LAB-ON-A-CHIP CARTRIDGE FOR PROCESSING OF IMMUNOASSAYS WITH INTEGRATED SAMPLE PREPARATION

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ABSTRACT

We present a novel centrifugal microfluidic lab-on-a-chip cartridge for the fully integrated processing of immunoassays including sample preparation and metering, incubation, washing and waste handling. From a sample of 10 µL human whole blood we extract 4 µL of blood plasma by sedimentation of blood cells driven by centrifugal forces with a CV of 6%. Routing of fluids is realized by capillary and volume triggered siphon valves. The reliability of the capillary-driven fluid channels is increased by surface coating with BSA for hydrophilization and blocking. The reaction chamber allows efficient mixing of fluids and dissolution of prestored reagents [1]. For demonstration purposes we quantified estradiol with a range of 25 pg/mL to 1 ng/mL using a chemiluminescent competitive immunoassay within 30 minutes [2].

KEYWORDS: Centrifugal Microfluidics, Diagnostics, Immunoassay, Blood Plasma Separation

FUNCTIONAL PRINCIPLE

Our modular setup consists of a microstructured polystyrene cartridge (Fig. 2A) which is spun by a frequency programmable rotary motor. Each cartridge features 4 fluidic structures for parallel immunoassay processing and the rotor can take up to 6 cartridges at a time allowing us to perform 24 assays. Prior to assay processing the surface of the fluidic chip is treated with a PBS blocking solution containing 0.3 % BSA and 0.1% Tween 20. Radially inward the cartridge includes a separation structure for extraction of blood plasma (Fig. 1A). The plasma is moved through a capillary siphon into the interconnected mixing chamber (Fig. 1B). There estradiol HRP conjugate molecules are being mixed homogeneously with the blood plasma within 30 s using a shakemode protocol [3]. The resulting fluid with a volume of 50 µL containing sample and competitor molecules enters the reaction chamber through another capillary siphon. In the reaction chamber we immobilized anti-estradiol antibodies for capturing the sample antigens. After an incubation time of 15 minutes three washing steps are being operated. The competitive estradiol immunoassay (Fig. 3A) can be processed with only one buffer used for dilution, incubation and washing.

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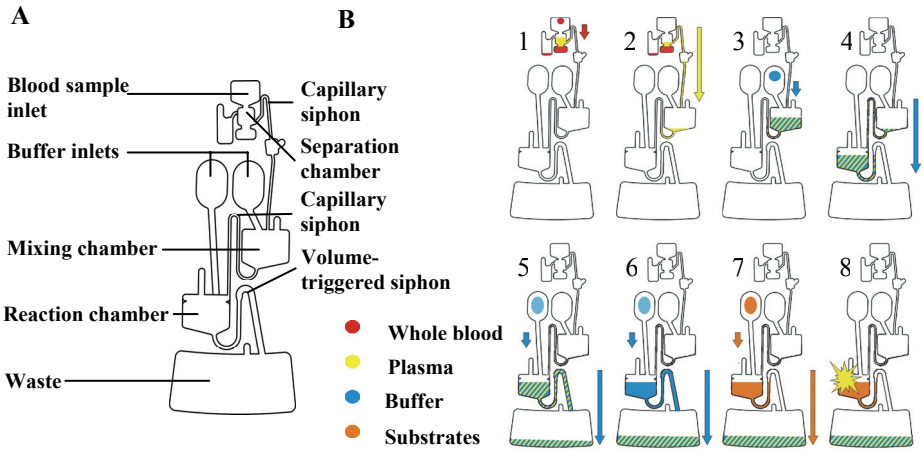

Fig. 1: (A) Schematic view of the fluidic structures integrated in our lab-on-a-chip cartridge. (B) 1: A sample of whole blood enters the cartridge through an inlet. The blood cells are separated at spinning frequencies of 50 Hz. 2: The blood plasma is moved into the mixing chamber due to capillary forces at spinning frequencies of 2 Hz. 3: The plasma sample gets mixed with the solution containing the competitor molecules. 4: The resulting fluid enters the reaction chamber with immobilized antibodies through a capillary siphon for incubation. 5: Sample and competitor solution is removed into the waste reservoir followed by three washing steps (6). 7: Substrate solution is incubated for 10 minutes. 8: The luminescent signal occurs.

EXPERIMENTAL RESULTS AND DISCUSSION

With our setup we are able to extract blood plasma from human whole blood within 2 minutes, by spinning the cartridge at a frequency of 50 Hz (Fig. 2B). From a blood volume of 10 - 15 µL we separate 4 µL of plasma with a small CV of 6%. The fraction of blood cells remaining in the extracted plasma is below 0.5 %. Therefore our separation structure provides the specifications regarding the plasma volume and purity for performing quantitative immunoassays

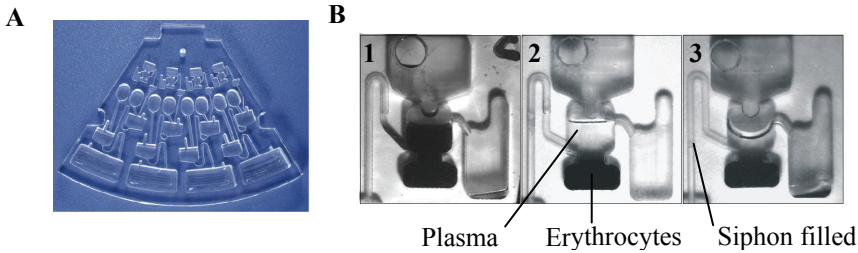

Fig. 2: (A) Photo of the centrifugal microfluidic cartridge for immunoassays. The cartridge consists of a polystyrene substrate. All fluidic elements are integrated by micromilling. (B) 1: Blood enters the separation chamber. 2: Erythrocytes are sedimented due to centrifugal forces. 3: Extraction of blood plasma through a capillary siphon.

With our lab-on-a-chip cartridge the detection of estradiol is shown for physiological relevant concentrations in the range of 25 pg/mL to 1 ng/mL (Fig. 3B). As time to result we achieve 30 minutes compared to 50 minutes using the standard protocol for the microtiterplate-based assay.

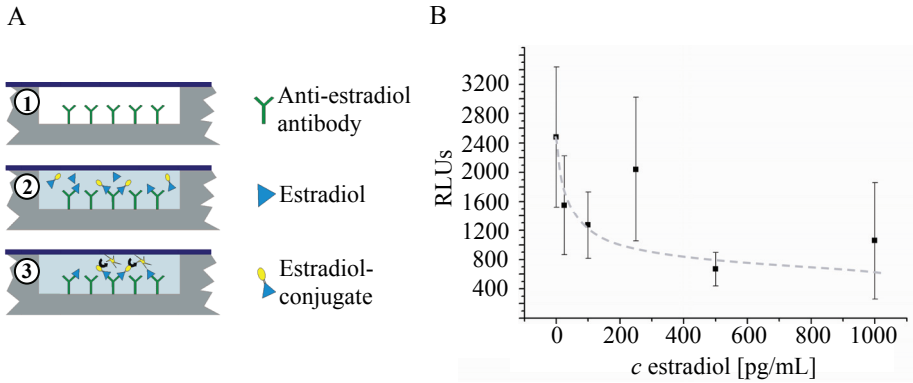

Fig. 3: A) Competitive estradiol immunoassay. 1: anti-estradiol antibodies immobilized on PS substrate. 2: Estradiol from sample is mixed with estradiol HRP conjugate molecules. Both compete for antigen binding sites. 3: After washing a substrate solution is added leading to light emission. (B) Results of the competitive estradiol immunoassay processed in the cartridge. The signal derived from the 250pg/mL estradiol sample is to high due to leakage problems of the cartridge.

CONCLUSIONS

We realized a highly integrated fluidic cartridge driven by centrifugal forces for the complete processing of competitive immunoassays including sample preparation of human whole blood, mixing, incubating, fluid routing and waste handling. It is shown that our plasma separation fits diagnostical demands for competitive immunoassays and the integrated assay can quantify estradiol in concentrations of pg/mL, meeting the physiological relevant range. In the future we will build a device with dispensers and optical detectors for the fully automated processing of the cartridge.

AKNOWLEDGEMENTS

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