RAPID ON-CHIP BLOOD/PLASMA SEPARATOR USING HETERO-PACKED BEADS AT THE INLET OF MICROCHANNEL

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ABSTRACT

A disposable rapid on-chip whole blood/plasma separator has been designed and fabricated by packing beads at an inlet of microchannel and characterized for the application of point-of-care (POC) clinical diagnosis. A hetero-packed beads at the inlet of microchannel was simply implemented by hetero-packing two different sizes of silica beads, where one was a large size of bead for blocking the inlet of microchannel, and the other was a small size of bead as a microfilter for blood cells. While whole human blood moved through the hetero-bead packed region by capillary force, the movement of blood cells was impeded by small pores between the packed-beads, and the plasma was finally separated from the whole blood. After dropping 5 µl of whole human blood, 350 nl of plasma has been successfully separated within 2 minutes by the developed blood/plasma separator, which was very desirable speed and amount for a POC clinical tests.

KEYWORDS: Blood/plasma separator, Point-of-care testing (POCT), Hetero-packed beads, Bead-packed inlet, Lab-on-a-Chip filter

INTRODUCTION

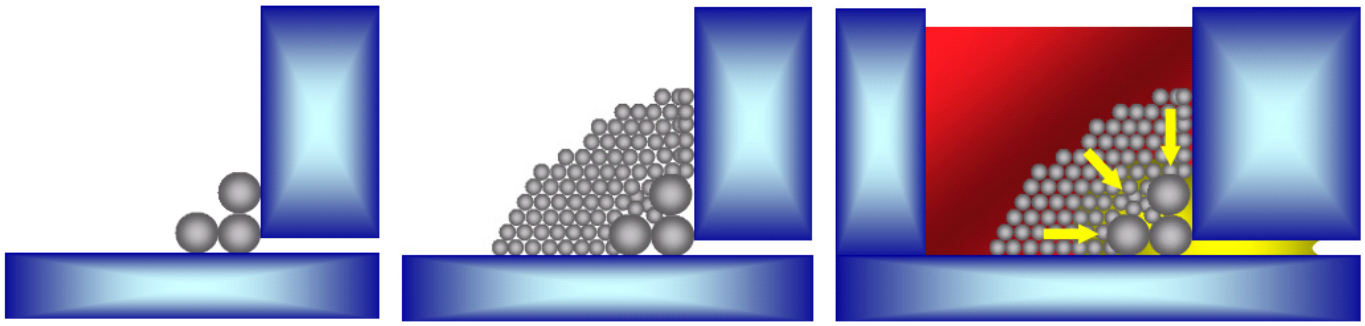
With the increasing applications of lab-on-a-chip (LOC), there has been a large demand for the development of microfilters for on-chip separation of the target sample. Specifically, for the point-of-care (POC) clinical diagnostics using LOC with whole human blood, an on-chip blood/plasma separator is desired as an integrated component with the LOC..

The microfilter for POC clinical tests is able to separate the plasma from undiluted whole blood within a short period of separation time to promptly diagnose urgent patients. Also, the amount of the separated plasma should be enough for a subsequent analysis. In addition, the LOC microfilter for blood cells is required to be built in a small area of chip and flexibly incorporated with surrounding microfluidic networks. Moreover, in many POCT applications for clinical tests, the devices are fabricated as a disposable type, so the blood/plasma separator is mass-producible in low cost. [1, 2]

In this work, an on-chip disposable blood/plasma separator has been realized by packing different size of beads as a heterostructure at the inlet of microchannel. The developed blood filter shows a rapid capillary separation of the plasma, producing enough volume for the POC clinical diagnosis with LOC..

PRINCIPLE and DESIGN

Figure 1 shows schematic diagrams for the structure of hetero-packed beads and the separation principle of blood/plasma separation. Two different sizes of beads (100 µm and 10 µm) are used to implement a blood/plasma separator at the inlet of microchannel. The first packed-beads have a large diameter of 100 µm to block the inlet of the microchannel, and the second packed-beads have a small diameter of 10 µm to induce a capillary separation of plasma from whole blood. When whole blood is dropped at the inlet composed of hetro-packed beads of the microchannel, the blood flows through the packed beads by capillary force. During this movement of blood, the blood cells are impeded by the hetero-packed beads, so plasma is separated from the whole blood by capillary force without any external power source.

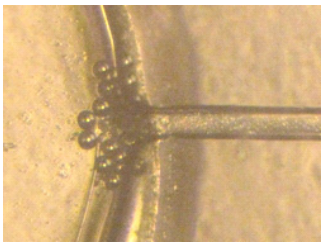


(a) Blocking inlet by packing large beads

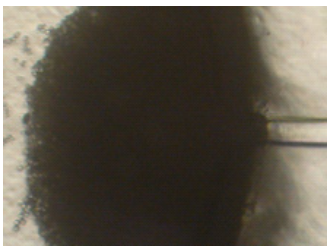
(b) Small beads packing for plasma separation

(c) Capillary separation of plasma from whole blood through bead-packed inlet of microchannel

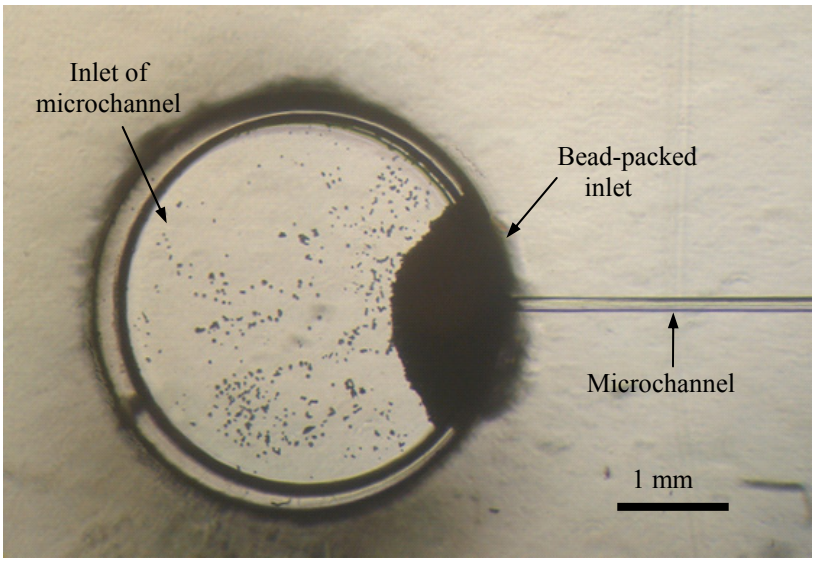
Figure 1. Schematic illustrations of hetero-packed beads and blood/plasma separation.



(a) Large bead packing



(b) Small bead packing



(c) Bead-packed inlet of microchannel

Figure 2. Microscopic picture of bead-packed inlet of microchannel for plasma extraction from whole blood.

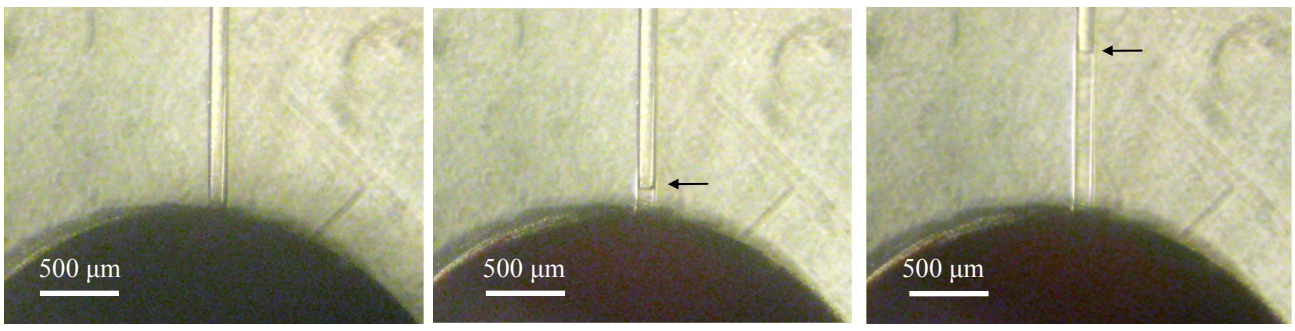
EXPERIMENTAL

A polymer injection molding was performed to fabricate the microchannel with Cyclic Olefin Copolymer (COC, TOPAS Advanced Polymers Inc.). The microchannel was designed to have 100 μm width, 3.5 cm length and 100 μm height. For the fabrication of the mold with microstructures, SU-8 2075 (Microchem Inc.) was lithographically patterned on a blank nickel (Ni) disk. Subsequently, Ni electroplating was performed with a guidance of the patterned SU-8. After injection molding of COC polymer on the Ni mold, the inlet and outlet of microchannel was drilled with 4.4 mm diameter of drill bit through 1 mm thickness of the injection-molded COC chip. Finally, the patterned chip was fusion bonded to the blank COC chip by applying high temperature and pressure. [3]

To pack the beads at the inlet of the microchannel, a negative pressure was applied at the outlet of the microchannel using a vacuum pump. To apply a high vacuum to the microchannel, the other inlets for inserting additional reagents were sealed by a detachable tape. While the suction vacuum was applied at the outlet of the microchannel, the glass beads with 100 μm diameter (Biospec Inc.) dispersed in DI water were dropped first at the inlet of the microchannel. After drying DI water by vacuum suctioning, the 100 μm beads blocked the inlet to prevent 10 μm beads passing through the microchannel. Then, the colloidal silica beads with 10 μm diameter (Discovery Scientific Inc.) were dropped at the blocked inlet by 100 μm beads, resulting in the formation of hetero-packed beads at the inlet of the microchannel as shown in Figure 2.

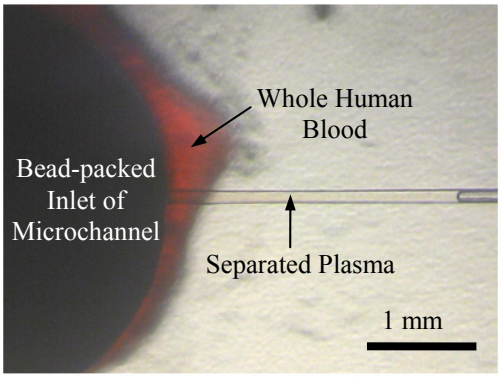
After the completion of packing, the hetero-packed beads and the microchannel were coated with Protein Blocking Solution (PBS, Thermo Fisher Scientific Inc.). By coating the microchannel with PBS, a hydrophobic surface of the COC microchannel was changed to a hydrophilic surface, which allowed the capillary flow of blood through the bead-packed inlet. The PBS-coated microchip was incubated for 5 minutes to make a uniform coating. Then, the negative pressure was applied at the outlet of microchannel for 30 minutes to dry out the PBS on the packed beads.

A whole human blood with 43 % of hematocrit was procured from the Medical Center in University of Cincinnati. 5 μl of blood was pipetted and carefully dropped at the inlet of microchannel. Without applying any external power, the blood flown through the packed beads and the plasma was rapid separated from the whole blood within 2 minutes. The hydrophilic surface of the packed beads and the microchannel induced the capillary flow of plasma. The movement of the separated plasma was monitored with an optical microscope using the reference marks alongside the microchannel.

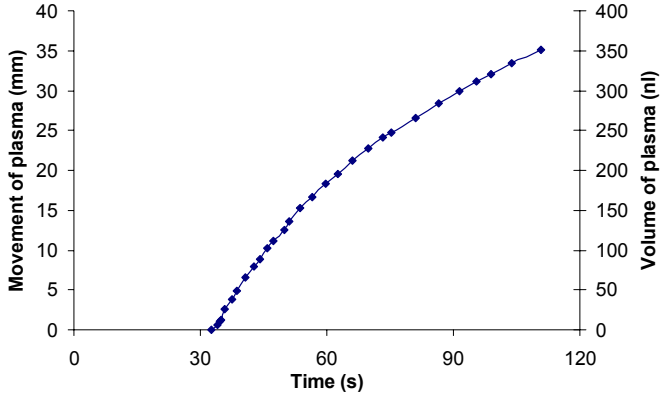


(a) Blood dropping (b) After 30 sec (c) After 33 sec

Figure 3. Rapid separation of plasma by the bead-packed inlet of microchannel (500 μm scale bar).



(a) Separated plasma from whole blood at the bead-packed inlet of microchannel



(b) Movement of separated plasma according to time

Figure 4. Movement of separated plasma according to the separation time (500 μm scale bar).

RESULTS AND DISCUSSION

As shown in Figure 3, the plasma was separated from the whole blood and flowed through the microchannel by the capillary force within 30 seconds after dropping the sample blood at the bead-packed inlet of microchannel. This fast extraction of the plasma from whole blood is very desirable for rapid clinical diagnostics of urgent healthcare in emergency room or intensity care units. This rapid separation was mainly attributed to a geometrical shape of the packed beads. When the beads were packed at the inlet by the suctioning pressure at the outlet of microchannel, the uniformly dispersed beads were swept to the entrance of microchannel, leading to the bead-packing with the geometrical shape of quarter-sphere. This spherical structure induced a focused flow of the filtered plasma, which accomplished a rapid extraction of the plasma from whole blood for rapid clinical testing.

The movement of the plasma over time was recorded as shown in Figure 4. The plasma separation was started within 30 seconds, and the extraction of plasma continued for 110 seconds. The separated plasma filled a microchannel of 35 mm long, where its cross-sectional area was 100 μm x 100 μm. This amount of filtered plasma was large enough to perform a subsequent clinical test on a LOC platform. Compared with the plasma separation by the bead-packed microchannel, the volume of the separated plasma was significantly increased. [3] This enhancement was attributed to the large contact area with the packed beads and the blood. When the beads were packed inside the microchannel, the contact surface between blood and the packed beads was approximately same with the cross-sectional area of microchannel. In case of the hetero-packed beads, however, the blood contacted to a quarter-spherical surface of the packed beads which was much larger than the contact surface of the blood with the bead-packed microchannel.

The developed on-chip blood/plasma separator showed no leakage of blood cells during the plasma separation. Because membrane type of filters are difficult to be integrated with microfluidic networks, the filters usually have the leakage of blood cells. Compared with the self-assembly of the beads previously developed [3], this hetero-bead packing method provides a practical and simple approach for implementing microfilter with LOC devices, achieving the successful filtration of the plasma from whole blood for POC clinical diagnosis.

CONCLUSION

In conclusion, the blood separator based on the hetero-packed beads has been realized by packing different sizes of beads at the inlet of microchannel. Also, the procedure of bead-packing does not require complex structures or procedures for assembling the beads. Thus, the developed bead-packing technique at the inlet of microchannel provides a wide range of adaptability in realizing microfluidic devices integrated with microfilters for blood/plasma separation. Furthermore, this blood/plasma separator achieves rapid separation of plasma from whole blood with enough volume for a subsequent analysis using LOC. Thus, the developed on-chip blood/plasma separator can be applied to numerous POC clinical analyses.

REFERENCES

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