A Silicon-Based Biosensor for Bacterial Pathogens Detection

Roberto Verardo, Salvatore Petralia, Claudio Schneider, Enio Klaric, Maria Grazia Amore, Giuseppe Tosto and Sabrina Conoci

Abstract The miniaturization of integrated nucleic acid testing devices represents a critical step toward the development of portable systems able to offer sample-in-answer-out diagnostic analysis. The conventional molecular testing workflow involves laboratory infrastructures and well-trained staff. Here we present a versatile, user-friendly miniaturized Lab-on-Chip device for the molecular diagnostics of infectious diseases. It is composed of a polycarbonate ring and a silicon chip; a customized reader integrating electronic and optical modules was developed for driving the thermal and optical processes. We report the results obtained using our device for the sample processing and detection of gram-negative opportunistic pathogenic bacteria.

Keywords Point-of-care diagnostics · Nucleic acid testing · Infectious diseases

1 Introduction

The detection of nucleic acids directly from biological samples and its integration into miniaturized biosensors represents a technical challenge in Point-of-Care molecular diagnostics [1]. Its clinical utility is particularly relevant in developing countries where infectious disease diagnosis is still a challenge due to poor laboratory infrastructures [2]. Great efforts have been made for the effective integration of all the main steps of nucleic acid testing (NAT) in miniaturized devices, including DNA extraction [3], PCR amplification and detection [4]. Many PCR-based NAT systems have been developed and reported in the literature; some of these were developed using plastic

R. Verardo · C. Schneider · E. Klaric
LNCIB, Laboratorio Nazionale del Consorzio Interuniversitario per le Biotecnologie, Padriciano 99, 34149 Trieste, Italy
e-mail: roberto.verardo@lncib.it

S. Petralia (✉) · M. G. Amore · G. Tosto · S. Conoci
STMicroelectronics, Stradale Primosole 50, 95121 Catania, Italy
e-mail: salvatore.petralia@st.com

B. Andò et al. (eds.), *Sensors*, Lecture Notes in Electrical Engineering 539,
https://doi.org/10.1007/978-3-030-04324-7_19

materials and include complex microfluidic modules [5], while a few PCR-free systems have been developed [6]. Plastic materials for integrated microfluidic devices have the advantage of low cost but the miniaturization, integration and automation required by the biological applications remain the main limitations towards the development of fully integrated point-of-care devices. Various miniaturized systems have also been reported for the detection of pathogenic bacteria such as Chlamydia trachomatis and Escherichia coli [7], Salmonella enterica [8, 9] and Mycobacterium tuberculosis (using surface plasmon resonance) [10].

In this study we have developed a fully integrated sample-in-answer-out system based on a silicon device able to perform sample processing and real-time PCR amplification in a single microchamber, without the need for nucleic acid purification or movement between different reaction chambers. The system is composed of a miniaturized silicon chip mounted on a polycarbonate ring to form microchambers [11]. A portable and easy-to-use reader integrates an electronic board to drive the silicon device and the optical components to capture the q-PCR fluorescence images. This work reports the experimental results for the detection of gram-negative (Pseudomonas aeruginosa) bacteria from cultured samples. The DNA extraction was performed by standard Lysozyme and Proteinase treatments, while the real-time PCR amplification was performed using species-specific PCR primers. For comparison, the same samples were analyzed using commercial q-PCR equipment (StepOnePlus, Applied Biosystems).

2 Materials and Methods

2.1 Chemicals and Biological Reagents

All reagents were purchased from Sigma-Aldrich and used as received. The proteinase reagent was purchased from ZyGEM (MicroGEM, United Kingdom). The PCR reagents were purchased from Kapa Biosystems and used as received. The biological target: Gram-negative (Pseudomonas aeruginosa) bacteria come from cultured samples internally prepared.

2.2 Biosensor Description

The biosensor system (developed by STMicroelectronics) is composed of a miniaturized hybrid silicon-polycarbonate chip with six microchambers (each microchamber with a total volume of 22 μl) (Fig. 1). The device, based on VLSI-technology, integrating heaters and temperature control sensors in its silicon part allows to perform PCR thermal cycles with an accuracy of 0.1 °C. A customized reader integrates an

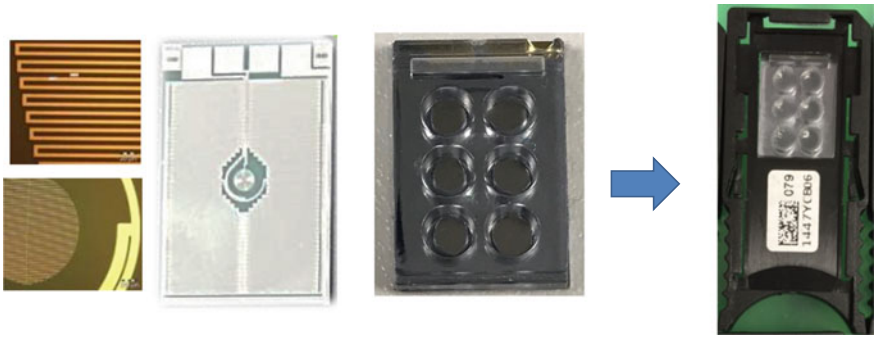

Fig. 1 Miniaturized silicon device composed of heaters and temperature sensors and 6 round microreactors

electronic board to drive the thermal and optical module for PCR amplification and detection, while a smart software was developed for data analysis.

3 Results and Discussion

3.1 Sample Processing and Detection

Gram-negative bacteria were lysed in a microchamber in the presence of lysozyme and proteinase using the following thermal protocol: 15 min at 37 °C (lysozyme activation), 15 min at 75 °C (proteinase digestion) and 15 min at 95 °C (proteinase inactivation). Next, 5 μl of lysate were transferred to a second microchamber and subjected to q-PCR with gram-negative specific primers using the following thermal protocol: 3 min at 95 °C (initial denaturation), 30 s at 60 °C (annealing-extension), for 45 cycles. For comparison, the same samples were run on a StepOnePlus Real Time PCR System (Applied Biosystems).

3.2 Real-Time PCR Experiments

Crude lysates were obtained from bacterial cultures and employed as described in materials and methods section. Five replicas were run for each sample, together with the negative control, NTC (No Template Control). The q-PCR experiments were performed on a miniaturized chip, which was thermally and optically driven by a miniaturized electronic board. The fluorescence signals were collected by the CCD detector inside the board and analyzed by a smart detection software. Figure 2 shows an example of the q-PCR curves while quantitative results as reported in Table 1.

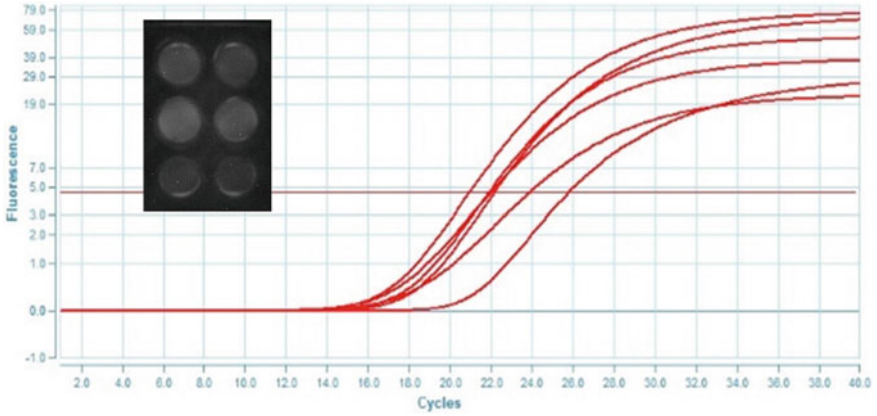

Fig. 2 q-PCR curves

Table 1 q-PCR quantitative results. The experimental results demonstrate the applicability of the new system based on the silicon biosensor compared to standard methods

Sample	Ct from device	Ct from standard instrumentation
PA1	21.1	20.8
PA2	20.8	20.0
PA3	22.2	21.5
PA4	23.2	22.8
PA4	19.8	20.8
Negative control (NTC)	33.3	32.6

4 Conclusion

Here we present a versatile, miniaturized Lab-on-Chip device useful for the molecular diagnostics of infectious diseases. The whole system is composed of a miniaturized silicon-based chip, a reader and a software for data analysis. We report the results obtained for the sample processing and detection of gram-negative opportunistic pathogenic bacteria. The q-PCR experiments revealed that the proposed system is able to perform the species-specific detection on the silicon-based chip. These results, together with the miniaturization and integration are important features towards the identification of an easy-to-use, Point-of-Care molecular diagnostic platform for rapid and sensitive detection of bacterial pathogens without requiring major hands-on time and complex laboratory instrumentation.

References

1. Petralia, S., Verardo, R., et al.: Sens. Actuators B Chem. **187**, 99 (2013)
2. Yager, P., Domingo, G.J., Gerdes, J.: Annu. Rev. Biomed. Eng. **10**, 107–144 (2008)
3. Petralia, S., Sciuto, E., Conoci, S.: A novel miniaturized biofilter based on silicon micropillars for nucleic acid extraction. Analyst **142**, 140–146 (2017)
4. Foglieni, B., Brisci, A., San Biagio, F., Di Pietro, P., Petralia, S., Conoci, S., Ferrari, M., Cremonesi, L.: Clin. Chem. Lab. Med. **48**, 329–336 (2010)
5. Petralia, S., Conoci, S.: PCR technologies for point of care testing: progress and perspectives. ACS Sens. **2**, 876–891 (2017)
6. Petralia, S., Sciuto, E.L., Di Pietro, M.L., Zimbone, M., Grimaldi, M.G., Conoci, S.: Innovative chemical strategy for PCR-free genetic detection of pathogens by an integrated electrochemical biosensor. Analyst **42**, 2090–2093 (2017)
7. Fernández-Carballo, B.L., McGuiness, I., McBeth, C., Kalashnikov, M., Borrós, S., Sharon, A., Sauer-Budge, A.F.: Low-cost, real-time, continuous flow PCR system for pathogen detection. Biomed. Microdevices **18**(2), 34 (2016)
8. Hsieh, K., Ferguson, S.B., Eisenstein, M., Plaxco, K.W., Soh, H.T.: Integrated electrochemical microsystems for genetic detection of pathogens at the point of care. Acc. Chem. Res. **48**, 911–920 (2015)
9. Rijal, K., Mutharasan, R.: A method for DNA-based detection of E. coli O157:H7 in proteinous background using piezoelectric-excited cantilever sensors. Analyst **138**, 2943–2950 (2013)
10. Hsu, S.H., Lin, Y.Y., Lu, S.H., Tsai, I.F., Lu, Y.T., Ho, H.S.: Mycobacterium tuberculosis DNA detection using surface plasmon resonance modulated by telecommunication wavelength. Sensors **14**, 458–467 (2013)
11. Guarnaccia, M., Iemmolo, R., Petralia, S., Conoci, S., Cavallaro, S.: Miniaturized real-time PCR on a Q3 system for rapid KRAS genotyping. Sensors **17**, 831 (2017)