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Droplet-based microscale colorimetric biosensor for multiplexed DNA analysis via a graphene nanoprobe

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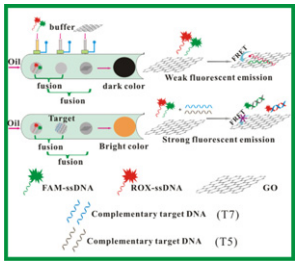
HIGHLIGHTS

- A quantitative detection for multiplexed DNA is first realized on droplet platform.
- The DNA detection is relied on a simple fluorescence-based colorimetric method.
- GO is served as a quencher for two different DNA fluorescent probes.
- This present work provides a rapid, sensitive, visual and convenient detection tool for droplet biosensor.

GRAPHICAL ABSTRACT

With a microvalve manipulate technique combined with droplet platform, a microscale fluorescence-based colorimetric sensor for multiplexed DNA analysis is developed via a graphene nanoprobe.



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ABSTRACT

The development of simple and inexpensive DNA detection strategy is very significant for droplet-based microfluidic system. Here, a droplet-based biosensor for multiplexed DNA analysis is developed with a common imaging device by using fluorescence-based colorimetric method and a graphene nanoprobe. With the aid of droplet manipulation technique, droplet size adjustment, droplet fusion and droplet trap are realized accurately and precisely. Due to the high quenching efficiency of graphene oxide (GO), in the absence of target DNAs, the droplet containing two single-stranded DNA probes and GO shows dark color, in which the DNA probes are labeled carboxy fluorescein (FAM) and 6-carboxy-X-rhodamine (ROX), respectively. The droplet changes from dark to bright color when the DNA probes form double helix with the specific target DNAs leading to the dyes far away from GO. This colorimetric droplet biosensor exhibits a quantitative capability for simultaneous detection of two different target DNAs with the detection limits of 9.46 and 9.67 $\times$ 10^{-8} M, respectively. It is also demonstrated that this biosensor platform can become a promising detection tool in high throughput applications with low consumption of reagents. Moreover, the incorporation of graphene nanoprobe and droplet technique can drive the biosensor field one more step to some extent.

1. Introduction

Hybridization analysis of DNA is of paramount importance in the detection of genetic diseases, gene expression profiling and biomedical studies [1]. The interest of DNA analysis has been increased in microfluidic field in recent years [2–5]. With the development of droplet technique, droplet-based microfluidics has become one of the most popular platforms for DNA analysis [6–8]. Compared with laminar flow-based microfluidics [9,10], as an isolated reactor, droplet can not only reduce reagents consumption, accelerate reagents mixing and shorten reaction time, but also greatly eliminate the dispersion, immobilization, incubation time and cross-contamination of reagents, as well as control solution volume precisely. Although there are many works of DNA analysis

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in droplet, such as DNA purification, high-throughput droplet PCR, single DNA molecule detection and amplification, etc. [11–14], the reports to use droplet technology for building DNA sensor are still very rare. Therefore, it is significant to apply this unique platform in developing a droplet biosensor for DNA analysis alternative to traditional DNA detection mode.

In recent years, some quick and accurate DNA sensors in droplet had been reported [15,16]. On one hand, most of assay methods rely on costly and sophisticated scientific instruments to monitor the high-speed movement of droplet and obtain fluorescence spectra. On the other hand, these DNA sensors mainly depend on FRET technique, which restrict the development of droplet sensor because of the distance limitation required by FRET. These drawbacks hinder the widespread applications of droplet in chemistry and biology. Therefore, it is necessary to develop a simple sensor manner for droplet platform. Douglas and Wang et al. proposed a luminescence-based colorimetric method for oxygen determination with satisfactory results [17,18]. This colorimetric concept provides a new path for droplet sensor, which is hard to realize by traditional colorimetric method. Moreover, this method could expect to enable droplet platform more applicable in biology and chemistry because the combination of fluorescence-based colorimetry can enhance assay efficiency and simplify detection procedure. With the development of droplet manipulation schemes [19–23], different reagents can be wrapped in droplet, fused in a droplet and the droplet can be trapped in any place of the chip. These unique functions are favourable for the realization of the colorimetric droplet DNA sensor. Compared to the DNA sensors reported in droplet, the application of droplet manipulation techniques in DNA sensor has some advantages: firstly, the colorimetric sensor process can be obtained via a common imaging device instead of the expensive high-speed camera and spectrum instruments; secondly, unlike the premixing the reagents by special cross-channel structure, the reagents in different channels can be mixed easily and selectively without cross-contamination.

Graphene oxide (GO) based biosensor has attracted lot of research interests recently because GO can be a super-quencher for some luminescent materials with the long-range nanoscale energy transfer property [24–26]. On the basis of this quenching mechanism, Fan and He et al. reported the multicolor fluorescent DNA analysis and virus detection in bulk solution [27,28]. However, simultaneous detection of multiplexed DNA with GO nanoprobe has not been reported in droplet to date. Inspired by these, a quantitative, colorimetric and multiplexed DNA droplet biosensor can be realized as well via the introduction of GO into droplet platform, which can drive the biosensor field one more step to some extent.

We herein demonstrate a fluorescence-based colorimetric droplet sensor that allows simultaneous DNA determination by using GO nanoprobe as a quencher for the ssDNA probes labeled with carboxy fluorescein (FAM) and 6-carboxy-X-rhodamine (ROX), respectively. The chip design and droplet sensor strategy for DNA analysis is depicted in Fig. 1. Our approach relies on the unique characteristic with the different affinities toward single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA) of GO. By adjusting the time on-state and switching the open or close of gas valve, the ssDNA probes droplet, target DNAs droplet and GO droplet are generated, fused, trapped and imaged on demand. A series of target DNAs with stepwise concentration gradient are introduced and created into droplets on a chip. Moreover, the quantitative determination of target DNAs is realized in visualization by analyzing the fluorescence intensities of the dyes in droplet. This sensor platform provides a universal and promising tool in high throughput applications with low consumption of reagents.

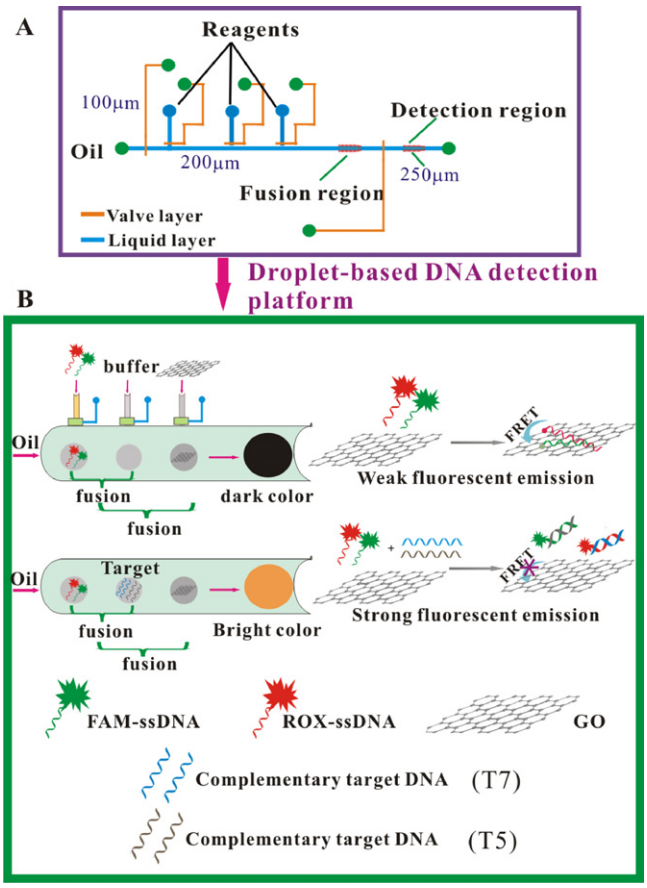

Fig. 1. (A) Schematic diagram of the microfluidic droplet platform and (B) schematic illustration of a fluorescence-based colorimetric biosensor for multiplexed DNA via a GO nanoprobe. The chip contains three layers. The bottom, middle and upper are glass, automatic gas microvalve and fluid layer, respectively. The main channel and three side channels of the fluid layer are 200 μm. The five channels of the valve layer are 100 μm. The width of fusion and detection region are 250 μm.

2. Materials and methods

2.1. Reagents and apparatus

Graphene oxide (GO) was purchased from Sinocarbon Materials Technology Co., Ltd., China. Mineral oil, Span 80 were purchased from Sigma–Aldrich (USA). DNA oligonucleotides were synthesized and purified by Invitrogen Biotechnology Co., Ltd. (USA). The sequences of the oligonucleotides were as follows: (1) probe P5: 5′-FAM-CAGAGGCAGTAACCA-3′; target DNA for P5 (T5): 5′-TGGTTACTGCCTCTG-3′; (2) probe P7: 5′-ROX -CCTGGTGCCGTAGAT-3′; target DNA for P5 (T7): 5′-ATCTACGGCACCAGG-3′; (3) single-base mismatched target DNA M1T5 for P5: 5′-TGGTTACGGCCTCTG-3′; (4) two-base mismatched target DNA M2T5 for P5: 5′-TGGTTATGGCCTCTG-3′.

The solutions of ssDNA probes were prepared in 10 mM Tris–HCl buffer (10 mM NaCl). Other aqueous solutions were prepared using ultrapure water (Mill-Q, Millipore, 18.2 MΩ resistivity). The droplet generation and size adjustment were controlled by a homemade instrument (SeriesZSE30A (F)/ISE30A, SMC, Japan). All droplet images were acquired by an inverted fluorescence microscope (Axio Observer.A1, Zeiss, Germany) with a 10× objective and a Spot RT3 charge-coupled device (CCD, Diagnostic Instruments, Inc., USA). The fluorescence intensity of dye in droplet was acquired by Image-Pro Plus software (USA).

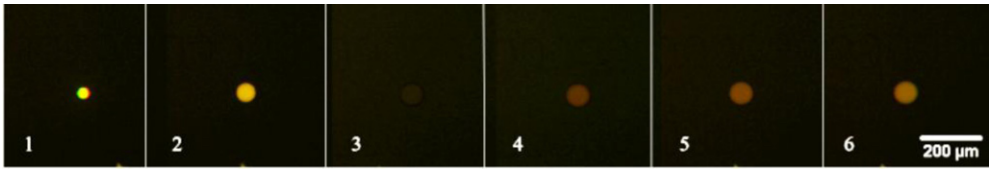

Fig. 2. Color responses of RF–GO droplet in the absence and presence of target DNAs: (1) and (2) presented the color of RF and RF–GO, respectively. The droplets showed the color responses of RF–GO droplet with the addition of buffer, T7, T7 + T5 and T5 from 3 to 6. The mixing order of the droplets is RF probe- buffer/target DNAs–GO mode. The VRs of FR-buffer/target-GO was 1:1:2. The concentrations of ROX and FAM of RF probe were 7.5 and 2.5×10^{-5} M. The concentrations of DNA and GO were 5×10^{-7} M and 0.8 mg mL^{-1} respectively. The pH of buffer was 5.4. The average size of droplets was 97 pL.

2.2. Procedure for DNA detection in droplet

A three-layer PDMS microfluidic chip was designed by soft-photolithography with five gas valves (Fig. 1A). There were three valves to control the side channels for droplet generation and the others were used to control main channel for droplet trap. The chip fabrication and the generation, control as well as fusion of droplets based on this microfluidic system referred to the previous work [23]. For this multiplex DNA sensor, firstly, the reagents with 0.2 μL were introduced into the side channels of the chip. And then different component droplets were generated and fused in the mixing region via adjusting the gas valve. Finally, the well-mixed droplet was immobilized and photographed at the detection region via a common imaging device.

3. Results and discussion

3.1. The principle of colorimetric droplet sensor for DNA analysis

For the fluorescence-based colorimetric droplet sensor (Fig. 1B), the fluorescent ssDNA probes (FAM-ssDNA and ROX-ssDNA) are premixed as RF probe before they are introduced into the chip, which are complementary with the sequence-specific DNAs (T5 and T7, respectively). In the absence of target DNAs, only the probe, buffer and GO droplet are fused together. As a result, the probes adsorb on GO via π–π interactions between DNA nucleobases and nucleosides and the π-rich GO. The fluorescence of RF is suppressed effectively through a long-range energy transfer. Accordingly, the droplet displays a dark color. In the presence of the target DNAs, the RF probe and target DNAs droplets are first fused resulting in the formation of dsDNA. When the well-mixed droplet combines with GO droplet, the RF probe is released from the surface of GO and the luminescence of RF is restored. Correspondingly, the droplet shows a mixed color of FAM and ROX. The increased fluorescence emission intensities will be positively related to the concentration of the target DNAs added in droplet.

The feasibility of the quenching and recovery experiments was evaluated on this colorimetric droplet platform. The results were shown in Fig. 2. Firstly, pairs of droplet containing RF probe and GO were generated and fused. In the absence of target DNAs T5 and T7, it was seen that the droplet displayed dark color, which indicated that the fluorescence of RF decreased greatly due to the occurrence of the FRET process. When target DNAs combined with the well-mixed RF–GO droplet, a distinct orange droplet was observed. The results suggested that the double helix formation of the RF probe and target DNAs had removed RF from the surface of GO successfully, leading to the fluorescence restoration of ROX and FAM. Moreover, this biosensor was also used to explore the color response with the addition of target DNAs T7, T7 + T5 and T5, respectively. Interestingly, when the target DNAs was fused into the RF–GO droplet, the droplet exhibited a gradually enhanced color response from T7 to T5. It suggested that the specific DNA hybridization could be achieved by droplet-based microfluidics in short time with high and good mixing efficiency. Thus, the strategy for simultaneous detection of DNA is feasible on droplet platform via fluorescence-based colorimetric method.

3.2. Optimization of the variables in droplet

In order to obtain high sensitivity and good selectivity, the variables of this sensor platform were further assessed. Owing to the strong adsorption of GO on PDMS, the droplet with high GO concentration was hard to form. The concentration of GO was thus fixed at 0.8 mg mL^{-1} in our system for an effective quenching. Firstly, the volume ratio (VR) of ROX and FAM of RF probe was optimized for good color change. As shown in Fig. 3, obvious color difference was observed in droplets between in the absence and presence of target DNAs T7 and T5 when the VR of ROX/FAM was up to 3:1. Therefore, the VR of ROX and FAM was chosen as 3:1 in the subsequent work. It was worthy to notice that the fluorescence of some fluorescence dyes is easily influenced by the pH of buffer. Then the effect of the pH of the buffer on the fluorescence of RF was explored and the results were shown in Fig. 4(1). Evidently, with the increase of pH value, the fluorescence of RF enhanced. The reason could be that the surface of graphene oxide is more negatively charged when pH value is increased, leading to electrostatic repulsion between DNA

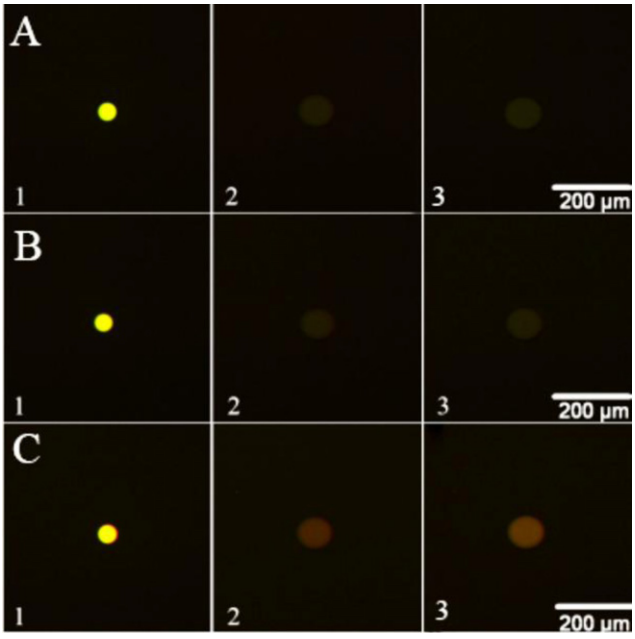

Fig. 3. Color responses of RF–GO droplet in the absence and presence of target DNAs under different VRs of ROX and FAM in RF probe. (A) color responses of 1:1 RF (A1), RF–GO droplet in the absence (A2) and presence of target DNAs T7 and T5 (A3). (B and C) Color responses of 2:1 and 3:1 RF (B1 and C1), RF–GO droplet in the absence (B2 and C2) and presence of target DNAs T7 and T5 (B3 and C3). The concentration of FAM of RF probe was 2.5×10^{-5} M. The concentrations of ROX from A to C were 2.5×10^{-5}, 5×10^{-5} and 7.5×10^{-5} M, respectively. The concentrations of target DNAs were 1×10^{-6} M. The pH value of buffer was 7.4. Other experimental conditions were the same as in Fig. 2.

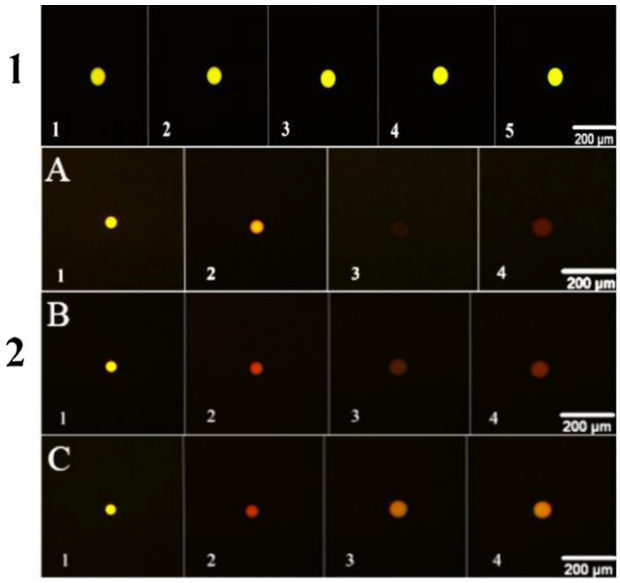

Fig. 4. (1) Effect of the pH value of buffer on the fluorescence of RF probe with the VR of RF/buffer at 1:1. The pH values from 1 to 5 were 4.4, 5.4, 6.4, 7.4 and 8.0; (2) the comparisons of color responses of RF–GO droplet in the absence and presence of target DNA under different pH values. The pH values of buffer were 5.4, 6.4 and 7.4 in (A)–(C), respectively. The VR of ROX/FAM in RF probe is 3:1. The images from 1 to 4 presented the color of the RF, RF–GO, RF-buffer-GO and RF-target DNAs-GO droplets, respectively. Other experimental conditions were the same as in Fig. 2.

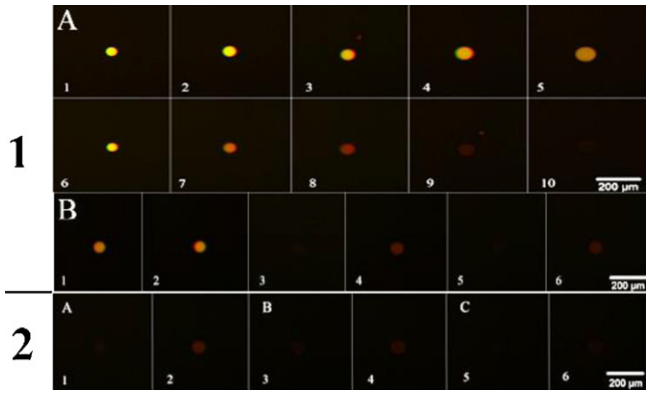

Fig. 5. (1) Quenching effect of the droplet size of GO on the fluorescence of RF probe. The VR of RF/GO were 1:1, 1:2, 1:3, 1:4 and 1:5 from 6 to 10 in (A). The droplets from 1 to 5 presented the color of RF fused with buffer with the corresponding VR. (B) The color responses of RF–GO droplet in the absence and presence of target DNAs under different VR of RF/GO (B1 and B2) 1:1, (B3 and B4) 1:2, and (B5 and B6) 1:3. (2) Effect of the droplet size of target DNAs on the fluorescence recovery of RF. The VR of RF/buffer (target)/GO were 1:1:2 (1 and 2), 1:2:2 (3 and 4), and 1:3:2 (5 and 6), respectively. Other experimental conditions were the same as in Fig. 2.

and graphene oxide [29]. As a result, the fluorescence of RF was hard to be quenched effectively. Moreover, the range of color change could also become narrow. To avoid the recovery processes of RF affected by the buffer, the pH was further investigated (Fig. 4(2)). With the addition of buffer and target DNAs to the RF–GO droplet, the difference in color change was observed clearly when the pH of buffer was 5.4. In order to facilitate the DNA hybridization, the pH 5.4 was chosen for the experiments without exploring the lower pH value. Besides, the amount of GO would affect the sensitivity of this experiment. It was necessary to investigate the VR between the RF and GO and the results were shown in Fig. 5(1A). It was seen that the fluorescence of RF was quenched obviously when the VR was up to 1:2. Although the RF was quenched completely with the increase of VR, the fluorescence of RF could be too weak to obtain good sensitivity. Thus the quenching and recovery processes were carried out together with the VR of RF/GO at 1:1, 1:2 and 1:3 (Fig. 5(1B)). Evidently, when the VR was 1:2, the color change of droplets in the absence and presence of target DNAs was greater than that of the VR at 1:1 and 1:3. The VR of RF and GO was chosen as 1:2. The fluorescence recovery of RF depended on the amount of target DNAs added in droplet because the specifically binding of RF probe and target DNAs could set the RF free from the surface of GO. Therefore, the effect of the droplet size of target DNAs on the fluorescence recovery was explored in Fig. 5(2). When the RF–GO droplet was fused into the buffer droplet and target DNAs droplet, respectively, a significant color change was found. On the contrary, the recovery effect became bad when the droplet size continued to increase. The reason might be that the concentration of RF probe were decreased greatly and caused weak fluorescence signals. Thus, the VRs of RF/GO/target DNAs or buffer were chosen as 1:2:1. In addition, the effect of mixing order of droplets was investigated on this platform. As shown in Fig. 6, a great difference in color change were observed by the RF-target DNAs-GO mode. The results might be caused by that the RF probe and the low concentration target DNAs cannot be fully hybridizated with the short time when RF probe was strongly absorbed on the surface of GO. In summary, the RF-target DNAs-GO mode was employed for this colorimetric droplet sensor with the VRs at 1:1:2.

3.3. The droplet-based colorimetric sensor for DNA analysis

To further demonstrate the viability of the colorimetric droplet biosensor, the experiments for the simultaneous determination of DNA in visualization were carried out and the results were shown in Fig. 7. With the increase of added target DNAs concentration into the RF–GO droplets, a series of color variation in droplet appeared (Fig. 7A). The light red color appeared firstly, and then became deep gradually, finally turned to bright orange. By

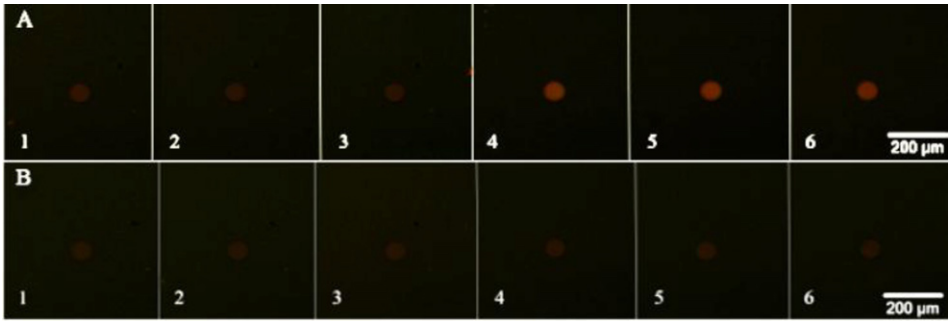

Fig. 6. Comparisons in color change of RF–GO droplet in the absence (1–3) and presence (4–6) of target DNAs with a different mixing order of reagents: RF-buffer/target-GO (A), and RF–GO-buffer/target (B). Each condition was repeated three times. Other experimental conditions were the same as in Fig. 5.

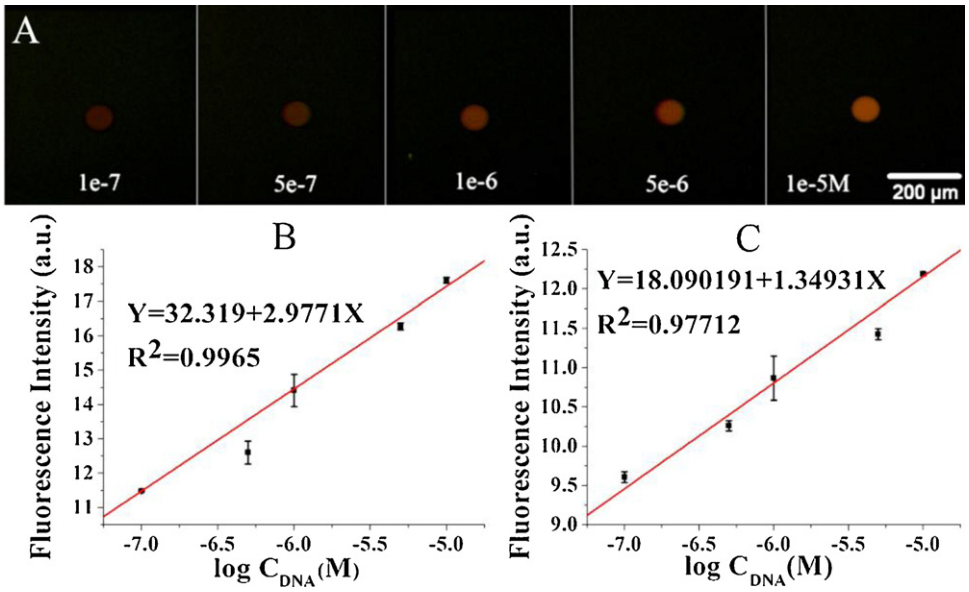

Fig. 7. The relationships between the fluorescence intensities and the concentrations of target DNAs: (A) the color responses of RF–GO droplet with different concentrations of target DNAs T5 and T7, (B) the relationships between the fluorescence intensities and the concentrations of target DNA T7 and (C) the relationships between the fluorescence intensities and the concentrations of target DNA T5. Other experimental conditions were the same as in Fig. 2.

comparing the color change of droplet intuitively, as low as 10^{-7} M target DNAs T7 and T5 could be identified. The relative standard deviation for 5 replicate measurements of target DNAs T7 and T5 at 5×10^{-6} M were 10% and 7.1%, respectively. Additionally, the fluorescence intensities were linearly related to the logarithm of target DNAs concentration ($\log C_{DNA}$) over the range from 10^{-7} to 10^{-5} M (Fig. 7B and C). The limits of detection for T7 and T5 were calculated as 9.46 and 9.67×10^{-8} M, respectively. These results suggested that this unique platform combining with a simple colorimetric method had great potential in quantitative detection of nucleic acid. Although the sensitivity of this DNA sensor is not enough good, it will be able to improve via coating GO with some chemical reagents and signal amplification method [29–31]. In a word, this platform could be expanded to apply in high throughput DNA analysis and simultaneous assay of other biomolecules.

3.4. The specificity of the colorimetric droplet sensor for DNA analysis

Finally, the specificity of this droplet sensor was evaluated in the presence of the two-based mismatched target DNA M2T5, single-base mismatched target DNA M1T5 and complementary target DNA T5. The corresponding results were shown in Fig. 8. It was seen that a deep orange color was found upon the fusion of T5 into RF–GO droplet. It was worthwhile to point out that the addition of M2T5 or M1T5 only lead to a light color. The significant color differences between MT and T5 were caused by the weak hybridization of mismatched target DNA with RF probe. The results had thus clearly illustrated that the colorimetric droplet sensor for DNA analysis could differentiate the mismatched target DNA and complementary target DNA. It had a promising in high throughput single-nucleotide polymorphism analysis.

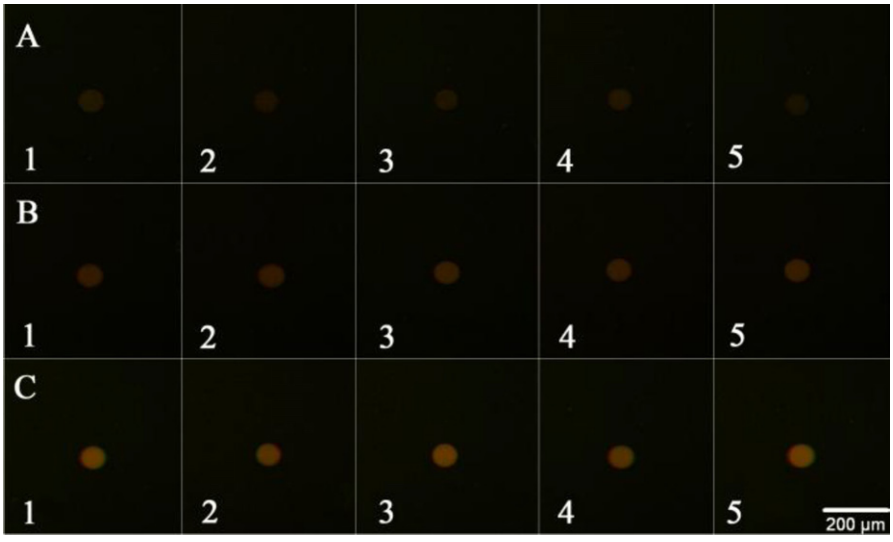

Fig. 8. Color responses of RF–GO droplet with the addition of the target DNAs: (A) two-based mismatched target DNA M2T5, (B) single-base mismatched target DNA M1T5 and (C) target DNA T5. The concentrations of DNA were 1×10^{-6} M. Each condition was repeated five times. Other experimental conditions were the same as Fig. 2.

4. Conclusions

In conclusion, a colorimetric biosensor for multiplexed DNA determination is first presented via a droplet platform based on the unique characteristic with the different affinities toward ssDNA and dsDNA of GO. Compared with those reports of DNA analysis in droplet, this platform can not only quantitatively detect multiplexed DNA but also provide a low-cost, visual and simple detection method for other droplet biosensor. Moreover, there are some ideal features in this droplet biosensor: short assay time, free of the immobilization of probe and incubation of target DNA, and low reagents consumption. On the basis of the principle mentioned above, the colorimetric droplet platform has a great potential as a detection tool in high throughput biosensor.

Acknowledgments

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References

[1] C.A. Mirkin, N.L. Rosi, D.A. Giljohann, C.S. Thaxton, A.K.R. Lytton-Jean, M.S. Han, Science 312 (2006) 1027–1030.
[2] R.K.P. Benninger, O. Hofmann, B. Onfelt, I. Munro, C. Dunsby, D.M. Davis, M.A.A. Neil, P.M.W. French, A.J. de Mello, Angew. Chem. Int. Ed. 46 (2007) 8536.
[3] J. Jung, L.X. Chen, S. Lee, S. Kim, G.H. Seong, J. Choo, E.K. Lee, C.H. Oh, S. Lee, Anal. Bioanal. Chem. 387 (2007) 2609–2615.
[4] K.H. Yea, S. Lee, J. Choo, C.H. Oh, S.H. Lee, Chem. Commun. 14 (2006) 1509–1511.
[5] L.B. Pinheiro, V.A. Coleman, C.M. Hindson, J. Herrmann, B.J. Hindson, S. Bhat, K.R. Emslie, Anal. Chem. 84 (2012) 1003–1011.
[6] Y. Zhang, S. Park, K. Liu, J. Tsuan, S. Yang, T.H. Wang, Lab Chip 11 (2011) 398–406.
[7] Y. Zhang, V. Bailey, C.M. Puleo, H. Easwaran, E. Griffiths, J.G. Herman, S.B. Baylin, T.H. Wang, Lab Chip 9 (2009) 1059–1064.
[8] L. Mazutis, A.F. Araghi, O.J. Miller, J.C. Baret, L. Frenz, A. Janoshazi, V. Taly, B.J. Miller, J.B. Hutchison, D. Link, A.D. Griffiths, M. Ryckelynck, Anal. Chem. 81 (2009) 4813–4821.
[9] J. Sun, Y. Zheng, X.J. Feng, W. Du, B.F. Liu, Anal. Chim. Acta 721 (2012) 104–109.
[10] H. Schroder, L. Hoffmann, J. Muller, P. Alhorn, M. Fleger, A. Neyer, C.M. Niemeyer, Small 5 (2009) 1547–1552.
[11] M.C. morales, J.D. Zahn, Microfluid. Nanofluid. 9 (2010) 1041–1049.
[12] A.L. Markey, S. Mohr, P.J.R. Day, Method 50 (2010) 277–281.
[13] M. Srisa-Art, D.A.J. Demello, J.B. Edel, Chem. Commun. 43 (2009) 6548–6550.
[14] Y.H. Zhang, P. Ozdemir, Anal. Chim. Acta 638 (2009) 115–125.
[15] M. Srisa-Art, D.A.J. Demello, J.B. Edel, Anal. Chem. 79 (2007) 6682–6689.
[16] A.T.H. Hsieh, P.J.H. Pan, A.P. Lee, Microfluid. Nanofluid. 6 (2009) 391–401.
[17] R.C. Evans, P. Douglas, Anal. Chem. 78 (2006) 5645–5652.
[18] X.D. Wang, X. Chen, Z.X. Xie, X.R. Wang, Angew. Chem. Int. Ed. 47 (2008) 7450–7453.
[19] J.H. Choi, S.K. Lee, J.M. Lim, S.M. Yang, G.R. Yi, Lab Chip 10 (2010) 456–461.
[20] K. Churski, J. Michalski, P. Garstecki, Lab Chip 10 (2010) 512–518.
[21] S.J. Zeng, B.W. Li, X.O. Su, J.H. Qin, B.C. Lin, Lab Chip 9 (2009) 1340–1343.
[22] F. Guo, X.H. Ji, K. Liu, R.X. He, L.B. Zhao, Z.X. Guo, W. Liu, S.S. Guo, X.Z. Zhao, Appl. Phys. Lett. 96 (2010) 193701.
[23] X. Xiang, L. Chen, Q.G. Zhuang, X.H. Ji, Z.K. He, Biosens. Bioelectron. 32 (2012) 43–49.
[24] J.H. Jung, D.S. Cheon, F. Liu, K.B. Lee, T.S. Seo, Angew. Chem. Int. Ed. 49 (2010) 5708–5711.
[25] C.H. Lu, H.H. Yang, C.L. Zhu, X. Chen, G.N. Chen, Angew. Chem. Int. Ed. 48 (2009) 4785–4787.
[26] M. Luo, X. Chen, G.H. Zhou, X. Xiang, L. Chen, X.H. Ji, Z.K. He, Chem. Commun. 48 (2012) 1126–1128.
[27] S.J. He, B. Song, D. Li, C.F. Zhu, W.P. Qi, Y.Q. Wen, L.H. Wang, S.P. Song, H.P. Fang, C.H. Fan, Adv. Funct. Mater. 20 (2010) 453–459.
[28] L. Chen, X.W. Zhang, G.H. Zhou, X.H. Ji, Z.H. Zheng, Z.K. He, H.Z. Wang, Anal. Chem. 84 (2012) 3200–3207.
[29] L.F. Sheng, J.T. Ren, Y.Q. Miao, J.H. Wang, E.K. Wang, Biosens. Bioelectron. 26 (2011) 3494–3499.
[30] S. Bi, J.L. Zhang, S.S. Zhang, Chem. Commun. 46 (2010) 5509–5511.
[31] A.X. Zheng, J.R. Wang, J. Li, X.R. Song, G.N. Chen, H.H. Yang, Chem. Commun. 48 (2012) 374–376.