HIGHLY EFFICIENT ON-CHIP PLASMA/SERUM GENERATION FOR DISPOSABLE POINT-OF-CARE DEVICES
Holger Becker1, Richard Klemm1, Cornelia Carstens2, and Claudia Gärtner1
1microfluidic ChipShop GmbH, Jena, GERMANY
2Laborgemeinschaft Elbracht und Carstens, Naumburg, GERMANY

ABSTRACT
We present a highly efficient method for the generation of plasma and serum from full blood on-chip. The use of a conventional separation membrane integrated in a flow-chamber simplifies chip manufacturing as well as regulatory approval aspects for a point-of-care diagnostic device. Thus handling was massively facilitated, serum yields were comparable with conventional methods.

KEYWORDS: Plasma generation, diagnostics, polymer chip

INTRODUCTION
Due to the continuous technological advances in the field of microfluidics [1] and the increasing application know-how, a trend towards a higher degree of functional integration in microfluidic devices can be noted in recent times. This development denotes an important step in the commercialization of microfluidics [2], as the potential for a full functional integration of all analytical process steps into a single device represents one of the early and for a long time unfulfilled promises of this technology. Especially the field of point-of-care-diagnostic (POC) applications which utilize methods of modern molecular biology e.g. for the rapid diagnostics of virus- or bacteria-induced diseases like respiratory tract inflammations or tuberculosis or an early diagnostics of cancer, promises to greatly benefit from the potential of full functional integration by miniaturization of all assay steps. The diagnostic process of these diseases so far suffered from the fact that many individual process steps had to be carried out in the clinical laboratory, making these tests slow, expensive and possibly error prone.

For a large number of diagnostic assays, the first step in sample preparation is the generation of plasma/serum from full blood. The analysis of the composition of blood plasma or serum and to carry out immunological testing of marker proteins or auto-immune antibodies in these liquids is still an essential tool to confirm or exclude a whole bunch of different diseases. Current developments in the lab-on-a-chip research area open new avenues for a rapid isolation of blood plasma or serum at the point of care. Blood plasma or serum can either directly be used during a chip-integrated processing inside downstream modules of the same chip or, alternatively, can be applied to other processes that are independent from the plasma/serum preparation unit. Separation of blood liquid from the solid components via both, centrifugation and filtration, has been described so far. A design of the next generation of disposable microfluidic cartridges for the filtration mediated isolation and purification of blood plasma is shown in the following.

EXPERIMENTAL SET-UP
The idea of the approach can be taken from figure 1. Figure 1 shows the schematical CAD drawing of such a separation unit, of which several are injection molded on a chip with the outer dimensions of a microscopy slide (75.5 mm x 25.5 mm). The chip has been realized in a variety of thermoplastic materials like cyclic-olefin copolymer (COC, tradename Topas), polystyrene or polycarbonate from a mold insert made using precision mechanical machining. One of the significant advantages of injection molding is the ability to integrate fluidic interfaces directly onto the chip at no extra manufacturing cost. In this case, the device has integrated mini Luer fluidic interfaces molded onto the chip, allowing for either a press-fit instrument interface or, using a special adapter, the use of standard tubing. The membrane material, which is a commercially available separation membrane, is cut to size and welded onto the chip.

The actual injection molded chip with 5 separation units is shown in Fig. 2a.

A single plasma generation unit from this chip is shown in Fig. 2b, with the numbers denoting functional elements as listed below. The blood plasma/serum preparation module consists of a mini Luer interface (1) for blood loading, a support channel with a cross section of 300 µm x 100µm (2) for the transfer of the blood on top of a separation membrane (3) that is fused into a chip-based chamber of 10 mm diameter, a plasma/serum collection channel (4) below the membrane, and a ventilation channel of 100 µm x 100 µm (5) – also below - the membrane. The vacuum is applied via the collection channel and a second interface (6) to the outer world. A third interface (7), which is closed during the sample loading, helps to smoothly release the slight vacuum after the membrane pores are blocked by the solid components of the blood such as erythrocytes, monocytes, platelets, or leucocytes.

Fig 1a: CAD drawing of the separation unit with integrated fluidic interfaces but without membrane
Figure 3 shows the outcome of a typical blood plasma preparation process. The yield of plasma/serum is dependent on the ratio between liquid and solid components of the blood, which varies between individuals and the history of food uptake. Reflecting these points, up to 80% of the plasma/serum can be obtained, a value comparable with conventional methods. Figure 4 displays the quantitative level of contribution of characteristic proteins to the composition of purified blood serum as analyzed in an off-chip electrophoresis system. This analysis as well as the results from tables 1 and 2 reveal that the composition of this preparation is practically identical to that blood serum obtained by the application of conventional techniques.
Table 1: Test results of immunoassay

<table>
<thead>
<tr>
<th>Serum generation</th>
<th>PSA [mg/ml]</th>
<th>IgG [mg/ml]</th>
<th>total protein [mg/ml]</th>
</tr>
</thead>
<tbody>
<tr>
<td>conventional</td>
<td>19.3</td>
<td>14.52</td>
<td>82.7</td>
</tr>
<tr>
<td>chip</td>
<td>17.4</td>
<td>14.67</td>
<td>84.6</td>
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</tbody>
</table>

Table 2: Densitometric analysis of serum protein separation via gel electrophoresis

<table>
<thead>
<tr>
<th>Serum generation</th>
<th>albumin [%]</th>
<th>α 1 [%]</th>
<th>α 2 [%]</th>
<th>β [%]</th>
<th>γ [%]</th>
<th>total protein [mg/ml]</th>
</tr>
</thead>
<tbody>
<tr>
<td>conventional</td>
<td>44.4</td>
<td>4.2</td>
<td>16</td>
<td>12.3</td>
<td>23.1</td>
<td>84.3</td>
</tr>
<tr>
<td>chip</td>
<td>45.2</td>
<td>5.1</td>
<td>17.8</td>
<td>12.3</td>
<td>20.4</td>
<td>84.3</td>
</tr>
</tbody>
</table>

CONCLUSION

We have been able to show that a relatively simple injection-molded module encompassing a membrane plus fluidic supply is able to prepare either blood plasma or serum in a quality that is comparable to that generated via application of classical methods. This functional element can either be used as a stand-alone or as an integral part of a more complex device encompassing several different functions such as ELISA analysis of clinical or biochemical marker molecules. This represents an important step in sample-prep for POC tests and shows a method suitable for high-volume production of such devices.

ACKNOWLEDGEMENTS

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REFERENCES