POTENTIAL CLINICAL RELEVANCE

Nanomedicine: Nanotechnology, Biology, and Medicine
10 (2014) 809–818

Original Article

nanomedjournal.com

An integrated microfluidic device utilizing vancomycin conjugated magnetic beads and nanogold-labeled specific nucleotide probes for rapid pathogen diagnosis

Chih-Hung Wang, PhD[a], Chia-Jung Chang[a], Jiunn-Jong Wu, PhD[b], Gwo-Bin Lee, PhD[a,c,d,*]

[a]*Department of Power Mechanical Engineering, National Tsing Hua University, Hsinchu, Taiwan*
[b]*Department of Medical Laboratory Science and Biotechnology, National Cheng Kung University, Tainan, Taiwan*
[c]*Institute of Biomedical Engineering, National Tsing Hua University, Hsinchu, Taiwan*
[d]*Institute of Nano Engineering and Microsystems, National Tsing Hua University, Hsinchu, Taiwan*

Received 30 May 2013; accepted 31 October 2013

Abstract

A PCR-free assay for rapid pathogen diagnosis was implemented on an integrated microfluidic system in this study. Vancomycin-conjugated magnetic beads were used to capture multiple strains of bacteria and nucleotide probes labeled gold nanoparticles were used to specify and detect a specific strain by hybridization-induced color change. The assay was entirely automated within an integrated microfluidic device that was composed of suction-type micropumps, microvalves, microchannels, and microchambers that fabricated by microfluidic technology. Multiple strains of bacteria could be captured simultaneously by vancomycin-conjugated magnetic beads, with capturing efficiency exceeding 80%. Subsequently, sensitive and strain-specific detection against target bacteria could be achieved by using nanogold labeled specific nucleotide probes. The limit of detection of 10^2 CFU bacteria was achieved. Importantly, nucleic acid amplification was not involved in the diagnostic procedures; the entire analytic process required only 25 min. The developed platform may provide a promising tool for rapid diagnosis of bacterial infections.

From the Clinical Editor: In this novel study, a PCR-free pathogen detection method is demonstrated. After vancomycin-conjugated magnetic beads captured bacteria, nucleotide probes-labeled gold nanoparticles were employed to specify and detect specific strains via hybridization-induced color change. Multiple strains of bacteria could be captured simultaneously with an efficiency exceeding 80%, enabling the detection of as low as 10^2 CFU of bacteria.
© 2014 Elsevier Inc. All rights reserved.

Key words: Vancomycin; Nanogold; Microfluidic; Bacteria

Infectious diseases have become a growing threat to the public health worldwide.[1] The emerging bacterial diseases and antibiotic-resistance bacteria are crucial issues since the mortality of the bacterial infections has gradually increased.[2] Conventionally, these bacteria can be detected and identified through pure culture of bacterium and several existing biological assays.[3,4] However, bacterial growth in cell culture may take several days to even weeks until a sufficient amount of bacteria can be obtained for biological and chemical analysis. In addition, excessive experimental procedures can easily cause artificial errors, leading to false diagnostic results. Therefore, rapid and accurate diagnosis plays an important role for infectious disease prevention and control. Recently, protein-based immunoassays (e.g. enzyme-linked immunosorbent assay) and nucleic acid amplification based methods (e.g. polymerase chain reaction (PCR), real-time PCR) have been widely applied for pathogen

Abbreviations: Bp, base-pair; CFU, colony forming unit; DNA, deoxyribonucleic acid; dNTP, deoxyribonucleotide triphosphate; *E. coli*, *Escherichia coli*; EDAC, (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide; EMVs, electromagnetic valves; ddH_2O, deionic distilled water; G(−), Gram-negative bacteria; G(+), Gram-positive bacteria; HCl, hydrogen chloride; LB, Luria-Betani; LOC, lab-on-a-chip; LOD, limit of detection; MEMS, micro-electro-mechanical-systems; MRSA, methicillin-resistant *Staphylococcus aureus*; PCR, polymerase chain reaction; PDMS, polydimethylsiloxane; SDS, sodium dodecyl sulfate; TBE, Tris-Bornate-EDTA; Tween 20, (polyoxyethylene (20) sorbitan monolaurate; Xcv, *Xanthomonas campestris* pv. *vesicatoria*.

The preliminary results from this paper were presented at the 2013 IEEE Micro Electro Mechanical Systems (IEEE MEMS 2013), Taipei, Taiwan, 2013.

The authors would like to thank the National Science Council in Taiwan (NSC 101-2120-M-007-014) and the "Toward a World-class University" Project for financial support of this study.

There is no any conflict of interest.

*Corresponding author at: Department of Power Mechanical Engineering, National Tsing Hua University, Hsinchu 300, Taiwan.

E-mail addresses: biowang2007@gmail.com (C.-H. Wang), jerry780620@gmail.com (C.-J. Chang), jjwu@mail.ncku.edu.tw (J.-J. Wu), gwobin@pme.nthu.edu.tw (G.-B. Lee).

1549-9634/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.nano.2013.10.013

detection.[5] These molecular methods can achieve highly sensitive and specific diagnostic results. However, these methods often require expensive reagents and equipments and well-trained operators for performing relatively complicated assays. In particular, nucleic acid amplification based techniques require extracted nucleic acid products from bacterial by performing sample pretreatment for differentiating pathogen DNA.[6–8] In other words, it was a labour-intensive, time-consuming and costly pretreatment in bacteria detection.

Vancomycin can be used as a capturing agent for bacteria because it can bind to the peptidoglycans on the bacteria cell wall for both Gram-positive and -negative bacteria.[9–11] Specifically, Gram-negative bacteria were considered to contain a thinner peptidoglycan layer than Gram-positive bacteria.[12] Thus these antibiotics could reversibly bind onto D-alanyl-D-alanine, terminal residues of mucopeptides on the cell wall of the Gram-positive bacteria since specific hydrogen bonds could be formed between the alanine methyl group of the cell wall and vancomycin.[13,14] In addition to Gram-positive bacteria, the vancomycin also had affinity to be bonded with L-lysine-D-alanine residues of peptidoglycans that was expressed on the outer membrane of Gram-negative bacteria.[15] Therefore, vancomycin could be used to capture various species of bacterial pathogens if they may be properly surface-conjugated on the magnetic beads.

A thiol chemically modified oligonucleotide linked with gold nanoparticles was reported for DNA selectivity detection.[16,17] The method was used for pathogen identification that provided simply detection for specific target DNA in the mixture of oligonucleotides by colorimetric changed observation.[18,19] The optical properties relied on the light absorption that depended on the distance of gold nanoparticles. When compared with the aggregated nanogold particles, the nanogold particle-target DNA complex had different distance in the tested dispersion that could cause color change by light spectra shift. The distinct optical properties of gold nanoparticles were demonstrated as sensitive and specific DNA biosensors that caused by red-shift effect in a gold nanoparticle aggregation process.[20,21] Nanogold was relatively biocompatible and enable facile surface immobilization chemistries for ready conjugation of oligosaccharides, nucleic acids, peptides or small biofunctional molecules.[22,23]

In this study, an integrated microfluidic device for performing the new protocol for rapid pathogen diagnosis was developed. The diagnostic system consisted several functional components in the developed devices, including a recognition element for binding pathogens by using vancomycin-conjugated magnetic beads and a sensing element by using gold nanoparticle labeled with specific nucleotide probes binding to target genes. Advantages of the micro-total-analysis-systems (μ-TAS) or lab-on-a-chip (LOC) platform include reduced consumption of samples and reagents, fast analysis speed, and integrated analytic and sensing techniques without the need of skilled operators. Micro-electro-mechanical-systems (MEMS)-based biomedical systems have recently been widely applied for molecular diagnosis.[24] For instance, an integrated microfluidic system performing sample pretreatment and rapid diagnosis of *Staphylococcus aureus* was reported.[25] Furthermore, suction-type micropumps, microchannels, microchambers and microvalves fabricated by polydimethylsiloxane (PDMS)-based processes have been developed to realize on-chip fluidic transportation and sample mixing, which greatly simplifies experimental processes and reduces the size of microfluidic chips.[24] Regulated by electromagnetic valves (EMV), fluid sample could be efficiently transported forward and backward to realize reagent exchange and incubation processes. In this study, these microfluidic devices were integrated on a single chip to automate the entire process for fast diagnosis of bacterial infections with minimal human intervention by using the new protocol described above.

Methods

Tested bacteria and primers

Gram-positive (G(+)) bacteria, including *Streptococcus agalactiae*, *Streptococcus pyogenes*, methicillin-resistant *S. aureus* (MRSA), and *Bacillus subtilis* and Gram-negative (G(−)) bacteria, including *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* were provided from National Cheng Kung University Hospital, Tainan, Taiwan. *Xanthomonas campestris* pv. *vesicatoria*, a G(−) plant pathogen, was provided by Dr. Hui-Liang Wang, from Department of Biotechnology, National Kaohsiung Normal University, Kaohsiung, Taiwan. All of the tested bacteria were freshly incubated in LB broth (Sigma-Aldrich Co. USA) at 37 °C for 16 hours. The sequences of primers for the multiplex PCR were selected from nucleotide database of NCBI website (National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov/nucleotide), designed by FastPCR software (ver 6.1, PrimerDigital Co. USA), and listed in Supporting Information Table 1.

Chip design and fabrication

A schematic layout of the integrated microfluidic device was shown in Figure 1. The device composed of suction-type micropumps, microvalves, microchannels, and loading chambers, is capable of performing eight independent diagnoses simultaneously. The complete microfluidic chip functions as a "cartridge" that integrates seamlessly in a custom-designed microfluidic machine that includes a temperature controller, electromagnetic valves, vacuum pumps, and waste chambers.[26] Samples and other reagents were loaded into respective chambers before testing. The micropumps could deliver all processing buffer and nanogold probes into the sample loading chambers to perform incubation, hybridization and nanogold aggregation processes in an automatic format.

The device was fabricated by polydimethyl siloxane (PDMS) molding and bonding processes as previously reported.[26,27] Briefly, PDMS layers were made with PDMS polymer and curing agent (Sylgard 184A/B, Sil-More Industrial Ltd., USA) at a weight ratio of 10:1. The two PDMS layers and a glass substrate were treated with oxygen plasma, aligned, and bonded together to form the integrated microfluidic chip.

Method of vancomycin-conjugated with magnetic beads

The protocol for coating vancomycin on magnetic beads was modified from a previous study.[28] Briefly, 100 μL of carboxylic modified magnetic beads (Dynabeads® MyOne™ carboxylic

Download English Version:

https://daneshyari.com/en/article/10436151

Download Persian Version:

https://daneshyari.com/article/10436151

Daneshyari.com