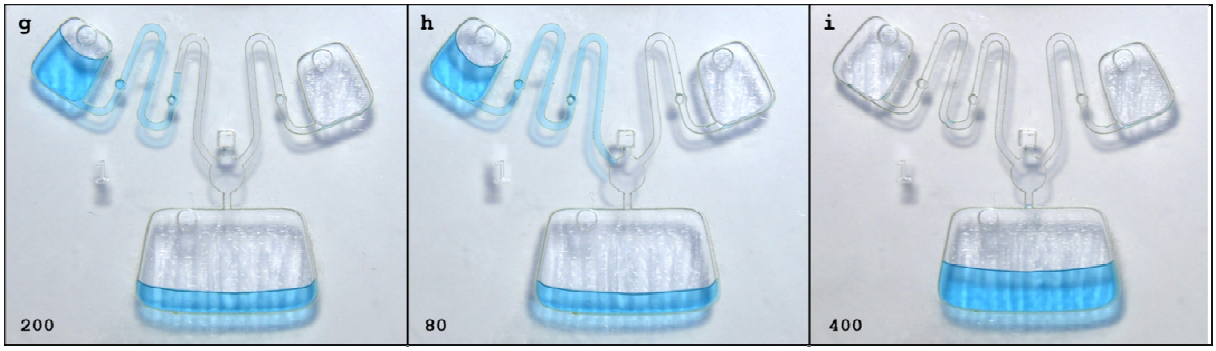

Figure 7. Capture of the left fluid first crossing the second capillary valve, then the second siphon valve and finally flowing into the big reservoir

The third high speed setting, as seen in Figure 7(g) is set to 200 rpm. Here, the liquid forefront ran over the second capillary valve and stopped at a comparable level as the fluid surface in the left reservoir lies. Figure 7(h) is taken at the third low speed setting at 80 rpm. It presents the liquid forefront, which ran over the second crest, through the rest of the channel and stopped at the diameter change at the detection area. The last picture (Figure 7(i)) shows the results of the fourth high speed setting at 400 rpm. The liquid from the left reservoir ran into the mixing chamber. The fluid height in the mixing chamber increased.

5 DISCUSSION AND OUTLOOK

The results show a successful realization of the disk and the valves. It was possible to reproduce the basic function of rotation-speed-dependent fluid mixing. The behavior of the fluid within the valves fits to the theory described in section 2.

The possibility of on-operation-management allows explicit discussion of the fluid behavior. The movement of the first three steps (Figure 5) displays the capillary force in the channels and the hydrophobic behavior of the engraved stop points. Figure 6(d) and (e) show the right fluid overcoming the crest, while the left fluid fails to break through the first obstacle and returns to the same state as Figure 5(b). This can be explained in different ways. The most probable explanation is the air pressure in the canal. While the right fluid has reached the detection area, the left fluid canal is sealed on both sides. The enclosed air might create a counterforce to the movement of the left fluid. Another possible explanation is a different crest height of the first left and right crest. Even though the crests were designed with an identical height in relation to the disk center, the rotational center fixation had a tolerance of up to 1 mm. An unfortunate interaction of the determining velocity range and the rotation center can result in different activation speeds of specific crests. The speed of 80 rpm can be sufficient for an activation of the right crest, while it can be insufficient for the left crest. A third explanation for the retreat of the left fluid can be dirt/dust in the canal. This might diminish the hydrophilic effect and the capillary force. Forgoing the detection area and directing the canals directly into the mixing camber should solve the problem of enclosed air. An improved mounting of the disk can ensure a robust crest radius. With these possibilities compared, the behavior of the right fluid points toward the first option, because the right fluid does not flow up the left channel, speaking for an increased air pressure.

With this approach, it was possible to realize and gain insights into the functionalities of lab-on-a-chip, especially lab-on-a-disk devices as a first step. The initial question was, if we are able to realize the critical function. Therefore the critical function was chosen by comparing existing lab-on-a-disk devices for similarities in respect to the selected task of PCR. For the majority, guiding and mixing fluids was a crucial part, as it was in our concept. With this function to realize, we were building and testing nearly 20 prototypes within two months' time and learned quickly about sealing, acceleration, effects of hydrophilicity and hydrophobicity, materials and material treatments. The idea of realizing one critical function within a certain amount of abstraction does not prove that the project would not fail, but it shows that it can be handled somehow. If the critical function could not be realized in any way and abstraction, it would end the project early and therefore possibly without big expenses.

With the presented results of the experiment, many important aspects were revealed. On one hand, the manufacturing process is not optimized for dust free manufacturing. The design of the final device

needs to be robust to small amounts of dust, dirt or humidity. This could be useful in the later product, since a robust process can be certified for the market easier. The centering of the disk and the symmetric design needs to be focused not only to make the mixing process reliable, but also to ensure a save centrifugation process. The used camera setup allowed real time observation, but effected a rather long delay of 20 seconds. In later tests, live tuning of the shutter time to the rotational speed might allow a digital real time observation without long delays. As an option for the near future, a stroboscope should be tested for direct manual readout.
Implied by the process of this design approach, several functions have not been developed and prototyped within this project. Some are particularly challenging. The most important design step for further development and decisions is to define the users needs and requirements. This includes not only the anticipated user experience, which is very important, but also requirements in terms of testing time, storage time and storage space. One difficulty is the lifetime of the reagents to be used. To create an easy product-user-interaction, the reagents should be stored in the device "ready-to-use". Some can be dried and stored easily at room temperature for several months, but others need to be kept at -20°C if stored for more than several days. Luckily, the other reagents do not suffer at this temperature, but this would either require a freezer function in the device or freezer space for the doctors' office, which most do not have. And additionally, this might be critical for the polymer. For another example, the mechanical forces needed to lyse (i.e. to destroy the cells and release the DNA) the different bacteria, imply a high accelerated shaking. The centrifugation on the other side needs to be very fast, creating around 10000 g on the probe. This results in conflicting goals in the requirements of the actuation motor. Temperature wise, different core temperatures at points of 27°C, 67°C and 98°C are needed for the chemical reaction of the DNA. Since the reaching of these core temperatures is needed one by one in cycles up to 30 times, the faster and more exact these are reached, the shorter the overall testing time will be, which has a substantial influence on the user experience. Finally, the safety of the user may not be left out. The presented prototype facilitates holes in the top layer as air vents to balance air pressure. This is not suitable for later application, since germs could emerge through these holes either in liquid or gaseous solutions. The cleaned disposable coming from manufacturing needs to be kept contamination free, e.g. by sealing. The contamination of the spinning device through emerging pathogens in operation would also make cross contamination of the following test disk possible. To address this problem, the possibility of enclosed contamination free air pressure buffering chambers will be evaluated and tested in the future. These chambers are relatively big and filled with air and allow the disk to be sealed completely before and after the insertion of the probe. The fluid could be moved easily. An alternative would be air permeable filtering membranes to be inserted in the vent holes. All of these functions are considered the next critical functions and need to be addressed by further development.
A big benefit of the described PCR method is that this procedure allows testing for all actual and upcoming future pathogens containing either DNA or RNA, just by adjusting the reagents that are included in the disposable. This includes all bacteria and viruses, like tuberculosis, Ebola, HIV and others. This creates new application fields, e.g. in third world countries and therefore it would be practical if not only well-trained medical personnel, but also untrained volunteers could use the device without risks of contamination or misinterpretation.

6 CONCLUSION

In conclusion, basic designs for centrifugally actuated microfluidic devices are presented, prototyped with laser cutter techniques and tested with real time visual observation. Two fluids, filled in different reservoirs are steered into a third reservoir one after the other and then mixed. The disk spinning motor is the only actuator. No manual intervention is needed, apart from the motor control, which indicates a fully automatic actuation possible. The shown valves and the prototyping method have demonstrated their practical feasibility for numerous possible applications for sample-to-answer lab-on-a-disk prototypes. A test setup for microfluidic lab-on-a-disk disposables with real time photo observation has also been developed. The created prototypes of the spinning device and the disposable polymer disk are successful first steps towards the development of fast and affordable point-of-care sample-to-answer devices for diagnosis of infectious diseases.

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