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Research paper

A disposable lab-on-a-chip platform for highly efficient RNA isolation

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ABSTRACT

Despite recent advances in lab-on-a-chip (LOC) technology, RNA-based miniaturized diagnostic platforms have been falling behind due to a lack of on-chip RNA isolation techniques. However, RNA analysis plays an important role in gene expression determination and pathogen detection. To meet the increasing need for the analysis of RNA with point-of-care (POC) testing, a reliable LOC platform for RNA isolation needs to be developed. Here, we present a method of RNA isolation using a LOC platform that provides high-quality RNA from a variety of biological samples. By taking advantage of pH-dependent reversible dimethyl adipimidate (DMA) binding to RNA, we were able to perform RNA isolation on a disposable LOC platform within 30 min without the need for chaotropic salts or solvent. Using this LOC platform, gene expression analyses were successfully carried out with RNA isolated from different cell lines (HEK293, Caco-2, MCF-7, and T24) by subsequent real-time reverse transcription PCR. In addition, we validated the utility of this LOC platform with viral RNA isolated from Dengue viruses (DENV-1, −2, −4). We demonstrated a high recovery rate and rapid processing with minimal contamination. Therefore, this novel method could be an ideal RNA isolation technique in gene expression research and for the diagnosis of infectious diseases caused by RNA viruses in POC testing.

1. Introduction

The development of miniaturized diagnostic systems that take advantage of lab-on-a-chip (LOC) techniques is becoming increasingly popular in molecular diagnostics [1]. One of the most active applications for miniaturized diagnostic systems is nucleic acid amplification testing (NAAT) due to its high sensitivity and specificity [2], which are critical for rapid and effective disease monitoring and successful identification of appropriate treatment, especially for acute infectious diseases such as malaria and Dengue fever [3]. The use of LOC platforms for NAAT offers many benefits over conventional laboratory-based techniques [4]. For example, point-of-care (POC) testing based on NAAT and using a LOC platform can offer rapid detection of pathogens (bacteria, viruses) for the diagnosis of infectious diseases in low-resource environments [5,6]. POC testing permits early detection without the need for the bulky instruments typically used in centralized laboratory-based testing. Hence, it can be a powerful tool for the effective control of infectious diseases by addressing access barriers to health care services experienced by patients in developing countries. Indeed, considerable effort has been expended to develop NAAT on LOC platforms for the identification of infections such as HIV to replace current screening systems in developing countries where insufficient health care facilities hinder early diagnosis and efficient treatment [7]. Additionally, NAAT on a LOC platform can be a low-cost and efficient tool for POC genetic analysis of diseases such as cancer in resource-limited settings [8].

Although a number of NAAT platforms have been developed, most are based on DNA. This is mainly due to the nature of RNA, including its lower structural stability compared with DNA, which results in a short half-life [9]. In addition, RNA is more vulnerable to degradation during cell lysis due to RNases ubiquitously present both in the cell lysate itself and the environment, which complicates RNA analysis and means that special precautions are required

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to avoid sample contamination when dealing with RNA samples [10]. Despite the challenges associated with sample handling, there is an increasing need for LOC platforms for RNA analysis because RNA investigation plays crucial roles in the identification of genetic diseases such as cancer, quantitative studies of gene expression, and detection of pathogens. Although numerous LOC techniques involved in RNA studies have been reported, most techniques are focused on a detection method that requires off-chip RNA isolation [11–17], with RNA isolation in the LOC platform remaining relatively unexplored. Lack of reliable LOC-based RNA isolation techniques has limited the development of an integrated analytical system for RNA analysis [3]. Therefore, a reliable LOC-based RNA isolation technique that facilitates RNA handling without compromising RNA quality is essential for the development of a practical RNA analysis platform for POC testing.

Many conventional RNA isolation methods have been used in molecular diagnostics, such as phenol-chloroform–based extraction [18], Oligo-dT–based affinity chromatography [19,20], and silica-based purification [10]. The first two conventional isolation methods have been widely used due to their high yield and rapid stabilization of RNA. However, these methods are restricted to the laboratory-based diagnosis level because they typically rely on laborious hands-on processes and centrifugation steps. In addition, in the case of viral RNA isolation, a low concentration of viral RNA extracted in a large volume complicates pathogen detection due to sample dilution during conventional sample processing. Moreover, carryover of contaminants such as phenol and chaotropic reagents may inhibit enzymatic reactions during PCR or other downstream analysis processes, making it difficult to fully realize an automated on-chip assay. In this respect, LOC techniques can solve the current issues associated with conventional isolation methods and address the needs for RNA analysis in POC testing.

To overcome the challenges of RNA isolation, there have been several attempts to perform it on microfluidic LOC platforms. Typically, total RNA is isolated from various biosamples and captured onto a solid-phase substrate or matrix under specific buffer conditions. After impurity removal, elution buffer flows through the matrix, releasing the RNA molecule by changing the binding affinity. One of the most common methods is silica-based solid-phase extraction (SPE). In the presence of chaotropic salt, RNA is bound to silica substrate as the lysates containing RNA are introduced into a microfluidic channel while other residual contaminants including proteins are removed during a washing step. This method has been used in a number of investigations [21–24] because the purification mechanism is similar to that of DNA isolation. However, typical silica-based isolation methods use alcohol to enhance nucleic acid binding, as in silica column-based purification, but the alcohol is likely to remain in the collected eluent despite thorough washing. Alternatively, mRNA isolation has been performed on a LOC platform by taking advantage of oligo(dT) affinity purification techniques that rely on the selective binding of oligo(dT) to the poly (A) tail of mRNA [25,26]. This protocol originated from an oligo(dT)-conjugated resin column for mRNA enrichment and a modified protocol has been implemented in microchips. This technique can achieve reproducible and highly efficient mRNA isolation but its use is limited to the analysis of the gene expression of eukaryotic cells. To circumvent the problems associated with the use of chaotropic agents, a LOC platform for RNA isolation in an aqueous solution has been devised [27,28]. For example, Hagan et al. [29] demonstrated RNA isolation from cancer cells using pH-dependent chitosan-RNA binding in a microfluidic device. Silica micro-bead surfaces were coated with chitosan for reversible RNA binding, with the bound RNA molecules released by simply adjusting the pH level of the buffer above the pKa of the amino group during the elution step. This method achieved a higher RNA recovery rate (71%) than the silica-binding method while avoiding the use of chaotropic salts or alcohol during the isolation process. However, a long processing time is required for the preparation of chitosan-coated silica and its integration into the microchip prior to isolation.

Most of these LOC-based RNA isolation techniques rely on an expensive fabrication process and involve complicated preparation procedures using microparticles [25,30–35] or porous structures [24,27,36] to enhance isolation efficiency. As an alternative, our group recently demonstrated a novel nonchaotropic DNA isolation technique involving dimethyl adipimidate (DMA) binding on microfluidic channel surfaces [37,38]. DMA was able to capture DNA onto either amine-modified silicon or polymer microfluidic channel surfaces, forming an amidine bond at pH 8–10 and releasing DNA molecules above pH 10. Neither chaotropic salt nor alcohol is required during the process, benefiting the downstream analysis procedure. Moreover, this method eliminated the need for a complicated fabrication process by using cost-effective rapid prototyping. It has proven its utility in DNA isolation from eukaryotic and prokaryotic cells as well as body fluids.

In this study, we report an RNA isolation method using a LOC platform that enables rapid sample preparation for subsequent gene expression analysis and pathogen detection. The platform exploits reversible DMA binding to capture and release RNA via adjustment of the buffer pH level in a microfluidic environment. The disposable LOC platform was fabricated with hydrophilic polyester film that allows for cost-effective manufacturing and rapid prototyping. The RNA isolated from the assay was suitable for reverse transcription and PCR detection with high purity and integrity and the results are comparable to that of a commercial kit but with a simpler procedure. We validated the utility of the platform in gene expression level analysis by isolating RNA from various human cancer cell lines. Additionally, identification of Dengue virus serotypes was successfully performed using the purified RNA, which suggests that this disposable platform has great potential to act as a diagnostic tool for RNA-based pathogen detection for POC application.

2. Materials and methods

2.1. Chip design and fabrication

The prototype of the on-chip RNA extraction device was designed using CorelDRAW software and constructed from Poly (methyl methacrylate) (PMMA) sheets by a laser cutting process (Universal Laser Systems, Scottsdale, AZ, USA) for rapid prototyping and low-cost manufacturing, as described previously [39]. The device consists of a 6×6 array for RNA binding connected to a single inlet and outlet for sample injection. A contraction-expansion array (CEA) was used as a binding reservoir for rapid mixing at high-volume flow rates during sample loading and washing. Microfluidic structures were patterned on a double-sided adhesive layer (3M 9475LE, USA). Clear hydrophilic polyester film (3M, USA) was used for the top and bottom layers. Through-holes were drilled on the top layer as inlet and outlet ports. Prior to assembly, the surfaces of both top and bottom layers were activated in a Femto oxygen plasma treatment system (80 W, 5–10 min, 2 sccm; Diener Electronic, Ebhausen, Germany).

After plasma treatment, protective layers attached to the double-sided adhesive layer were immediately removed and the layer was sandwiched between the top and bottom layers. Then, inlet and outlet connectors were aligned and attached to through-holes on the top layer and Tygon tubing (AAC02548; Cole-Parmer, Vernon Hills, IL) was inserted into the holes. Epoxy glue was then applied to prevent leaks around the port. After assembly, microfluidic reservoirs were functionalized in 2% (3-Aminopropyl)