Real-time DNA Amplification and Detection System Based on a CMOS Image Sensor

Tiantian Wang,* Jasmine Pramila Devadhasan,* Do Young Lee,** and Sanghyo Kim*,***†

*Department of Bionanotechnology, Gachon University, San 65, Bokjeong-Dong, Sujeong-Gu, Seongnam-Si, Gyeonggi-Do 461-701, Republic of Korea
**Optolane Inc., 633 Sampyeong-Dong, Bundang-Gu, Seongnam-Si, Gyeonggi-Do 463-400, Republic of Korea
***Graduate Gachon Medical Research Institute, Gil Medical Center, Inchon, 405-760, Republic of Korea

In the present study, we developed a polypropylene well-integrated complementary metal oxide semiconductor (CMOS) platform to perform the loop mediated isothermal amplification (LAMP) technique for real-time DNA amplification and detection simultaneously. An amplification-coupled detection system directly measures the photon number changes based on the generation of magnesium pyrophosphate and color changes. The photon number decreases during the amplification process. The CMOS image sensor observes the photons and converts into digital units with the aid of an analog-to-digital converter (ADC). In addition, UV-spectral studies, optical color intensity detection, pH analysis, and electrophoresis detection were carried out to prove the efficiency of the CMOS sensor based the LAMP system. Moreover, Clostridium perfringens was utilized as proof-of-concept detection for the new system. We anticipate that this CMOS image sensor-based LAMP method will enable the creation of cost-effective, label-free, optical, real-time and portable molecular diagnostic devices.

Keywords CMOS image sensor, isothermal amplification, LAMP technique, photon detection

(Received December 24, 2015; Accepted February 5, 2016; Published June 10, 2016)
real-time disposable LAMP system for gene amplification and detection using a saliva sample within 30 min. The study demonstrated an ion-sensitive field-effect transistor integrated to a complementary metal oxide semiconductor (CMOS) sensor for DNA amplification and detection by measuring the hydrogen ion release.

Recently, CMOS image sensor technology has been found to be less expensive and an alternative to PMT and CCDs. Recent advances in this technology offer promise of realizing hand-held molecular imaging technologies. In addition to achieve sensitivity close to that of CCDs, they consume lower power, operate at higher speeds and offer an unmatched level of integration. Moreover, CMOS has proven its use in DNA analysis as a DNA microarray, sequencing, DNA detectors, and other bio-sensing and bio-analytical approaches with a real-time approach. A CMOS image sensor has been widely developed for the biological and chemical detection. Our group has successfully carried out CMOS sensor based immunodiagnostics, glucose monitoring and chemical detection.

In the present study, we developed a disposable CMOS sensor based LAMP reaction with the real-time photon detection technique. The analysis carried out on Clostridium perfringens DNA at concentrations ranging from 10 ng/μL to 1 fg/μL and the results showed that this method enabled the detection of 1 fg/μL target DNA. C. perfringens is the main pathogenic bacteria of human gas gangrene and enteric diseases. The whole detection, which is based on the CMOS sensor without additional integrated technology, largely cut down the cost, and prompted a new system with high portability and low cost. Therefore, the CMOS sensor-based LAMP reaction can be used in the diagnosis of infectious diseases, especially in low-income countries due to its low cost compared to others. This setup can also be used for the detection of pathogens from minimally processed samples, such as immune-NASBA assays or in clinical sample matrices.

**Experimental**

**Preparation of purified DNA standards**

*Clostridium perfringens* (ATCC#13124) was cultured and extracted DNA by a QIAamp DNA Mini Kit. A thermo nanodrop 2000 spectroscopic measurement method was used to estimate the DNA concentration.

**Reagents and chemicals**

Six oligonucleotide primers targeting the *C. perfringens* were designed using the LAMP designer software (Optigene, Horsham, UK): Forward inner primer (FIP), 5′-TTTCACAACCTGCTGTGTTTTGGTTACGTGCCTGCCGTTGATAAC-3′; backward inner primer (BP), 5′-AGCATGAGTCATAGTTGGGATGATAGATACTCCCGCTGTTCCTTT-3′; forward outer primer (FP), 5′-AGATACTCATCATCCTCTGCT-3′; backward outer primer (BP), 5′-CCTCTGATACATCGTGTAAGAA-3′; loop forward primer (LF), 5′-TCTCAAAACCTTAAACATGTCTTGTCG-3′; and loop backward primer (LB), 5′-GGGATTATGCAGCAAAGGTAAC-3′ (Genotech, Daejeon, South Korea). *Bst* DNA polymerase buffer (10X), deoxynucleoside triphosphates (dNTPs) (10 mM; New England BioLabs, Ipswich, MA), *Bst* DNA polymerase (8 U/μL; New England BioLabs, Ipswich, MA), betaine (250 mM), MgSO₄ (150 mM), hydroxynaphthol blue (HNB) (3 mM; Sigma-Aldrich, St. Louis, MO, USA) were used for the LAMP assay.

**Apparatus**

The NOON130PC20L is 110000 active pixel single (APS) chip CMOS image sensor used for LMAP reaction and detection, which was sponsored by Optolane Inc., Republic of Korea. The sensor chip has 376 × 314 multi-pixel photon arrays with dual top header and the chip was connected to 10 bit range of analog-to-digital converter (ADC) circuits to convert the photon number to digital units. In addition, the circuitry controller program RS-232 maintained the temperature and light intensity throughout the reaction. For the LAMP analysis, a K-type temperature controller provided 63°C on a thermal block and a red LED with 600 - 650 nm spectra for the light intensity.

**Procedure**

The reaction area was developed on a disposable CMOS sensor array surface. A 1-mm thickness of transparent polypropylene cylindrical tube (Biosigma®, supplied by Human Science, Seoul, Republic of Korea) with a diameter of 5 × 5 mm was fixed on the surface of the CMOS sensor using silicon epoxy. Before fixing, the polypropylene cylindrical tube was autoclaved at 121°C for 15 min to remove any impurities. In addition, so as to avoid sample evaporation in the reaction well, a polypropylene well cap was used to close the reaction well tightly. The polypropylene well is sufficient to accommodate 20 μL of the sample. Figure 1 (a, b) shows the polypropylene well integrated CMOS sensor for the LAMP reaction. Indeed, we fixed the two wells for the right and left sensor’s surface to carry out the two reactions simultaneously on a single chip. On the other hand, it is possible to build up a multiple well on the sensor surface that enables several parallel experiments to reduce both the reaction volume and cost. The CMOS sensor surface contact angle (Fig. 1c) was measured by using Phoenix 300 contact angle measuring system (Surface Electro Optics, Republic of Korea). The contact angles were further analyzed with Image Pro 300 software.

Generally, the LAMP assay was performed in a one-step reaction by mixing 6.5 μL of a reaction buffer containing 1 mM dNTPs, 10 mM betaine, 6 mM MgSO₄, 120 μM HNB, 0.5 μL of each primer (FP and FB, 5 pmol/μL; BIP and FIP, 40 pmol/μL; and LF and LB, 20 pmol/μL), 1 μL *Bst* DNA polymerase,
μL DNA samples and 1 μL distilled water. Then, the mixtures were incubated at 63 °C for 60 min. Figure 2 (a, b) clearly illustrates the whole set up of the CMOS sensor based real-time gene amplification system and CMOS sensor based reaction system. Usually, the one-step LAMP reaction was carried out on the polypropylene well-integrated CMOS sensor with 12.5 μL of reaction sample. However, in this study, we used various concentrations of target DNA sample containing 10 ng/μL, 100 pg/μL, 1 pg/μL, 10 fg/μL, 10 fg/μL, and 1 fg/μL to confirm the amplification sensitivity. Then, the CMOS sensor reaction well was kept on a thermal block for amplification on the thermal platform for 55 min at 63 °C. During the reaction, the circuitry controller provided a sufficient temperature for amplification and light intensity for photon detection. Subsequently, CMOS sensor measured the amplification by observing the photon number as quantitatively. In addition, the negative control was analyzed on the CMOS well following the aforementioned procedure without a DNA sample. After 55 min of reaction, the temperature was increased to 80 °C for 5 min to inactivate the enzyme and terminate the reaction. Further, optical detection, UV-spectral analysis, pH analysis, and electrophoresis were explored to authenticate the CMOS sensor based LAMP amplification.

Results and Discussion

LAMP assay on CMOS sensor platform

Each CMOS sensor has a photodiode that observes the photons and converts them into electrical energy by the photoelectric effect. The electrical energy is converted to digital units with the aid of ADC. Also, the CMOS sensor has a setup to fix the standard calibration value by adjusting the exposure time and the analog gain value in order to change the background light intensity. In the present study, the circuitry controller, RS-232, was used to provide a standard light source to fix the calibration value. Generally, the photon number will be changed when exposed to objects over the CMOS sensor surface. The nanometer thickness of the substrates could be analyzed by the CMOS sensor as photon detection techniques. Based on this concept, we carried out the LAMP analysis using a CMOS sensor. For the LAMP assay, various concentrations of DNA samples were added into the polypropylene well integrated CMOS sensor, which was kept on the thermal block at 63 °C, and provided an LED light source for the photon count. The calibration value was fixed at 345 (A.U.), and the photon number was monitored by a CMOS image sensor. The amplification was monitored from 0 to 60 min. During the LAMP reaction, pyrophosphate ion is released from the dNTP and this pyrophosphate ion reacts to the magnesium ion in the buffer which leads to the precipitated magnesium pyrophosphate (Mg₂P₂O₇). Moreover, the reduction of magnesium ion concentration leads to the color change of the HNB dye from violet to sky blue. On the other hand, the use of red light increases the color intensity of the final assay solution (blue). Figure 3a demonstrates the LAMP reaction, and Fig. 3b shows the LAMP sample for optical detection; the LAMP sample color has changed from purple to sky blue during the amplification and the color was stable for 40 min. In quantitative analysis, the photon number decreased when amplification started. Since, the Mg₂P₂O₇ precipitation generates the turbidity in the well and the increment of color intensity that block the photons pass through it. The turbidity decreases when the DNA concentration decreases, which produces an increasing photon number.
Figure 4a shows the CMOS sensor based digital output value according to the concentration. In the present study, every curve reaches its own plateau at about 30 min. In addition, huge photon number reduction has been observed from 3 - 20 min for all concentration of DNA sample, and there was no photon number reduction after 40 min, which proved that the amplification had stopped at 40 min. The decreasing photon number was observed up to 1 fg/μL of the target DNA, and no photon number changes were observed in negative control representing that there was no DNA amplification occurred. Based on the CMOS data, the cumulative value was demonstrated as a decrease of the photon value shown in Fig. 4b by subtracting the calibration value to the amplified value. The decrease of the photon value shows a higher amplification level in high concentration of the DNA sample and lower amplification level in low concentration of the DNA sample. The CMOS sensor based LAMP reaction proves that the CMOS sensor is a sensitive amplification tool for real-time molecular diagnosis. On the other hand, DNA extracted from *E. coli O157* was used as to compare and to clarify the specificity of designed LAMP primers and the sensitivity of the device. Figure 5 showed that the *E. coli O157* DNA is incapable to an amplified positive result by using the designed primers for *C. perfrigens*.

**Sensitivity analysis of LAMP**

After the LAMP reaction, the reacted samples were independently characterized based on a UV spectrophotometer, pH analysis, and electrophoresis. Various concentrations of
amplified samples were measured by a UV-spectrophotometer at between 200 – 800 nm. Figure 6a shows the UV-absorbance for various concentrations of LAMP products. The different concentrations of the amplified samples showed the peak at 260 nm, and the absorbance decreased as the concentration decreased. The negative control and 1 fg/μL showed almost the same absorbance at 260 nm, which proved that the spectral absorbance could not identify the least amplification. Further, the absorbance level corresponded to a previous report.29 On the other hand, the previous report says that DNA amplification can be confirmed based on the hydrogen ion release.17 The pH level has changed before and after amplification. Therefore, we confirmed the DNA amplification level by pH analysis that is shown in Fig. 6b. Various concentrations (10 ng/μL to 1 fg/μL) of amplified DNA were dropped on pH paper. The negative control showed around pH 8 and the pH level changed to nearly neutral at 10 ng/μL. The study proved that the more hydrogen ions released at high amplification LAMP production and the negative control and 1 fg/μL shows the similar pH.

To confirm and quantify of LAMP amplification, series concentrations of the LAMP products were analyzed by electrophoresis on 3% (w/v) agarose gel stained with ethidium bromide and photographed under a UV transilluminator which was shown in Fig. 7. Particularly, the electrophoresis proved that the amplified products exist in 1 fg/μL of the C. perfringens DNA. In addition, the result proved that various lengths of the final products had been amplified after the LAMP assay. The positive products exhibited a ladder-like band, while there was no band in negative control. The electrophoresis showed clear bands at 10 ng/μL, 100 pg/μL, 1 pg/μL, 100 fg/μL, 10 fg/μL, 1 fg/μL, and no band in the negative control after amplification. The previous report proved real-time LAMP amplification up to 10 fg/μL and no amplification products was observed at 1 fg/μL.30 Interestingly, our CMOS sensor based photon number changes was identified at 1 fg/μL; also electrophoresis proved the amplified LAMP products in 1 fg/μL. The above result confirmed that the CMOS integrated platform could be used to amplify the product up to 1 fg/μL. Even though the sensitivity and specificity of LAMP assay mainly depends on the primer design as well as the quality of template DNA. The detection of the amplified products at a low template concentration is not usual. The detection system used in this device is so sensitive that it detects the photon number changes even at 1 fg/μL template DNA. This is mainly due to the sensitivity of the developed device.

Conclusions

In conclusion, a disposable polypropylene well integrated CMOS chip was developed to carry out nucleic acid amplification and detection simultaneously. This is a new attempt for on-chip LAMP reactions and detection that shows a great amplification potential in a real-time LAMP assay up to 1 fg/μL. The sensitivity of the CMOS sensor based gene amplification was confirmed by UV-spectral absorbance, pH analysis, and electrophoresis. Moreover, the CMOS sensor based photon number detection is simple and user-friendly to identify the amplification. Also, the sensor could be available in mobile phones and possibly to integrate into the portable diagnostic devices. Based on the obtained data, we anticipate that the CMOS sensor-based isothermal LAMP assay can make it possible to create high sensitivity, specificity, rapid detection, high portability, user friendly and real-time amplification system for the molecular diagnosis.

Acknowledgements

This research was supported by the R&D Program for Society of the National Research Foundation (NRF) funded by the Ministry of Science, ICT & Future Planning, Republic of Korea (2013M3C8A3078806 and 2015M3A9E2031372).
References

1. K. B. Mullis, *Sci. Am.*, 1990, 262, 56.
2. M. A. A. Valones, R. L. Guimaraes, L. A. C. Brandao, P. R. E. d. Souza, A. d. A. T. Carvalho, and S. Crovela, *Braz. J. Microbiol.*, 2009, 40, 1.
3. Q. Li, J. Liang, G. Luan, Y. Zhang, and K. Wang, *Anal. Sci.*, 2000, 16, 245.
4. N. W. Lucchi, A. Demas, J. Narayanan, D. Sumari, A. K. Kabanywanyi, S. P. Kachur, J. W. Barnwell, and V. Udhayakumar, *PLoS One*, 2010, 5, e13733.
5. H. D. VanGuilder, K. E. Vrana, and W. M. Freeman, *BioTechniques*, 2008, 44, 619.
6. P. Craw and W. Balachandran, *Lab Chip*, 2012, 12, 2469.
7. T. Notomi, H. Okayama, H. Masubuchi, T. Yonekawa, S. Amino, and T. Hase, *Nucleic Acids Res.*, 2000, 28, e63.
8. M. Yi, L. Ling, S. B. Neogi, Y. Fan, D. Tang, S. Yamasaki, L. Shi, and L. Ye, *Food Control*, 2014, 41, 91.
9. N. A. Tanner, Y. Zhang, and T. C. Evans, *BioTechniques*, 2012, 53, 81.
10. K. Nagamine, T. Hase, and T. Notomi, *Mol. Cell. Probes*, 2002, 16, 223.
11. Y. Duan, C. Ge, X. Zhang, J. Wang, and M. Zhou, *PLoS One*, 2014, 9, e11094.
12. T. D. Rane, L. Chen, H. C. Zec, and T. H. Wang, *Lab Chip*, 2015, 15, 776.
13. A. Gansen, A. M. Herrick, I. K. Dimov, L. P. Lee, and D. T. Chiu, *Lab Chip*, 2012, 12, 2247.
14. J. Luo, X. Fang, D. Ye, H. Li, H. Chen, S. Zhang and J. Kong, *Biosens. Bioelectron.*, 2014, 60, 84.
15. F. Wang, L. Jiang, Q. Yang, W. Prinyawiwatkul, and B. Ge, *Appl. Environ. Microbiol.*, 2012, 78, 2727.
16. S. Xie, Y. Chai, Y. Yuan, L. Bai, and R. Yuan, *Biosens. Bioelectron.*, 2014, 55, 324.
17. C. Toumazou, L. M. Shepherd, S. C. Reed, G. I. Chen, A. Patel, D. M Garner, C. J. A Wang, C. P. Ou, K. A. Desai, P. Athanasiou, H. Bai, I. M Q Brizido, B. Caldwell, D. C. Alford, P. Georgiou, K. S Jordan, J. C Joyce, M. L. Mura, D. Morley, S. Sathyavruthan, S. Temelso, R. E. Thomas, and L. Zhang, *Nat. Methods*, 2013, 10, 641.
18. M. Davenport, A. H. Titus, E. C. Tehan, Z. Tao, Y. Tang, R. M. Bukowski, and F. V. Bright, *IEEE Sensors J.*, 2004, 4, 180.
19. G. Giraud, H. Schulze, D. U. Li, T. T. Bachmann, J. Crain, D. Tyndall, J. Richardson, R. Walker, D. Stoppa, E. Charbon, R. Henderson, and J. Arlt, *Biomed. Opt. Express*, 2010, 1, 1302.
20. H. J. Chao and N. Uzun, *IEEE J. Solid-State Circuits*, 1992, 27, 1634.
21. J. Musayev, Y. Adlguzel, H. Kulah, S. Eminoglu, and T. Akln, *IEEE Sens. J.*, 2014, 14, 1608.
22. J. P. Devadhasan and S. Kim, *BioChip J.*, 2013, 7, 258.
23. J. P. Devadhasan, S. Kim, and C. S. Choi, *Analyst*, 2013, 138, 5679.
24. J. P. Devadhasan, M. Shao, and S. Kim, *JOLST*, 2014, 2, 20.
25. I. Kaneko, K. Miyamoto, K. Mimura, N. Yumine, H. Utsunomiya, S. Akimoto, and B. A. McClane, *Appl. Environ. Microbiol.*, 2011, 77, 7526.
26. J. P. Devadhasan, M. Marimuthu, S. Kim, and M. G. Kim, *Anal. Bioanal. Chem.*, 2012, 402, 813.
27. J. P. Devadhasan and S. Kim, *Analyst*, 2012, 137, 3917.
28. J. P. Devadhasan and S. Kim, *Anal. Sci.*, 2012, 28, 875.
29. C. H. Wang, K. Y. Lien, J. J. Wu, and G. B. Lee, *Lab Chip*, 2011, 11, 1521.
30. X. Fang, Y. Liu, J. Kong, and X. Jiang, *Anal. Chem.*, 2010, 82, 3002.