Supplementary Materials

A Microfluidic Biosensor Based on Magnetic Nanoparticle Separation, Quantum Dots Labeling and MnO2 Nanoflower Amplification for Rapid and Sensitive Detection of *Salmonella* Typhimurium

Li Hao 1, Li Xue 2, Fengchun Huang 2, Gaozhe Cai 2, Wuzhen Qi 1, Miao Zhang 2, Qing’an Han 3, Zengli Wang 3 and Jianhan Lin 1,2,\*

1 Key Laboratory of Agricultural Information Acquisition Technology, Ministry of Agriculture and Rural Affairs, China Agricultural University, Beijing 100083, China; [haoli123@cau.edu.cn](mailto:haoli123@cau.edu.cn) (L.H.); [wuzhen.qi@cau.edu.cn](mailto:wuzhen.qi@cau.edu.cn) (W.Q.)

2 Key Laboratory of Modern Precision Agriculture System Integration Research, Ministry of Education, China Agricultural University, Beijing 100083, China; [li\_xue@cau.edu.cn (L.X.)](mailto:li_xue@cau.edu.cn%20(L.X.)); [fengchunhuang@cau.edu.cn (F.H.)](mailto:fengchunhuang@cau.edu.cn(F.H.)); gaozhe@cau.edu.cn (G.C.); [zhangmiao@cau.edu.cn (M.Z.)](mailto:zhangmiao@cau.edu.cn%20(M.Z.))

3 Veterinary Laboratory, Hebei Animal Disease Control Center, Shijiazhuang 050035, China; [hanqingan2004@sina.com (Q.H.)](mailto:hanqingan2004@sina.com%20(Q.H.)); [wangzlh891220@163.com](mailto:wangzlh891220@163.com) (Z.W.)

**\*** Correspondence: jianhan@cau.edu.cn, Tel.: +86-10-6273-7599

Received: 15 February 2020; Accepted: 05 March 2020; Published: date

1. Preparation of the bacteria

In this study, *Salmonella* Typhimurium (ATCC 14028) was used as target bacteria, *E. coli* O157:H7 (ATCC 43888), *Listeria monocytogenes* (ATCC13932), *Staphylococcus* aureus (CICC10001), *Bacillus cereus* (CICC10041) and *Vibrio cholera* (CICC23795) from China Center of Industrial Culture Collection (CICC) were used as the non-target bacteria for evaluating the specificity of the proposed biosensor. All bacteria were stored at −20 °C with 15% glycerol, which were first revived in Luria-Bertani (LB) medium at 37 °C with shaking at 180 rpm for 12 hours respectively. Then, they were serially 10-fold diluted with the sterile PBS to obtain concentrations from 101 to 106 CFU/mL.

2. Preparation of the MnO2 NFs

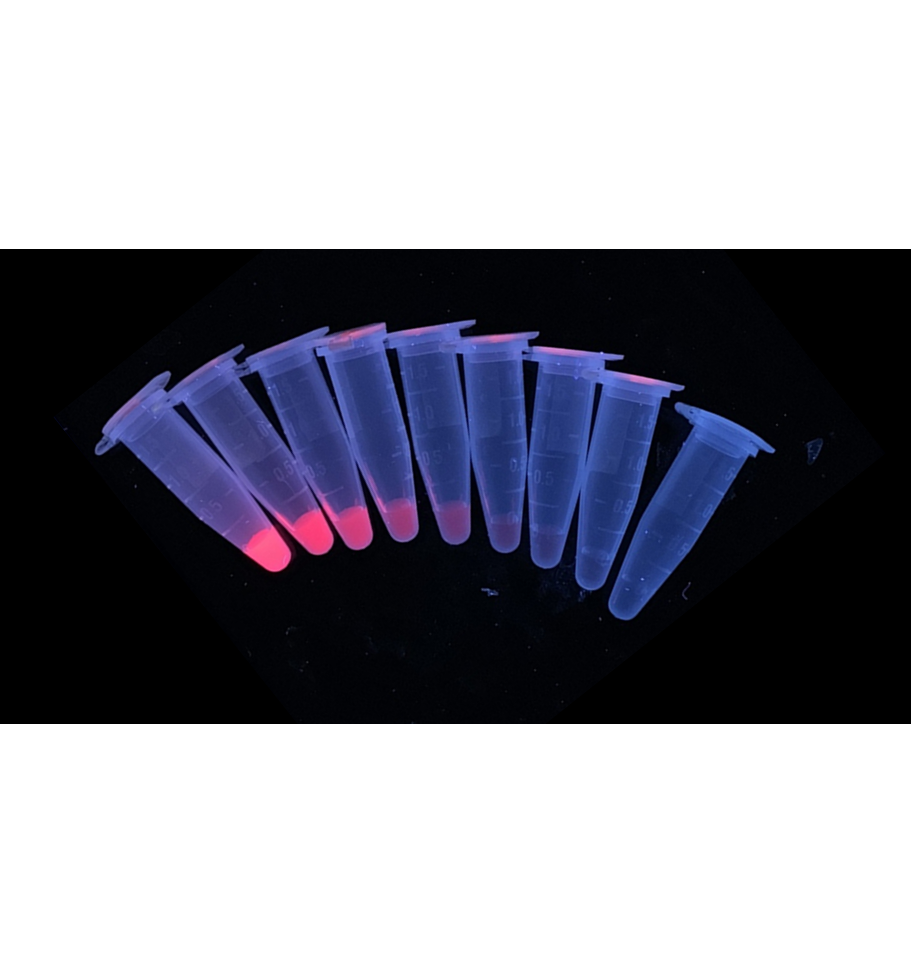
Briefly, 100 mL 0.1 mol/L of KMnO4 solution and 0.0261 g of polyvinyl pyrrolidone (PVP) were dissolved in 10 mL of distilled water under vigorous stirring, then HCl (100 mL, 0.2 M) was added into the mixture when the temperature rose to 90 °C. The precipitates were synthesized and cooled to room temperature after reacting for 1 h. Then, the precipitates were washed with distilled water for several times and dissolved in deionized water. The MnO2 NFs were stored at 4 °C for further use.

3. Preparation of the MnO2 NFs

Different amounts of quantum dots (QDs) were used to synthesize the MnO2-QD NFs. The QDs were released by the same glutathione (GSH) and measured to optimize the amount of QDs. As shown in Figure S2a, the mean fluorescent intensity of the released QDs increases from about 300 counts to about 800 counts when the amount of QDs changes from 0 to 50 pmol. Further increase on the amount to 70 pmol does not result in obvious increase on the intensity. Thus, the optimal amount of 50 pmol for the QDs was used to synthesize the MnO2-QD NFs in this study.

Different amounts (MnO2 NFs: from 0.02 mg to 0.06 mg) of the MnO2-QD-pAb NFs were used to detect the target bacteria with the concentration of 1.0 × 105 CFU/mL at the optimal conditions and the fluorescent intensity of the released QDs was measured at the characteristic wavelength of 661 nm. As shown in Figure S2b, the mean fluorescent intensity increases from about 200 counts to about 560 counts when the amount of the MnO2-QD-pAb NFs changes from 0 μg to 50 μg, and the intensity remains at the same level when the amount further increases to 60 μg. Thus, the optimal amount of 50 μg for the MnO2-QD-pAb NFs was used in this study.

The MnO2-QD-pAb NFs and the mixture of the immune MNPs and the target bacteria at the concentration of 1.0 × 105 CFU/mL were injected into the microfluidic chip with different flow rates from 20 μL/min to 40 μL/min at the optimal conditions. As shown in Figure S2c, the mean fluorescent intensity increases from about 400 counts to about 670 counts when the flow rate decreases from to 40 μL/min to 25 μL/min and does not increase obviously when the flow rate further decreases to 20 μL/min. Thus, the optimal flow rate of 25 μL/min was used in this study.



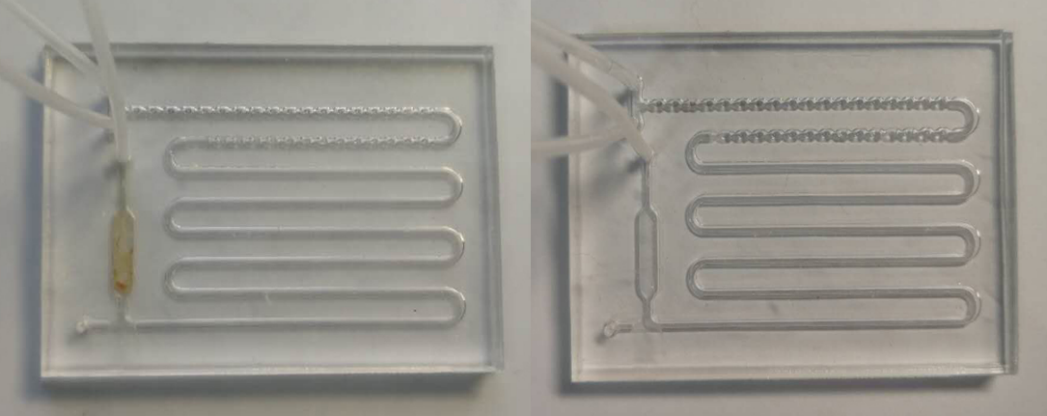
**Figure S1.** The fluorescence of the released quantum dots (QDs) on 2-fold dilutions of the MnO2-QD NFs.

(**a**)

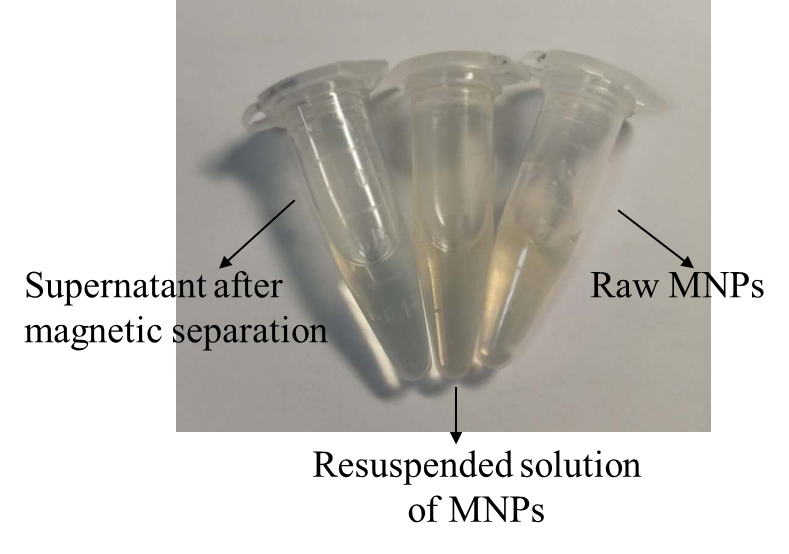
(**b**)

(**c**)

**Figure S2.** (**a**) Optimization of the amount of the QDs; (**b**) Optimization of the amount of the MnO2-QD-pAb NFs; (**c**) Optimization of the flow rate.



(**a**)



(**b**)

**Figure S3.** (**a**) Difference in separation chamber with or without magnets; (**b**) The color of supernatant of magnetic nanoparticles (MNPs), suspended MNPs after separating and original MNPs.

|  |  |
| --- | --- |
| copyRight | © 2020 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/). |