VIRUS DETECTION BY ON-CHIP HYDROXYAPATITE CHROMATOGRAPHY

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ABSTRACT

We have succeeded in isolating virus from the particle laden solution like body fluid. Because many of virus infectious diseases occur in the developing region, compact and low-cost device for virus detection from the patients' body fluid is required. We proposed a microfluidic chip for virus isolation by hydroxyapatite chromatography. This microfluidic chip utilized a column and a pair of switching valves. In the upstream and downstream part of the column, cylindrical micropillars are placed to hold the hydroxyapatite particles in the column. Chromatography was carried out in this column and the viruses were successfully isolated by buffer displacement.

KEYWORDS

Virus detection, Hydroxyapatite, Chromatography.

INTRODUCTION

Many of virus infectious diseases occur in the developing countries in Asia and sub-Saharan Africa, where it is very difficult to diagnose virus infection because of poor medical technology and poverty. In order to diagnose virus infection accurately at an early stage, it is indispensable to isolate virus from body fluid and detect in situ.

Tiselius et al. first introduced protein purification by hydroxyapatite liquid chromatography in 1956 [1]. Hydroxyapatite is a very complex crystalline compound with the sum-formula $Ca_{10}(PO_4)(OH)_2$ and a variety of substances adsorb to its surface. Many researchers have reported that the interaction between hydroxyapatite and electrically-charged adsorbate is due to ion exchange or static attraction [2]. Because hydroxyapatite has specific isolation property, it has been used extensively as a matrix for the purification and fractionation of an array of biochemical substances, including enzymes, nucleic acids, hormones, and viruses [3]. However, in the developing region, where many of virus infectious disease occur, it is difficult to carry out hydroxyapatite chromatography, because the conventional chromatography systems need large and expensive equipments [4].

In this paper, we proposed a novel microfluidic chip for virus isolation and detection from the particle laden solution like body fluid by using hydroxyapatite chromatography. On-chip virus detection will enable to diagnose virus infectious disease in the developing region.

THEORY

Figure 1 shows the concept of the microfluidic chip for virus isolation. This microfluidic chip has a column for hydroxyapatite chromatography and a pair of switching valves. In the upstream and downstream part of the column, 50-μm diameter cylindrical micropillars are placed at 20-μm intervals to hold the hydroxyapatite particles in the column. Hydroxyapatite particles are introduced into the column through inlet 1. The sample and the elution buffers are introduced into the column through inlet 2. As shown in figure 1, the sample is introduced in the column and both viruses and impurities in the sample are adsorbed on the hydroxyapatite

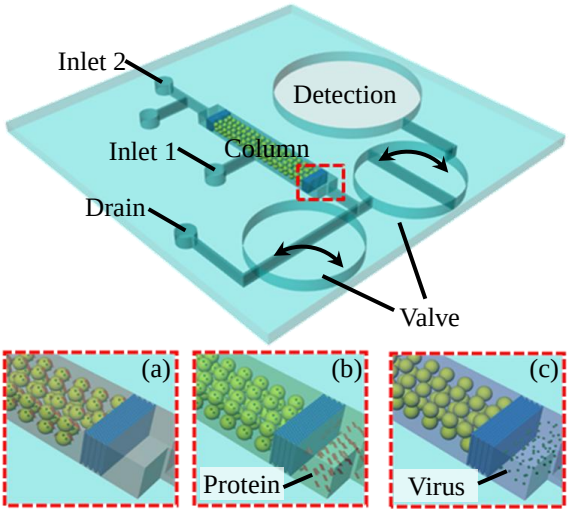

Figure 1: Concept of the microfluidic chip for on-chip hydroxyapatite chromatography.
(a): Sample adsorption on hydroxyapatite.
(b): Elution of the impurities such as proteins.
(c): Elution of the viruses.

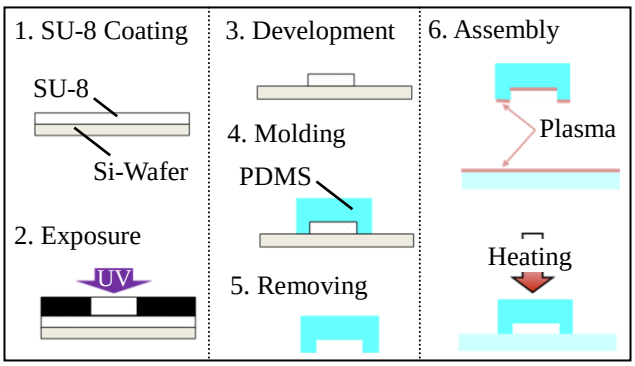

Figure 2: Fabrication process of the proposed microfluidic chip. The microfluidic chip consists of a PDMS microchannel and a PDMS substrate.

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particles. Then, an elution buffer is introduced in the column to elute the impurities such as proteins. Finally, another elution buffer is introduced in the column to elute the viruses.

The impurities and the viruses are guided towards the drain port and the detection port respectively by a pair of switching valves.

EXPERIMENTAL

The proposed microfluidic chip consists of a PDMS (polydimethylsiloxane) microchannel and a PDMS substrate. Figure 2 shows the fabrication process of the PDMS microchannel and assembly method of the microchannel and the substrate. The PDMS microchannel was produced by replica molding using a master mold fabricated by photolithography. The negative-type photoresist (SU-8 3050, Kayaku Microchem, Co., Ltd.) was spin coated on the silicon substrate. After prebaking, ultraviolet light was exposed through a photomask to produce a microchannel pattern using a mask aligner. After exposure, the substrate was developed and rinsed. Then the PDMS was molded by patterned substrate. Finally, the PDMS microchannel and the substrate were bonded by air plasma. The height of channel was 100 μm.

Figure 3 shows the fabrication process of a pair of switching valves. Two holes 8 millimeters in diameter were punched in the microfluidic chip using a biopsy punch. The PDMS residue parts in the biopsy punch after punched out were reinserted in the holes in the microfluidic chip as a pair of switching valves. The valves are opened or closed by rotation of the reinserted PDMS residue parts. Our switching valve has a great advantage that it is very easy to fabricate. In order to decrease the risk of leakage from the side of the valve, parylene thin film with the thickness of about 800 nm was coated on the side. Pressure test of the valves uncoated and coated with parylene was conducted to demonstrate the effect of parylene thin film.

Ceramic hydroxyapatite particles (CHT, particle diameter: 40 μm, Bio-rad Laboratories) were used as the adsorbent material of chromatography. First, the left valve was opened and the right valve was closed. CHT particles were introduced into the column, and then NDV (Newcastle disease virus) suspension containing 5 % FBS proteins was introduced into the column. In the column NDVs and FBS proteins were adsorbed to CHT particles. Then 500 mM potassium chloride solution was introduced into the column to elute FBS proteins. Next, the left valve was closed and the right valve was opened. Then 1 M phosphate buffer (pH: 7.0) were introduced into the column to elute NDVs. The flow rate of each fluid was 1.0 mL/hour. The phosphate buffer was collected at the detection port after passing through the column, and presence or absence of NDVs was diagnosed by using hemagglutination(HA) reaction [5].

RESULTS AND DISCUSSION

Figure 4(a) shows the fabricated switching valve. From figure 4(b), it can be seen that the proposed valves enabled to switch microchannels. First, the right valve was closed and the left one was opened and blue buffer was guided toward the left channel. Next, the left valve was closed and the right one was opened and red buffer was guided toward the right channel.

Figure 5 shows the results of the pressure test of the

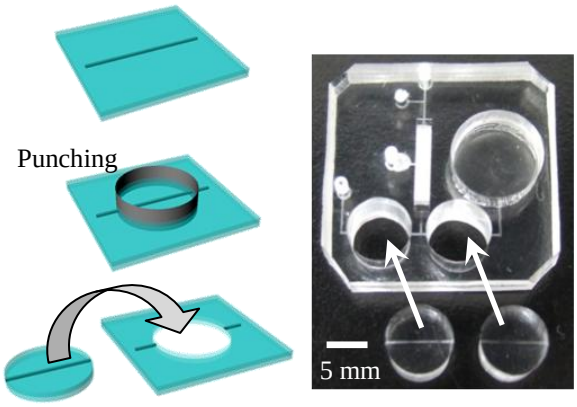

Figure 3: Fabrication process of a pair of switching valves. The PDMS residue parts in the biopsy punch after punched out were reinserted in the holes in the microfluidic chip as a pair of switching valves.

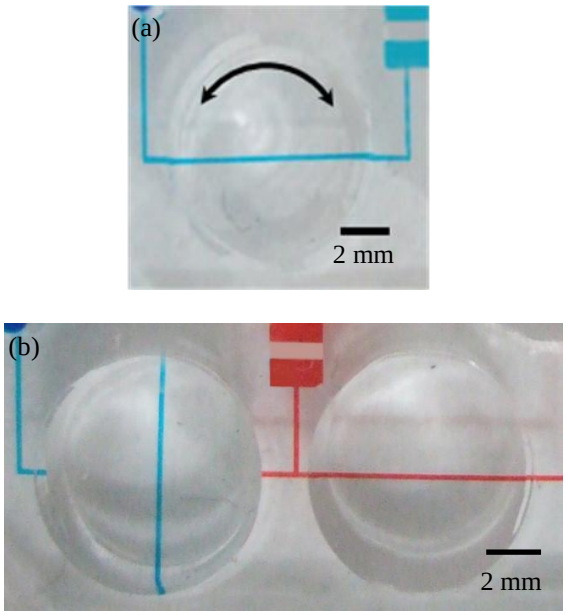

Figure 4: A pair of switching valves. The valves are opened or closed by rotation of the reinserted PDMS residue parts.

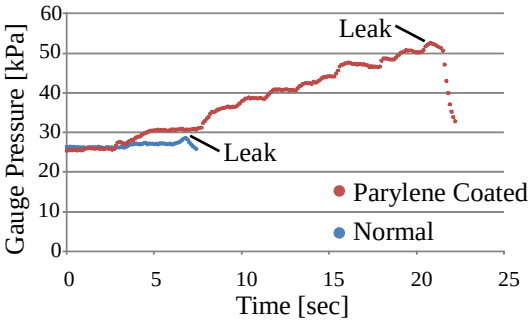

Figure 5: The results of the pressure test of the valve. The leak pressure of the valves uncoated and coated with parylene were 28.6 kPa and 52.6 kPa, respectively.

valve. As shown in this data, the leak pressure of the valves uncoated and coated with parylene were 28.6 kPa and 52.6 kPa, respectively. These results mean that coating the side of the valve with parylene thin film was effective to decrease the risk of leakage.

Figure 6(a) shows the fabricated microfluidic chip. As shown in figure 6(b), packed CHT particles were held in the column by the cylindrical micropillars placed in the upstream and downstream part of the column.

Figure 7 shows the results of HA reaction after hydroxyapatite chromatography using the proposed microfluidic chip. Figure 7(a) and 7(b) are negative control (PBS) and positive control (NDV suspension), respectively. Positive result forms a uniform reddish color across the well because each of the agglutinating molecule of virus attaches to multiple red blood cells and they form a bridged structure. Meanwhile, negative result appears as a red dot in the center of the round-bottomed plate. Figure 7(c) and 7(d) show the results of the HA reaction of NDV suspension containing 5 % FBS and 1 M phosphate buffer eluate, respectively. Figure 7(c) gives a negative result even though the suspension contains NDV. It is because the suspension contains not only NDVs but also FBS proteins. Figure 7(d) gives a positive result, which means NDVs were dissolved out with 1 M phosphate buffer and collected. In summary, these results show that our microfluidic chip can improve the sensitivity for detection of NDV by removing FBS proteins by hydroxyapatite chromatography.

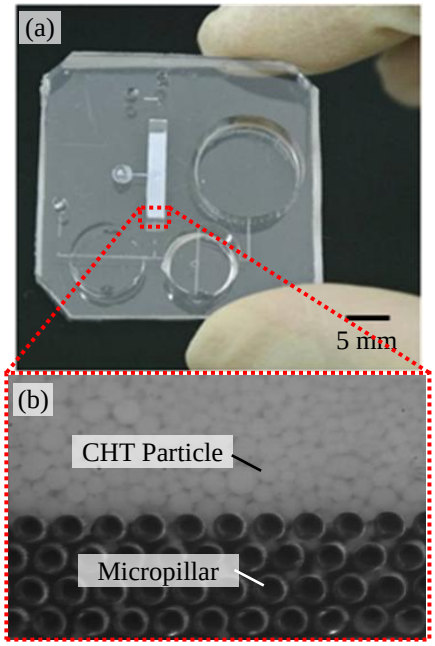

Figure 6: The fabricated microfluidic chip. CHT particles were held in the column by the cylindrical micropillars placed in the upstream and downstream part of the column.

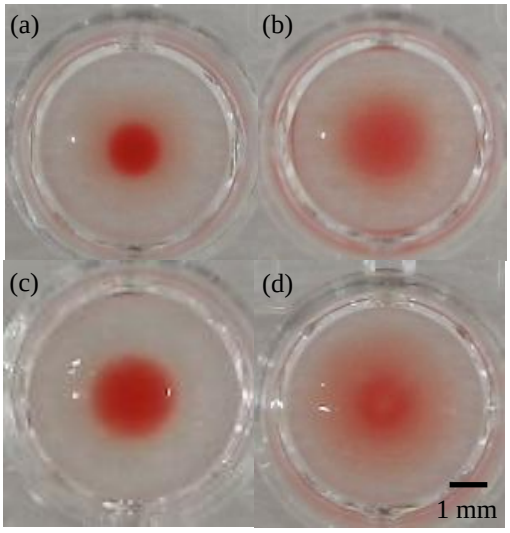

Figure 7: Results of hemagglutination (HA) reaction. (a) and (b) are negative control and positive control, respectively. (c) is NDV suspension containing 5 % FBS, (d) is 1 M phosphate buffer eluate.

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