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A distance-based capillary biosensor using wettability alteration†

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Distance-based detection methods with a quantitative readout are of great significance to point-of-care testing (POCT), are low-cost and user-friendly, and can be integrated into portable analytical devices. Here, we submit a visual quantitative distance-based sensor by capillary force alteration in a capillary tube. This sensor converts the wettability alteration caused by the target molecules into a capillary rise height signal. Moreover, the sensor profits from isothermal amplification technology, achieving the detection of miRNAs with high sensitivity and specificity by visually reading the height of the water in the capillary tube. The proposed biosensor shows great potential in routine clinical diagnosis as well as POCT in resource-limited settings.

1. Introduction

In the past few decades, point-of-care testing (POCT) has achieved rapid development and provides real-time diagnostic approaches in home health care, accident points and emergency situations.[1,2] Compared with traditional POCT devices, a distance-based measurement is an alternative for a quantitative readout, which relies on reading a visual signal length that is developed linearly with a target concentration.[3,4] In distance-based measurements, one common method is translating a molecular signal into a coloured band relying on biochemical reactions, and the length of the coloured band is related to the amount of the target.[5,6] Another way to integrate a distance-based readout is based on volumetric expansion from gas generation reactions.[7,8] Moreover, the dynamic range can be adjusted by changing the amount of a specific reagent and the design of the chip, further adding to the flexibility of devices with a distance-based readout.[5,9] However, the existing distance-based detection methods still rely on high color resolution (coloured band method) or very high specification air tightness (volumetric expansion method).[10,11] Therefore, it is highly desirable to continue the development of devices with a distance-based readout applicable in a variety of fields.

Capillary action describes the ability of a liquid flowing against gravity in a narrow space such as a capillary tube, and the capillary force is affected strongly by the wetting angle.[12,13] The wettability variation in the capillary inner wall will change the surface tension of the solution in the vertical direction, achieving control of the capillary rise height.[14] External stimuli, such as light illumination, temperature, solvents, electrical potential, pH, and others, can change the surface conformation and/or morphology of stimuli-sensitive materials, which results in the change of wettability.[15,16] DNA is one of the most stimuli-sensitive materials because of its programmability, which derives from the predictable and stable pairs formed between the bases of complementary DNA strands. In addition, DNA is structurally stable, and its specific identifying characteristics have been widely studied in biosensors.[17]

In this work, we propose a distance-based capillary biosensor using editable DNA to control the capillary force. The DNA molecule modified with hydrophobic molecules at the end (hydrophobic DNA) is grafted on the inner wall of the capillary. When the target molecule is present, the neck loop structure of auxiliary DNA will be opened by the target to form a double-stranded DNA structure. The cleavage site in the hydrophobic DNA chain is recognized by the double-stranded DNA structure and enzyme. Then, the hydrophobic group is cut off by the enzyme, which causes the wettability variation of the inner wall of the capillary. The wettability variation could change the capillary force of the vertical capillary tube and results in different capillary heights. The capillary sensor device has a series of technical advantages such as strong practicability, low price, and simple

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operation, which provides a new idea for the design and manufacture of POCT devices.

2. Results and discussion

2.1. The design strategy of the sensor device

Herein, microRNA-21 is used as a target molecule model to verify the sensing device. Small regulatory RNA microRNA-21 (miRNA-21) plays a crucial role in a plethora of biological functions and diseases including cancer, cardiovascular diseases and inflammation.[18–20] In recent years, researchers have developed many highly sensitive detection methods for miRNA-21 and the development of portable and easy-to-use miRNA sensors is still one of the directions of researchers.[21–23] As shown in Fig. 1, we design a cyclic amplification experiment to cut the hydrophobic DNA (a single DNA strand functionalized by a hydrophobic fluorescent group) on the capillary inner wall to control the its wettability, and then use pure water to test the capillary rise height. This concept is a highly promising method for point-of-care assay and field applications without a bulky conventional analyzer.

The procedure of making and using the capillary biosensor is shown in Fig. 2. In order to avoid the influence of proteins and cells in the complex sample system on capillary behavior, the use of the sensing device is divided into two steps. Firstly, a solution system containing targets and auxiliary DNA molecules is filled into the capillary and reacts with the hydrophobic DNA on the inner wall of the capillary tube. The target molecule acts as a trigger for cleaving the hydrophobic DNA on the inner wall of the capillary tube and changes its infiltration properties. Subsequently, the solution in the capillary tube is rinsed off, and the capillary tube is blown dry with nitrogen. Secondly, the capillary tube is vertically in contact with pure water, and the capillary rise height is recorded to achieve the purpose of detection.

2.2. Feasibility analysis of the sensor device

We assess the factors that influence the capillary rise height. According to the Young–Laplace equation,[24] we can calculate the capillary rise height as:

$$h = \frac{2\gamma \cos\theta}{\rho g r}, \tag{1}$$

where γ and ρ are the surface tension and density of the liquid, respectively, θ is the advancing contact angle of the liquid on the solid, r is the mean static radius of the pores, g is the acceleration due to gravity, and h is the height reached by the liquid.[25,26] According to the formula of the capillary rise height, the contact angle is one of the critical parameters to adjust the capillary rise height.

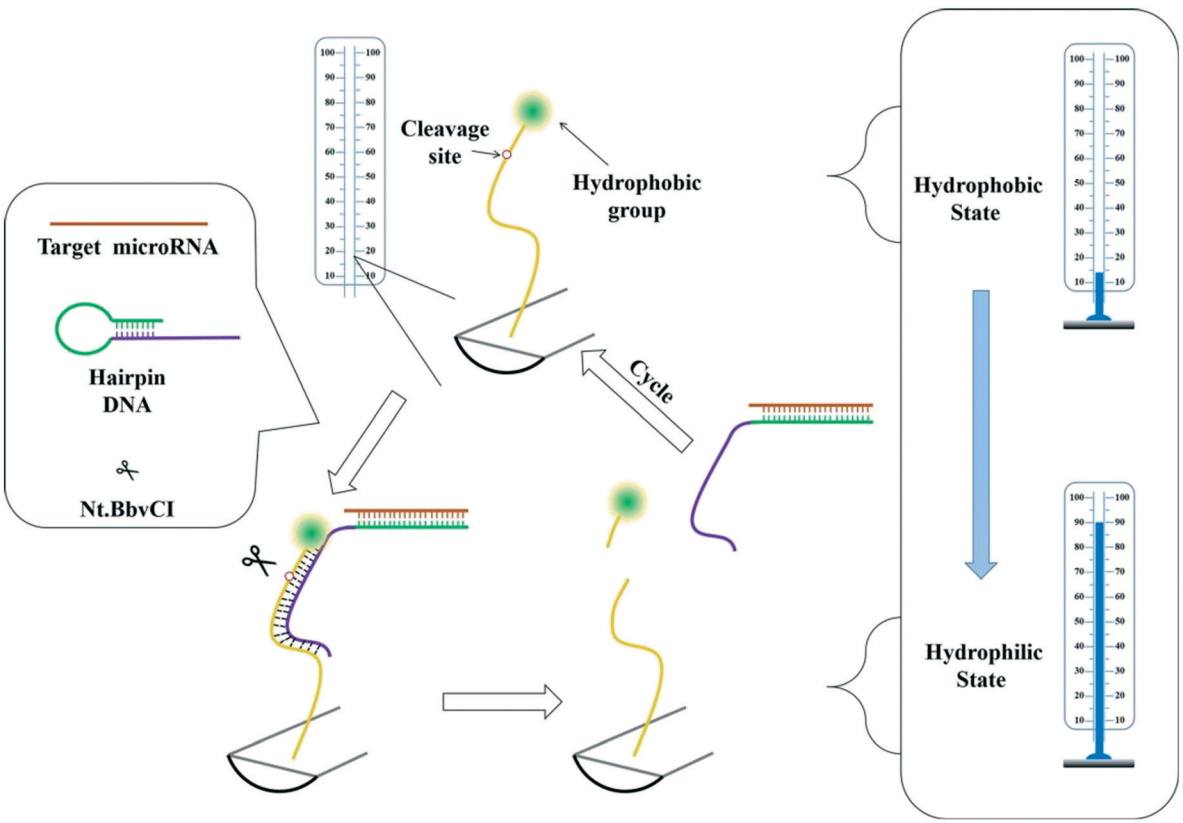

Fig. 1 The principle of visible quantitative detection of miRNA using the capillary sensor.

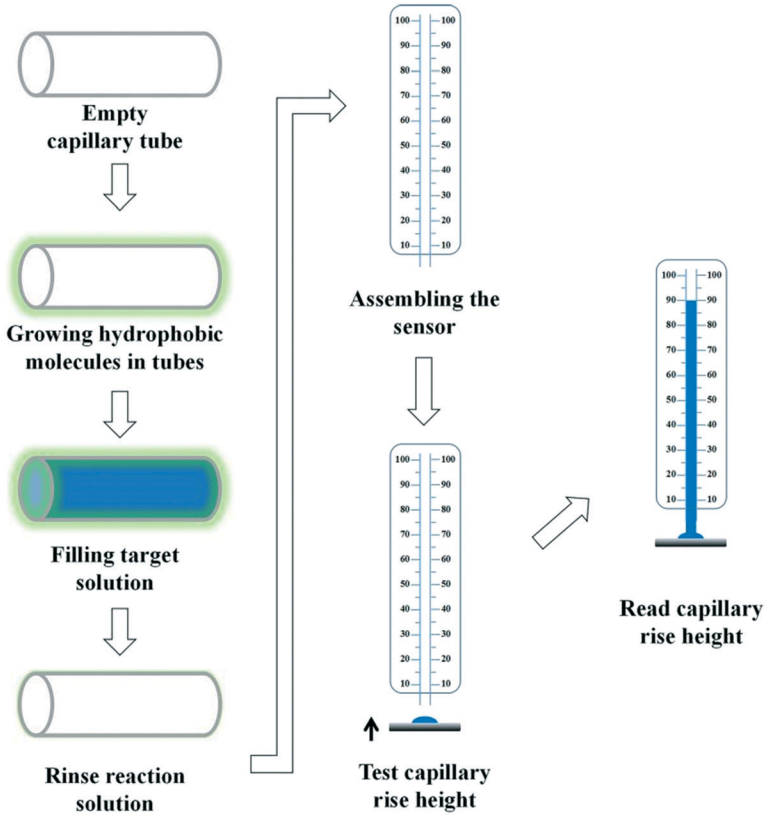

Fig. 2 The preparation process of the capillary sensor. First, hydrophobic DNA molecules are modified on the inner walls of a clean glass capillary. Then the target solution is filled into the capillary. The capillary is subsequently cleaned and blown dry with N_2 after reaction. Finally, the capillary tube is fixed on a scale substrate to complete the preparation of the capillary sensor and then the capillary rise height is recorded as the final detection signal.

2.3. Hydrophobic DNA is modified into the capillary tube

Grafting hydrophobic DNA on the inner wall of the capillary is a critical step in the capillary rise experiment. The process of synthetic grafting on the capillary tube is shown in Fig. S1.† In order to allow more hydrophobic DNA strands to be grafted on the inner wall of the capillary, we optimize the pH and time of the grafting reaction. In the experiment of optimizing the pH, we use a sodium carbonate/sodium bicarbonate buffer system to prepare a buffer system with pH = 7, 9, and 11. Different hydrophobic DNA buffer solutions are filled into an epoxy-modified capillary. Then, the capillary is transferred to an oven at 60 °C for the reaction for 30 min. The fluorescence photos of the modified capillary in Fig. S2† indicate that functional DNA is modified on the capillary inner wall. And the fluorescence intensities of the capillary reflect the modified density of functional DNA. Lastly, the pH of the buffer system for modification of functional DNA is determined to be 9.

We further optimize the grafting reaction time. The capillary tube is filled with hydrophobic DNA reaction solution and then washed after a certain time. The fluorescence intensities of the capillary are measured using a fluorescence imaging system. As shown in Fig. S3,† the fluorescence intensity is the strongest when the reaction time is 60 min. So we choose pH = 9 and 60 min as the conditions of the grafting reaction.

Then we verify that the hydrophobic DNA is successfully grafted on the inner wall of the capillary through the capillary rise height (Video S1 and S2†) and fluorescence images. The modified capillary tube is fixed on a graduated cardboard to complete the preparation of the capillary biosensor. Compared with the capillary rise height before modification (Fig. 3A, 6.7 cm), the height is significantly reduced (Fig. 3B, 1.9 cm) after grafting hydrophobic DNA. The modified capillary tube is placed under a confocal microscope, and it is found that a layer of green fluorescent substance is attached to the capillary inner wall (Fig. 3C). This phenomenon indicates that hydrophobic DNA has been successfully grafted on the capillary inner wall, and it can cause changes in the capillary rise height.

2.4. Optimization of the detection conditions

Nb·BbvCI is a kind of endonuclease that can specifically recognize the corresponding DNA sequence.[27,28] In this work, the added miRNA-21 can be paired with the neck loop structure of auxiliary DNA to form a double-stranded DNA structure. The opened strand of auxiliary DNA can be combined with hydrophobic DNA fixed on the capillary inner wall to form the structural site that can be recognized by the Nb·BbvCI enzyme. After the cleavage is completed, the double-stranded composite structure is separated from the

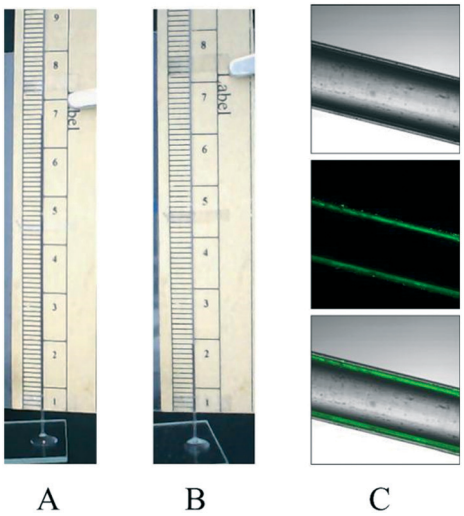

Fig. 3 Characterization of hydrophobic DNA grafted on the glass capillary wall. (A) Capillary tube before modification by hydrophobic DNA and (B) the capillary tube after modification by hydrophobic DNA. (C) Fluorescence images of the capillary tube modified by hydrophobic DNA (top: no excitation light, middle: dark field fluorescence and bottom: composite figure).

hydrophobic DNA and Nb·BbvCI continues to combine with the hydrophobic DNA strands to cut more hydrophobic DNA strands to achieve the effect of signal amplification.

The triggering of the enzyme digestion reaction requires the addition of the target molecule, and the efficiency of the enzyme digestion reaction will be affected by many conditions. Therefore, we optimize the time of enzyme digestion reaction and the amount of enzyme. 10 nM miRNA-21 is used as the detection solution to optimize the conditions. It can be observed in Fig. S4A† that as the reaction time increases, the capillary height rises gradually until the reaction time is 60 min, where it basically rises to the highest position. During the amplification reaction of the enzyme-assisted chains, the amount of enzyme will have a significant effect on the shear result. It can be seen from Fig. S4B† that in the 50 μL reaction solution when the additional amount of the cleaving enzyme (10 000 U mL^{-1}) is 1 μL, the capillary rise height basically reaches the highest position. Therefore, we used 60 min and 1 μL of the enzyme as the detection conditions.

2.5. Quantitative detection performance of the capillary sensor device

To further verify the practicability of this method, we prepare glass slides and capillary tubes modified with hydrophobic DNA. The target miRNA-21 (1 nM) is used to react with the modified glass slides, and the results of the contact angle are shown in Fig. S5.† The contact angle of the slides is 82° (before the reaction) and 52° (after the reaction), indicating that miRNA-21 could change the infiltration properties of the glass slide surface modified by hydrophobic DNA. After that, we use capillary tubes to react with miRNA-21 solutions at different concentrations. Then, these capillary tubes are vertically in contact with 10 μL pure water, and the capillary rise height is recorded after 5 minutes (Fig. 4A). The capillary rise height is linearly related to the miRNA-21 concentration in the range of 10^{-13} M to 10^{-8} M, as shown in Fig. 4B. Different concentrations of miRNA will cause various degrees of wettability variation of the capillary inner wall, and this change is clearly reflected in the visible distance signal of the capillary rise. These results confirm the feasibility of quantitative detection of miRNA based on the wettability variation of the capillary inner wall.

The specificity of the signal response is an essential factor in evaluating the sensors. The specificity of this method is verified by measuring the signals of the other three miRNA sequences (miRNA16, miRNA24, and miRNA26a) using the capillary biosensor. We found that the capillary rise height of other three miRNAs (10 nM) is basically consistent with that of 0 nM miRNA (Blank). However, the capillary rise height of 4.8 cm (10 nM miRNA21) is 2.4 times that of other miRNAs (Fig. 5). Therefore, this capillary sensor based on wettability response achieves good specificity, which is conducive to its stable work in complex environments.

2.6. Serum sample evaluation

To further evaluate the practicality of this biosensor, we test miRNA21 in serum. First, the serum is diluted with a 9-fold volume of buffer. And then, miRNA21 is added at different concentrations, which is used as a sample solution to be detected. The concentration of miRNA21 in the serum is detected using the capillary biosensor, which is basically consistent with the real concentration of miRNA21 (Fig. S6†). The result indicates that the biosensor has successfully achieved the detection of miRNA21 in the serum and has great potential in clinical applications.

3. Conclusion

In this work, we realize a distance-based biosensor through capillary action. The behavior of the capillarity self-driving rise is effectively regulated by the wettability alteration of the capillary inner wall. There are several advantages of the biosensor. First, the biosensor no longer relies too much on good air tightness and the user's color recognition ability. Second, the developed biosensor uses an easily available glass capillary without requiring additional manufacturing technology. And the cost of a glass capillary is less than 0.01 US dollars, which is much cheaper than that of other precision chips. Third, the new method provides quantitative results without any energy consumption. Moreover, as more and more smart molecules with wettability alteration are discovered, this biosensor will be further developed. Therefore, the biosensor is expected to be widely applied in visual quantitative analysis.

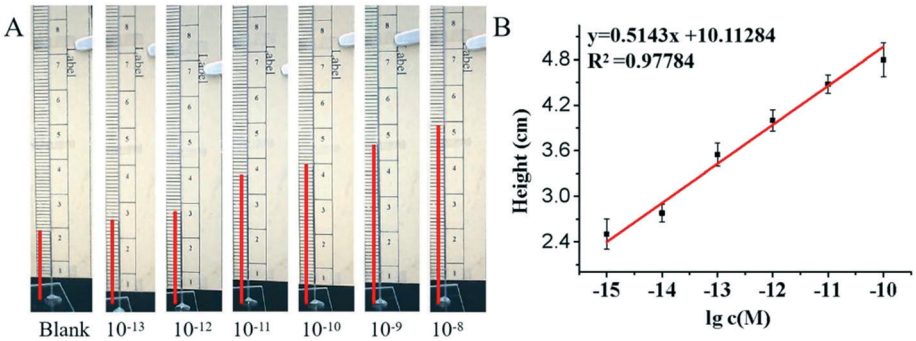

Fig. 4 (A) The result of 10^{-13}–10^{-8} M miRNA detection using the capillary biosensor. (B) Linearity between the capillary rise height and the miRNA concentration, with an R^2 value of 0.98.

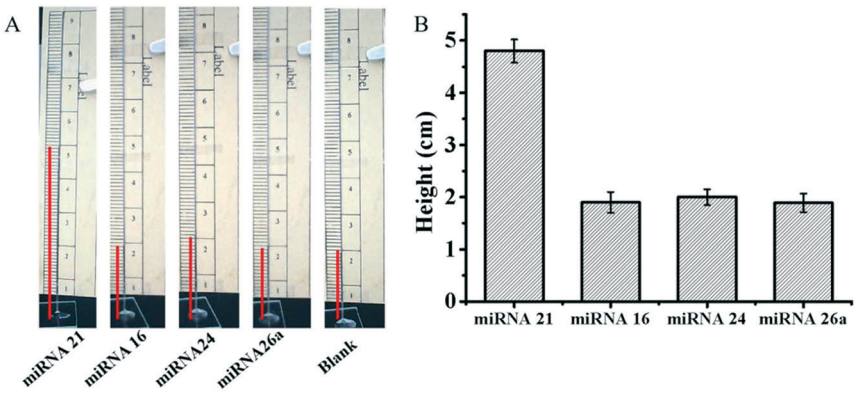

Fig. 5 Specificity of the capillary sensor with miRNA21 (10 nM), miRNA16 (10 nM), miRNA24 (10 nM), and miRNA 26a (10 nM). Representative photographs (A) and statistical results (B) of the capillary rise height.

Experimental section

Materials

KH560 (γ-(2,3-glycidoxy)propyltrimethoxysilane), MTMS (methyltrimethoxysilane), and toluene were purchased from Sigma; ethanol was purchased from Sinopharm Holding Co., Ltd. (Shanghai); and sodium chloride and magnesium chloride were purchased from Beijing Chemical Factory (Beijing). The hydrophobic DNA strands used in the experiment (referring to the functional DNA strands modified with the hydrophobic groups at one end) were purchased from Bao Bioengineering (Dalian) Co., Ltd. Other nucleic acid chains were purchased from Biotech Bioengineering (Shanghai) Co., Ltd. The base sequence was shown in Table S1.† The water employed in the experiment was ultra-pure Milli-Q water (resistance >18 MΩ cm^{-1}). Glass capillaries produced by Changzhou Kejing Glass Co., Ltd. were utilized. The inner diameter of these capillaries was 200 ± 5 μm.

Preparation of the functional glass capillary

The capillary glass was cleaned with piranha solution (a mixture of 7 : 3 (v/v) 98% H_2SO_4 and 30% H_2O_2). Then the cleaned capillary tube was immersed into a solution of 1% volume ratio of KH560 and MTMS absolute ethanol. After reacting at 80 °C for 4 h, an epoxy-modified glass capillary was prepared, then washed with absolute ethanol, dried with high-purity nitrogen and sealed for storage.

Capillary modified by hydrophobic DNA

The sequences of the DNA strands used in the experiments are shown in Table S1.† We used a sodium carbonate/sodium bicarbonate buffer system as a solvent for hydrophobic DNA strands to prepare a 1 μM nucleic acid solution, filled it into a capillary tube, and then let it react at 60 °C for a specific time to develop a functional capillary tube with hydrophobic DNA. The chemical grafting process is illustrated in Fig. S1.†

MiRNA-21 detection procedure using the capillary sensor

With the addition of the target miRNA-21, the cleavage of the hydrophobic DNA fragments was triggered. The specific operation is as follows: first, we added 1 μL of neck loop DNA solution, 1 μL of Nb·BbvCI cleaving enzyme, 5 μL of CutSmart buffer and 31 μL of 100 mM PBS buffer solution, and then added 10 μL of the target miRNA-21 solution. Then, we filled the above solution into the functional capillary prepared in the previous step, sealed it and then stored it in a 35 °C environment for a specified period of time. After the reaction was completed, the solution in the capillary was

blown out and cleaned with pure water, and then we dried it with nitrogen. Afterwards the functional capillary tube was attached on a graduated cardboard to complete the capillary sensor, and we used the capillary reacted with the target RNA to perform the capillary rise experiment. The capillary rise height after 5 min was recorded as the final detection signal.

Author contributions

Conceptualization: Y. Li; data curation: Y. Li and Y. Wen; methodology: Y. Li, X. Men, G. Gao, Y. Tian, Y. Wen and X. Zhang; software: Y. Li; validation: Y. Li and Y. Wen; formal analysis: Y. Li and Y. Wen; investigation: Y. Li and Y. Wen; original draft preparation: Y. Li; writing, review and editing: Y. Li and Y. Wen; supervision: Y. Wen and X. Zhang; and funding acquisition: G. Gao, Y. Tian and Y. Wen.

Conflicts of interest

There are no conflicts to declare.

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References

1 S. Ghosh, K. Aggarwal, T. Vinitha, T. Nguyen, J. Han and C. H. Ahn, *Microsyst. Nanoeng.*, 2020, **6**, 1–18.
2 B. Gao, Y. Yang, J. Liao, B. He and H. Liu, *Lab Chip*, 2019, **19**, 3602–3608.
3 J. Xu, X. Hu, H. Khan, M. Tian and L. Yang, *Anal. Chim. Acta*, 2019, **1071**, 1–7.
4 T. Tian, J. Li, Y. Song, L. Zhou, Z. Zhu and C. J. Yang, *Lab Chip*, 2016, **16**, 1139–1151.
5 K. Maejima, Y. Hiruta and D. Citterio, *Anal. Chem.*, 2020, **92**, 4749–4754.
6 C. T. Gerold, E. Bakker and C. S. Henry, *Anal. Chem.*, 2018, **90**, 4894–4900.
7 D. Liu, T. Tian, X. Chen, Z. Lei, Y. Song, Y. Shi, T. Ji, Z. Zhu, L. Yang and C. Yang, *Analyst*, 2018, 1294–1304.
8 Y. Huang, L. Fang, Z. Zhu, Y. Ma, L. Zhou, X. Chen, D. Xu and C. Yang, *Biosens. Bioelectron.*, 2016, **85**, 496–502.
9 Y. Xie, X. Wei, Q. Yang, Z. Guan, D. Liu, X. Liu, L. Zhou, Z. Zhu, Z. Lin and C. Yang, *Chem. Commun.*, 2016, **52**, 13377–13380.
10 T. Tian, Y. An, Y. Wu, Y. Song, Z. Zhu and C. Yang, *ACS Appl. Mater. Interfaces*, 2017, **9**, 30480–30487.
11 Y. Chen, W. Chu, W. Liu and X. Guo, *Sens. Actuators, B*, 2018, **260**, 452–459.
12 E. W. Washburn, *Phys. Rev.*, 1921, **17**, 273.
13 N. Ichikawa and R. Maeda, *Microscale Thermophys. Eng.*, 2005, **9**, 237–254.
14 C. Lin, Z. Xiang, C. Song, Y. Lin, M. Xu, X. Wang, C. Wu and X. Y. Liu, *Adv. Mater. Interfaces*, 2018, 1701326.
15 F. Xia, Y. Zhu, L. Feng and L. Jiang, *Soft Matter*, 2009, **5**, 275–281.
16 S. H. Jung, K. Y. Kim, A. Ahn, M. Y. Choi, J. Jaworski and J. H. Jung, *ACS Appl. Mater. Interfaces*, 2016, **8**, 14102–14108.
17 H. Sun, Y. Qiu, Q. Liu, Q. Wang, Y. Huang, D. Wen, X. Zhang, Q. Liu, G. Liu and J. Kong, *Biosens. Bioelectron.*, 2019, **131**, 193–199.
18 J. A. Chan, A. M. Krichevsky and K. S. Kosik, *Cancer Res.*, 2005, **65**, 6029–6033.
19 V. P. Dave, T. A. Ngo, A.-K. Pernestig, D. Tilevik, K. Kant, T. Nguyen, A. Wolff and D. D. Bang, *Lab. Invest.*, 2019, **99**, 452–469.
20 F. Meng, R. Henson, H. Wehbe–Janek, K. Ghoshal, S. T. Jacob and T. Patel, *Gastroenterology*, 2007, **133**, 647–658.
21 H. Wang, H. Wang, Y. Li, C. Jiang, D. Chen, Y. Wen and Z. Li, *Sens. Actuators, B*, 2020, 128036.
22 R. Kumarswamy, I. Volkmann and T. Thum, *RNA Biol.*, 2011, **8**, 706–713.
23 J. Lu, J. Wang, X. Hu, E. Gyimah, S. Yakubu, K. Wang, X. Wu and Z. Zhang, *Anal. Chem.*, 2019, **91**, 7353–7359.
24 T. Chen, M.-S. Chiu and C.-N. Weng, *J. Appl. Phys.*, 2006, **100**, 074308.
25 B. Zhmud, F. Tiberg and K. Hallstensson, *J. Colloid Interface Sci.*, 2000, **228**, 263–269.
26 J. Cai, B. Yu, M. Mei and L. Luo, *Chin. Phys. Lett.*, 2010, **27**, 054701.
27 A. Zheng, J. Wang, J. Li, X. Song, G. Chen and H. Yang, *Chem. Commun.*, 2011, **48**, 374–376.
28 H. Dong, X. Meng, W. Dai, Y. Cao, H. Lu, S. Zhou and X. Zhang, *Anal. Chem.*, 2015, **87**, 4334–4340.

