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MICRO MEMBRANE FILTERS FOR PASSIVE PLASMA EXTRACTION FROM WHOLE HUMAN BLOOD USING SILICON NITRIDE-BASED MICROFILTERS AND PLAMA COLLECTION USING AGAROSE GELS

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Abstract

The novelty of this study resides in the fabrication of a passive, operating on capillary force, penetration-flow microfluidic device for plasma separation, based on both silicon nitride combination (SiN-SiO-SiN)-based microfilters and agarose gels, and its characterization for plasma separation from whole human blood. The fabrication processes are compatible with IC process protocols, with merits of mass productions and precise size control. The fabrication process for silicon nitride membrane was reported at Lab Chip [1], and quantification its applications to affinity-based protein separation on the silicon nitride was reported at MicroTAS'07 [2]. Our method differs from that of group Yobas [3] in the specific separation method and materials, and of group Pizziconi [4] in the geometry of the filter, and fluidic components with the structure.

Keywords: Membrane filter; Passive plasma extraction; Silicon nitride complex; agarose gels;

1. Introduction

Plasma separation from the whole human blood, which is accessible easily and representative of complex patient pathologic states, is essential in performing diagnostic tests. Therefore, an efficient sample preparation technique that could be quickly and easily integratable on chip with extracting plasma reproducibly from whole non- or few diluted human blood is very required. Microfabrication technology facilitates the developed miniaturized diagnosis devices to analyze the blood and other body fluids effectively and precisely with minimum sample requirements. By employing the microfluidic devices, it is feasible to separate complex sample like whole blood on chip. There are needs to acquire nano-liter volumes of plasma from a drop of whole blood, while enhancing the microdevice operation times from a few seconds to several minutes using a novel microfluidic design strategy. Among them, a passive filter would be reasonable as a blood plasma separation if the delay time caused by cells clogging was

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shortened. Therefore, we here propose a silicon-based microfilter membrane which was prepared combining depositions of the stress-reduced silicon nitride-silicon oxide-silicon nitride thin films, compatible with IC-based processes, with agraose gel, for the separation speed enhancements and precise fabrication reproducibiltiy.

2. Experimental

The silicon-based microfilter membrane was microfabricated by deposition of silicon nitride-silicon oxide-silicon nitride thin films on silicon wafer by a typical low pressure CVD processes and etching processes with stress-reduced structures for membrane using KOH both wet etch and RIE etch. Plasma separation from the whole human blood, which is accessible easily and representative of complex patient pathologic states, is essential in performing diagnostic tests. Therefore, an efficient sample preparation technique that could be quickly and easily integratable on chip with extracting plasma reproducibly from whole non- or few diluted human blood is very required. The silicon-based microfilter membranes was prepared combining depositions of the stress-reduced. The silicon-based microfilter consists of holes with 1 μm diameter and the membrane with 0.6 μm thickness (Fig. 1, 2).

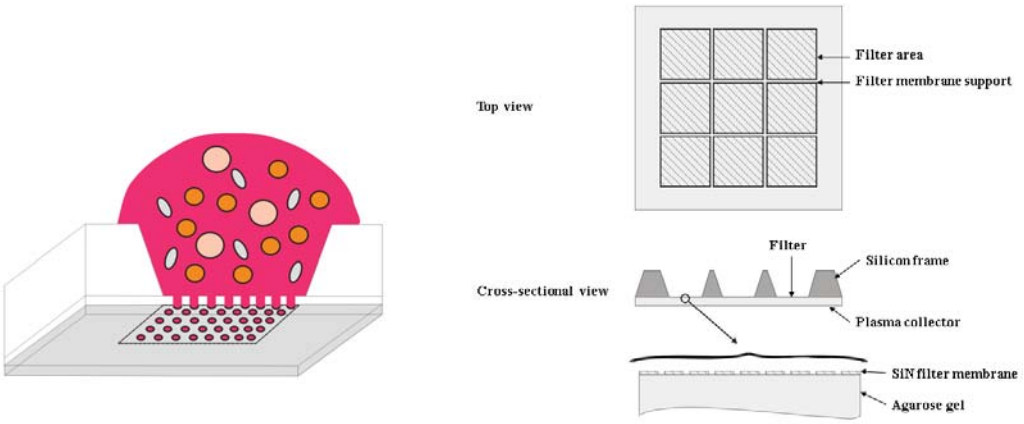


Fig. 1. Schematic diagram showing working principles of silicon membrane filter and its cross-sectional view.

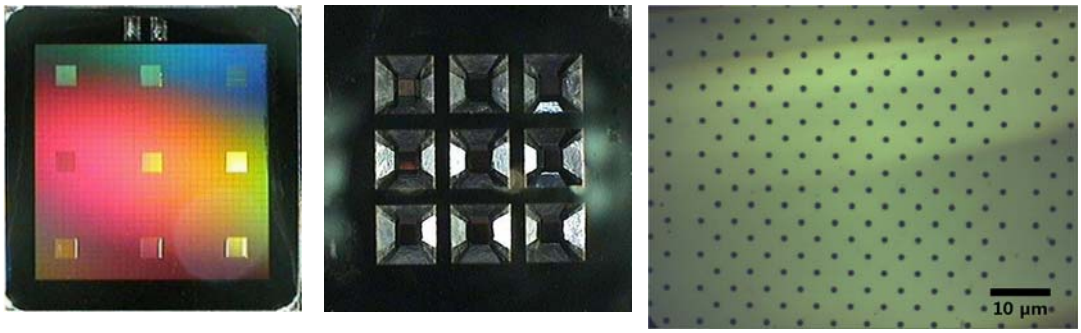


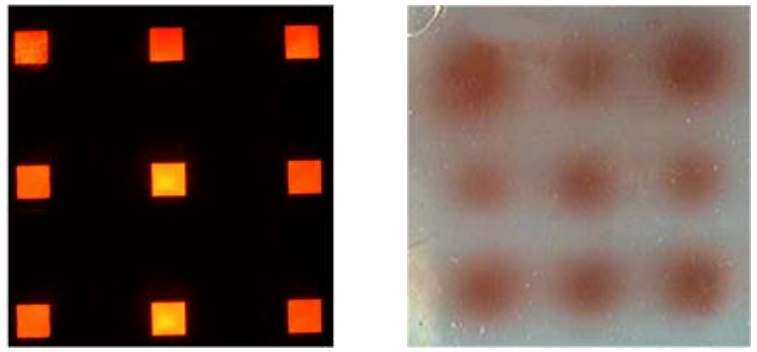
Fig. 2. Photographs of the SiN membrane filter (12x12 mm^2); top membrane part (a), bottom well part (b) and membrane hole with one micrometer diameter (c).

3. Results and Discussions

In order to remove the flow resistance from the surface and holes' end, oxygen plasma treatment for 5 minutes in a plasma equipment was carried out. Resulting wetting angle was nearly zero, and the wetting speed was enhanced to two times comparing before treatment (Fig. 3). By combining agraose gel with the membrane filter, the separation speed which can be delayed by clogging caused by accumulating cells and fluidic stopping caused by structural sudden expansion in holes' end, is increased. First of all, we tested the permeability of agarose gel to particle separation with red dye solutions as shown in Figure 4. After two minutes, the red dyes were permeated to the agarose gel with large amounts. Based on the results, we can investigate separation ability of plasma from the l0 μL whole human blood (Fig. 5). We can find out separation ability of plasma from the l0 μL whole human blood within twenty minutes (Fig. 5(a)), the separation speeds were so enhanced with help of agarose gel as shown in Fig. 5(b)). Furthermore, the agarose gel containing separated plasma can be possibly used a portable plasma carriers. The device is easily fabricated on silicon with IC-standard process compatibility and can be integrated with electronic control.

(a) (b)

Fig. 3. Numerical simulation on penetrating of plasma through holes at the wetting angles of 0° (a) and 70° (b).

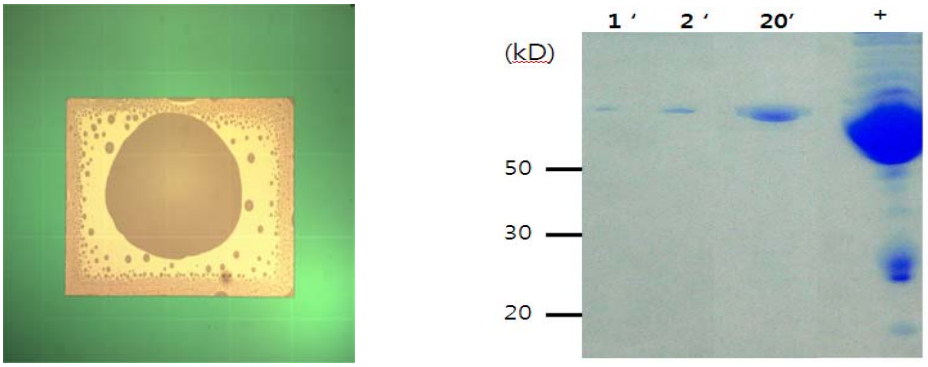


(a) (b)

Fig. 4. Photograph of SiN membrane filter containing red dye solutions (a), and patterns on the Agarose gel attached to the silicon membrane filter for plasma collection after 2 minutes.

The selective passing through of plasma using the fabricated micro-hole plate filter was confirmed by the identification of plasma proteins. 1 % (w/v) of agarose gel block was laid on the back of the filter plate before the application of blood. The gel block was harvested after different time intervals and boiled with an SDS-PAGE

(sodium dodecyl sulfate polyacrylamide gel electrophoresis) loading buffer for analysis [5]. Fig. 5(b) shows the SDS-PAGE analysis of the proteins absorbed in the gel block. The proteins were stained with Coomassie Brilliant Blue R-250 (Sigma) for visualization. Serum albumin protein, the most abundant one among the plasma proteins, was clearly visualized, and it was evident that the gel block absorbed the plasma proteins through the micro-hole filter. This silicon nitride membrane filter plus agarose gel collections can applicable to the nanofluidic analysis or human plasma separation systems.



(a) (b)

Fig. 5. Photograph showing plasma isolation through the silicon membrane filter after 20 min (a), and SDS-PAGE analysis of proteins absorbed in gel block. Agarose gel block was taken after different time intervals. The gel was mixed with equal volume of loading buffer, boiled for melting, and loaded along with 1 μl of human plasma (lane +) (b).

Acknowledgements

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