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Versatile microfluidic complement fixation test for disease biomarker detection

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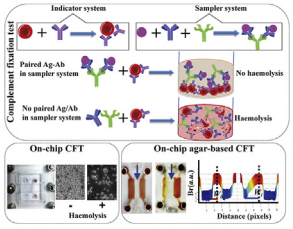
HIGHLIGHTS

- Microfluidic system was developed to set up sampler and indicator system of complement fixation test (CFT).
- Two types of on-chip CFT were demonstrated for biomarker detection.
- On-chip CFT exempts from protein immobilization, blocking, washing steps and enzyme/fluorescein conjugates.

GRAPHICAL ABSTRACT



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ABSTRACT

The complement fixation test (CFT) is a serological test that can be used to detect the presence of specific antibodies or antigens to diagnose infections, particularly diseases caused by microbes that are not easily detected by standard culture methods. We report here, for the first time, a poly(dimethylsiloxane) (PDMS)/glass slide hybrid microfluidic device that was used to manipulate the solution compartment and communication within the microchannel to establish sampler and indicator systems of CFT. Two types of on-chip CFT, solution-based and solid phase agar-based assays, were successfully demonstrated for biomarker carcinoembryonic antigen (CEA) and recombinant avian influenza A (rH7N9) virus protein detection. In addition, the feasibility of the on-chip CFT in assaying real biopsy was successfully demonstrated by specifically detecting rH7N9 and CEA in human serum. The results demonstrated that the miniaturized assay format significantly reduced the assay time and sample consumption. Exemption from protein immobilization, blocking, complicated washing steps and expensive enzyme/fluorescein conjugates highlights the merits of on-chip CFT over ELISA. Most attractively, the on-chip agar-based CFT results can be imaged and analysed by smartphone, strengthening its point-of-care application potential. We anticipate that the on-chip CFT reported herein will be a useful supplemental or back-up tool for on-chip immunoassays such as ELISA for disease diagnosis and food inspection.

1. Introduction

Millions of people around the world face the risk of infectious diseases. The recent outbreak of Ebola virus disease, avian influenza A and cholera has threatened various populations in undeveloped regions. Portable, easy-of-operation and low-cost disposable

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diagnosis tools are extremely important for on-site epidemic supervision and early diagnosis of those diseases and infections [1–3]. In clinical practise and epidemiology, serological techniques play a critical role in various aspects of infection detection, vaccine development, and evaluation [4,5]. Serology refers to the diagnostic identification of antibodies (Abs) in plasma serum and other body fluids [6]. The complement fixation test (CFT) is a classical serological method for the detection of Abs against infectious pathogens and has proven itself as a standard diagnostic method in many medical laboratories [6–9]. The key feature involved in CFT is the complement system, a component of the innate immune system that is characterized by serum proteins that react with antigen–antibody (Ag-Ab) complexes. In the classical activation pathway, the complement cascade (C1 to C9) is initiated by complexes of antigens and IgG1, IgG2, IgG3, and IgM antibodies. Since neither Ab nor Ag by itself triggers the activation cascade of complement, CFT is specifically suitable for detecting Ag-Ab complexes. Activation of the complement system on the cell surface results in the formation of membrane attack complex (MAC) leading to the destruction of the cell (Scheme 1D). In principle, there are two systems that participate in CFT (Scheme 1). One is a sampler system that includes the unknown sample to be tested, probe Ab/Ag and complement. The other one is an indicator system (haemolytic system), which contains Ab-coated erythrocytes. If a target molecule exists in the sampler system, the recognition and binding of Ag and Ab leads to the formation of Ag-Ab complexes that will fix/consume the complement (Scheme 1B). According to complement activation theory, free Ag or Ab (Ag or Ab alone) does not react with complement. Therefore complement will be conserved when there is no paired Ag and Ab to form Ag-Ab complexes in the sampler system. The conserved complement will react with the Ab-coated erythrocytes in the indicator system and lead to the lysis of the erythrocytes (Scheme 1C). Thus the occurrence of haemolysis is dependent upon the non-activated complement in the sampler system. Conversely, the lack of haemolytic phenomena indicates no complement is reserved to lyse Ab-coated erythrocytes, thus the existence of target molecule. Based on the principle illustrated in Scheme 1, it is equally possible to test for the presence of Ag and Ab by CFT.

The ability of CFT in detecting IgG and IgM Abs has allowed its wide use in screening tests for acute primary infections and therapy successes [9]. CFT is also an important tool for monitoring the status of a person with a known autoimmune disease. In this case, the Ag-specific complement activity can be a good way to measure the efficacy of a certain treatment. In addition, CFT is useful in veterinary practices [10]. Compared with other serological tests such as enzyme-linked immuno-sorbent assay (ELISA) and immunofluorescence (IF), although with an intermediate sensitivity, the merits of CFT over other assays are that costly conjugates are not needed, ease of reading and interpreting results, high specificity, and good reproducibility [7,8,11–13]. Specifically, no protein immobilization, washing and blocking steps were involved in CFT. Protein immobilization in heterogeneous ELISA sandwich format requires that an Ag possess two or more epitopes; therefore, CFT is appropriate for the analysis of small molecules, such as toxins and short peptides.

There is an incredible innovation in the filed of point-of-care detection, which has been propelled by tremendous processes in Micro-Electro-Mechanical Systems (MEMS), micro-total analysis systems and microfluidics [14–17]. Immunoassay has been demonstrated in microarrays and microfluidic platforms for protein detection [18–20], revitalizing this old assay towards low-cost, high throughput point-of-care applications. Unfortunately, in the progress of miniaturization of detection systems, CFT has been largely overlooked. Owing to the feasibility of microfluidics to manipulate the solution compartment and communication within the microchannel, the sampler system and indicator systems of CFT can be set up in a microfluidic platform. It is rationally anticipated that an on-chip CFT would inherit the merit of fast assays and low sample consumption from a microfluidic platform. In addition, the compartmentalization reaction-chamber largely reduces the biohazard waste, such as infection sera samples contamination. To fulfil this aim, we developed a simple and user-friendly microfluidic system to demonstrate the versatility of CFT as an important tool for biomarker testing.

2. Materials and methods

2.1. Materials

Complement (purified from guinea pig serum), erythrocyte (sheep red blood cell, SRBC) and its antibody (anti-SRBC antibody, haemolysin) were obtained from Nanfang Reagent Inc., Shanghai, China. Carcinoembryonic antigen (CEA) and anti-CEA antibody were purchased from Sigma Aldrich (St. Louis, USA). Recombinant protein of avian influenza A virus (rH7N9, A/Anhui/1/2013) and its specific antibody (anti-rH7N9 antibody) were obtained from Sino Biological Inc., China. Low melting point agar was obtained from Genview Scientific Inc. China. PDMS elastomer kits (Sylgard 184) were obtained from Dow Corning (Midland, USA). Ultraviolet (UV) light sensitive dry film (thickness = 40 μm) was purchased from Shenzheng Semiconductor Company (China). All other chemicals were purchased from Sigma–Aldrich (St. Louis, USA) and used without further purification unless otherwise indicated. All solutions were prepared with deionized water produced by PURELAB flex system, ELGA Corporation.

2.2. Microstructure design and fabrication

The proposed device for complement fixation test was a poly(-dimethylsiloxane) (PDMS)/glass slide hybrid structure. The fabrication of PDMS microchannel followed a standard soft lithograph protocol (Fig. 1A). Specifically, to generate a multi-depth structure, a two-steps photolithography was conducted with dry photosensitive (thickness = 40 μm) (Fig. 1A). Step 1: one layer of photosensitive film (40 μm) was laminated on a cleaned silicon wafer. Standard UV-lithography was conducted to pattern a strip (250 μm × 40 μm X 4000 μm), which defined the gap structure in the final device. Step 2: another five-layer of photosensitive dry film (5 × 40 μm = 200 μm) was firmly laminated on the patterned wafer and the second-time photolithography was conducted to reveal the feature of chambers, inlet and outlet channels. Next, the obtained mould was used to generate PDMS replica. In brief, PDMS precursor (1:10 w/w, base: crosslinking reagent) was poured onto the featured silicon wafer, degassed and baked at 80 °C for 2 h. The obtained PDMS replica was treated by oxygen plasma and firmly bonded with a clean-glass slide. Finally, the PDMS/glass slide hybrid device was assembled in a polymethyl methacrylate (PMMA) clamp set. Fig. 1B illustrates the structures and sizes of the PDMS microchannel and PMMA clamp set. The middle frame on PMMA clamp was specifically located on the gate-channel of PDMS microstructures. When the screws were fastened, the force on the PMMA clamp was imposed on gate-channel and the gap between the PDMS and glass was closed. The fluidic characters were demonstrated with the assistance of water-based liquid food dyes. Prior to solution injection, the fully assembled device was treated by oxygen plasma. The green and red solution were pipetted into the inlet reservoir and allowed to flow to the outlet reservoir. The flow characters were directly imaged by a smartphone camera (Apple iPhone 5).