ERYTHROCYTE SEDIMENTATION AND AGGLUTINATION ASSAYS IN A MULTI-BIFURCATING MICROFLUIDIC CARTRIDGE

Holger Becker, Cornalia Carstens, and Claudia Gärtner
microfluidic ChipShop GmbH, Jena, GERMANY

ABSTRACT

We have developed a polymer microfluidic cartridge for agglutionation assays based on a microfluidic network of bifurcation microchannels. Upon coagulation, the microchannels are clogging, preventing the sample from migrating further through the network. An alternative application are erythrocyte sedimentation tests, where the sedimentation time correlates with erythrocyte agglomerates which have different fluidic resistances and are thus travelliong slower through the microchannels than erythrocytes from healthy subjects.

KEYWORDS: Agglutination assay, cartridge, polymer chip

INTRODUCTION

For a first rapid health status check, simple diagnostic tests using a full-blood sample from the patient have been used for many decades in clinical laboratories. Amongst them is the determination of the erythrocyte sedimentation rate (ESR [1]) and agglutination reactions for direct blood typing or the indirect antiglobulin test (Column agglutination cartridges/cards, "Coombs" test [2]). In state-of-the-art IAT cartridges, which outer dimensions we have adopted also for our cartridge, small columns are filled with a gel that has the relevant antibodies incorporated in the gel matrix. The sample application and reaction chambers above the columns are then filled with the blood sample. Upon centrifugation, the red blood cells migrate through the gel and in case of a positive response, haemagglutinate on top of the gel or during passage as a result of the antibody–antigen reaction. Negative samples simply pass through the gel uninhibited to the bottom of the column.

We have developed a microfluidic cartridge to perform these tests yielding the following advantages:

1. Faster diagnostic result
2. Higher reproducibility due to the replacement of the gel by a bifurcating microfluidic network
3. Longer shelf-life due to absence of the gel
4. Transportability of the cartridge (gel filled cartridges develop air bubbles etc. upon movements; cartridges have to be transported upright)

EXPERIMENTAL

The layout of the cartridge is shown in Fig. 1. A full blood sample (1 µl) is pipetted in from the top into the buffer-filled reservoir at the to of the cartridge and travels

through the microchannel network in the lower half of the cartridge which has a sequence of 5 bifurcating branches, with the smallest channels having a cross-section of 40 by 40 µm. The fluidic driving mechanism is either gravity for the ESR test or centrifugal force in case of the IAT. The cartridge is manufactured out of PMMA using injection molding. The challenge in microfabrication [3] was the generation of a mold with up to 10 different heights. It was realized using precision mechanical micromachining. Figure 2 shows a detail of the injection molded channel structure (blood flow from the bottom to the top). The channels were closed with a thin (100 µm) PMMA foil.

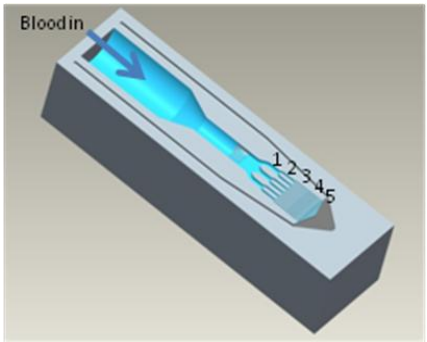

Figure 1. Schematic drawing of one cartridge element. The numbers denote the bifurcation level.

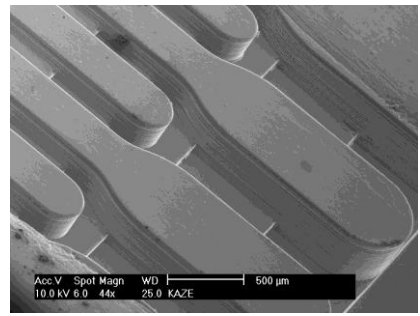

Figure 2. SEM of a bifurcation area. The blood flows in from the bottom.

RESULTS AND DISCUSSION

Figure 3. Agglutination assay for bloodgroup determination. Left side no agglutination (negative sample), right side agglutination (positive sample).

Figure 3 shows the result for an agglutination assay (blood-group determination). The left image shows the negative sample (no agglutination), the right image the positive sample (agglutinate is retained in microstructure). The big advantage of this test is the ability to extract the diagnostic result without the need for a complex detection system simply by naked eye. Figure 4 shows the cartridge in ESR-mode, 2 mins after depositing the blood sample. The sedimentation rate of the erythrocytes is a function of the presence of proteins generated by inflammatory reactions, shown for a healthy and a diseased sample in Fig. 5.The negative (healthy) sample sediments slower than the positive (dis-

eased) sample as . Although unspecific, this ESR is widely used, particularly in low-resource settings, as it can be performed without complex instrumentation.

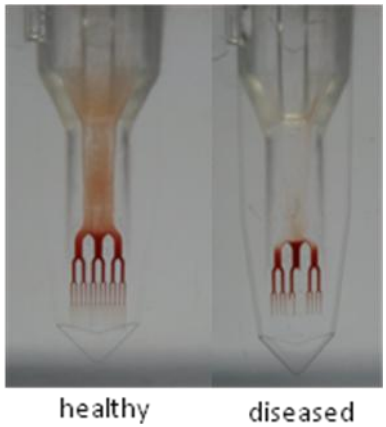

Figure 4. ESR measurement in cartridge. Left side healthy (negative sample), right side diseased (positive sample). The sedimentation rate of the diseased sample is increased.

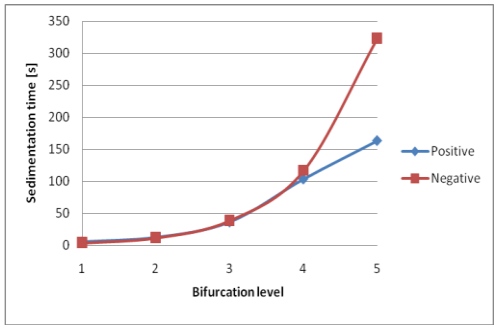

Figure 5. Sedimentation time per bifurcation level (see Fig. 1) in case of healthy (negative sample) and diseased (positive sample).

CONCLUSIONS

We have been able to eliminate two of the major drawbacks of conventional IAT cartridges, namely the gel-induced variability and shelf-life limitations by the replacement of the gel by a multiply bifurcation microchannel network. Results with clinical samples show comparable quality to conventional methods.

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