Sensors and Actuators B 201 (2014) 185–190

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Virus purification and enrichment by hydroxyapatite chromatography on a chip

Miyako Niimi [a], Taisuke Masuda [b,*], Kunihiro Kaihatsu [c], Nobuo Kato [c], Shota Nakamura [d], Takaaki Nakaya [e], Fumihito Arai [b]

[a] Department of Mechanical Science and Engineering, Graduate School of Engineering, Nagoya University, 1 Furo-cho, Chikusa-ku, Nagoya 464-8603, Japan
[b] Department of Micro-Nano Systems Engineering, Graduate School of Engineering, Nagoya University, 1 Furo-cho, Chikusa-ku, Nagoya 464-8603, Japan
[c] The Institute of Scientific and Industrial Research, Osaka University, 8-1 Mihogaoka, Ibaraki, Osaka 567-0047, Japan
[d] Department of Infection Metagenomics, Osaka University, 3-1 Yamadaoka, Suita, Osaka 565-0871, Japan
[e] Department of Infectious Diseases, Kyoto Prefectural University of Medicine, 465 Kajii-cho, Kawaramachi-Hirokoji, Kamigyo-ku, Kyoto 602-8566, Japan

ARTICLE INFO

Article history:
Received 15 January 2014
Received in revised form 4 April 2014
Accepted 7 April 2014
Available online 6 May 2014

Keywords:
Microfluidic chip
Virus purification
Virus enrichment
Hydroxyapatite chromatography
Infectious disease

ABSTRACT

The spread of infectious diseases has become a global health concern. In order to diagnose infectious diseases quickly and accurately, next-generation DNA sequencing techniques for genetic analysis of infectious viruses have been developed rapidly. However, it takes a very long time to pretreat clinical samples for genetic analysis using next-generation sequencers. We have therefore developed a microfluidic chromatography chip that can purify and enrich viruses in a sample using hydroxyapatite particles packed in a micro-column. We demonstrated the purification of virus from a mixture of virus and FBS protein, and enrichment of the virus using this novel microfluidic chip.

1. Introduction

The spread of infectious diseases has become a global health concern. In 2002, an outbreak of atypical pneumonia, referred to as severe acute respiratory syndrome (SARS) and first identified in Guangdong Province, China, occurred and spread to several countries [1,2]. In total, 8273 cases and 775 deaths attributed to SARS were reported in multiple countries according to the World Health Organization (WHO). In April 2009, the outbreak of a novel influenza A H1N1 virus in Mexico spread globally and developed into the first human influenza pandemic in 40 years [3,4]. To diagnose infectious diseases quickly and accurately, DNA sequencers for genetic analysis of infectious viruses have been developed rapidly. Newly developed "next-generation" sequencing technologies for massively parallel DNA sequencing are in fairly widespread use at present [5,6]. However, in order to diagnose infectious diseases using DNA sequences, the virus in the clinical sample must be purified and enriched in concentration before DNA sequencing [7]. These pretreatment processes are labor-intensive, cumbersome and time consuming. There is therefore need to speed up the pretreatment of clinical samples before DNA sequencing to complement the throughput of DNA sequencers. We here describe a novel microfluidic chip for purifying and enriching viruses. The microfluidic chip has many advantages; it requires only a small work place, a small sample volume, an enclosed region, and is comparatively low cost.

Tiselius et al. [8] first described protein purification by hydroxyapatite liquid chromatography in 1956. Since then, hydroxyapatite chromatography has been used extensively for the purification and fractionation of various biochemical substances such as protein [9–11], bacteriophages [12], and viruses [13,14]. Hydroxyapatite ($Ca_{10}(PO_4)(OH)_2$) is a very complex crystalline compound to which a variety of substances adsorb. 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 [15]. Many researchers have reported that the interaction between hydroxyapatite and an electrically charged adsorbate is due to ion exchange or static attraction [16,17].

We here describe a microfluidic chip developed for hydroxyapatite chromatography. Hydroxyapatite particles were packed in a micro-column and on-chip chromatography has been conducted to purify and enrich Newcastle disease virus.

* Corresponding author. Tel.: +81 52 789 5026; fax: +81 52 789 5027.
E-mail address: masuda@mech.nagoya-u.ac.jp (T. Masuda).

http://dx.doi.org/10.1016/j.snb.2014.04.011
0925-4005/

2. Materials and methods

2.1. Design of and theory behind the microfluidic chip

Fig. 1(a) shows the concept of the microfluidic chip for virus purification and enrichment by on-chip hydroxyapatite chromatography. Fig. 1(b) shows the microfluidic chip fabricated using photolithography. The hydroxyapatite used was Ceramic Hydroxyapatite Type II Support (CHT; BIO-RAD, Hercules, CA, USA), with a particle diameter of 40 μm. The microfluidic chip has a column for hydroxyapatite chromatography and a pair of switching valves. Upstream and downstream of the column, 50-μm-diameter cylindrical micropillars placed at 20-μm intervals hold the hydroxyapatite particles in the column, as shown in Fig. 1(b). Hydroxyapatite particles are introduced into the column through inlet 1. The sample and the elution buffers are introduced into the column through inlet 2.

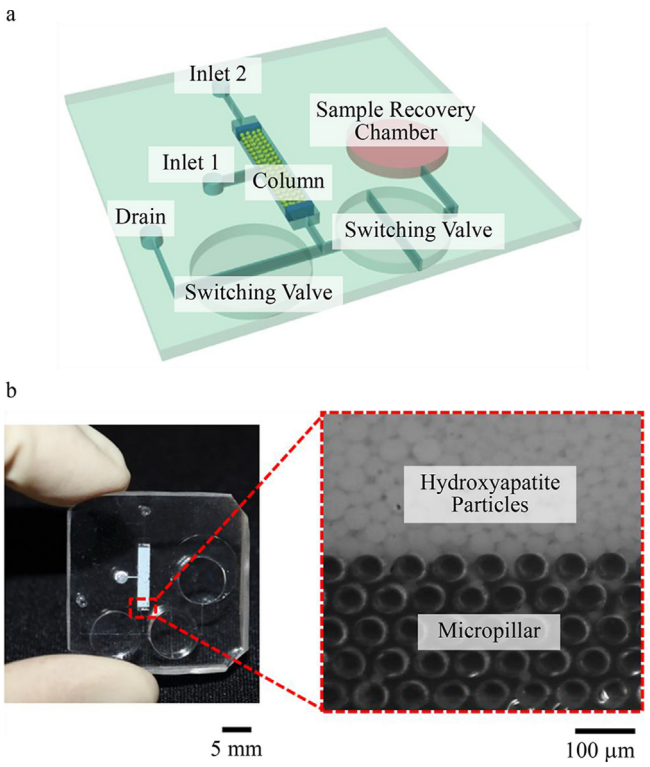

Fig. 1. (a) Concept of the microfluidic chip for purification and enrichment of virus using hydroxyapatite chromatography. The microfluidic chip has a column for hydroxyapatite chromatography and a pair of switching valves. Hydroxyapatite particles are introduced into the column through inlet 1. The sample is introduced into the column through inlet 2 and both viruses and impurities in the sample are adsorbed onto the surface of hydroxyapatite particles. Then, an elution buffer is introduced into the column to elute the impurities such as proteins. Then, another elution buffer is introduced into the column to elute the viruses.
(b) The fabricated microfluidic chip. Upstream and downstream of the column, 50-μm-diameter cylindrical micropillars placed at 20-μm intervals hold the hydroxyapatite particles in the column. The diameter of the hydroxyapatite particles is 40 μm.

2.2. Fabrication of the microfluidic chip

The microfluidic chip consists of a polydimethylsiloxane (PDMS) microchannel and a PDMS substrate. Fig. 2 shows the processes used to fabricate a PDMS (Silpot 184, Toray-Dow Corning Co., Tokyo, Japan) microchannel and bond it to a PDMS substrate. A PDMS microchannel was produced by replica molding using a master mold fabricated by photolithography [18]. A negative-type photoresist (SU-8 3050, Kayaku MicroChem Co. Ltd., Tokyo, Japan) was spin-coated on a silicon substrate. Spin-coating condition was 1000 rpm for 30 s. After prebaking at 95 °C for 3 h, ultraviolet light was irradiated through a photomask to produce a microchannel pattern using a mask aligner (MJB-3, Suss Microtec, Garching, Germany). After post-exposure baking at 65 °C for 30 s and at 95 °C for 60 s, the substrate was then developed in PM thinner for 8 min and rinsed in ethanol. The channel was 100 μm high. Unpolymerized PDMS (resin:catalyst = 10:1) was poured into the mold and cured in an oven at 90 °C for 20 min. The replica was then peeled from the master and the PDMS microchannel was ultrasonically rinsed in ethanol. The PDMS microchannel with outlet, inlet, drain and sample recovery chamber holes and the PDMS substrate were then treated with oxygen plasma (CUTE-MP, UVOTECH Systems, Concord, CA, USA) to increase its adhesion to the PDMS substrate. After assembling the PDMS microchannel and the PDMS substrate, it was heated in oven at 145 °C for 20 min. Then silicone microtubes with an inside diameter of 1 mm and an outside diameter of 2 mm were assembled into the holes in the PDMS layer to connect the microchannel.

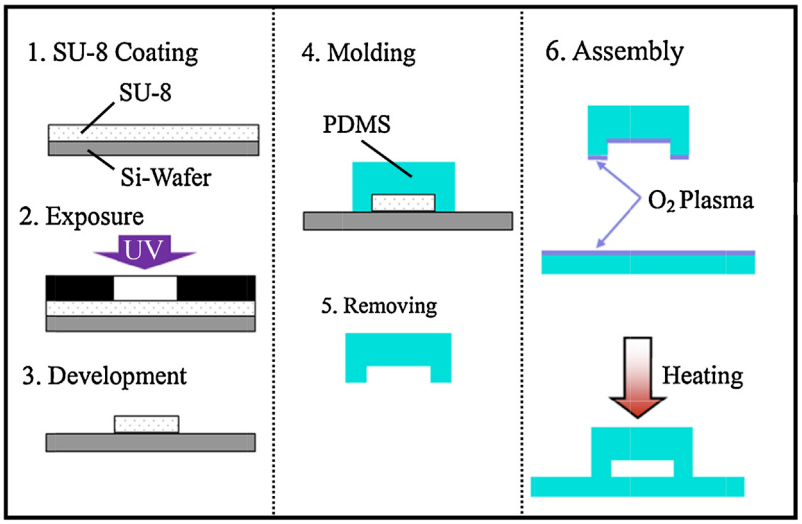

Fig. 2. Fabrication process of the proposed microfluidic chip. The chip consists of a PDMS microchannel and a PDMS substrate. The PDMS microchannel was produced by replica molding using a master mold fabricated by photolithography.