Electronic Supplementary Material (ESI) for Analyst.
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Supplemental Information

Capture and detection of urine bacteria using microchannel silicon nanowire microfluidic chip coupled with MALDI-TOF MS

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1. Supplementary Experimental Methods

Chemicals and materials. Silicon wafers with oxide layer (p-type, 1-10 Ω·cm, <100>, SiO_2 thickness 1 μm) were purchased from Suzhou Research Material Microtech (Suzhou, China). Photoresist (AZ4620) was purchased from AZ Electronic Materials (Japan). Hydrofluoric acid (HF) (40%), hydrogen peroxide (H_2O_2) (30%) was obtained from Sinopharm Chemical Reagent Co. (Shanghai, China). Potassium hydroxide (KOH) (90%), ammonium fluoride (NH_4F) (98%), and silver nitrate ($AgNO_3$) were purchased from Aladdin Co. (Shanghai, China). Bis(N-succinimidyl) carbonate (99%) was purchased from J&K Scientific Ltd (Beijing, China). Concanavalin A (Con A), (3-Aminopropyl) triethoxysilane (APTES) (≥98.0%), were from Sigma-Aldrich Inc (Saint Louis, USA). Alpha-cyano-4-hydroxycinnamic acid (α-CHCA) was purchased from Bruker Daltonics Inc. (Billerica, USA). Ndimethylpropane-1,3-diamine monohydrochloride (EDC) was obtained from Aladdin Co., (China). Vancomycin, hydrochloride was from Macklin Biochemical Co., Ltd (Shanghai, China).

Preparation of McSiNWs substrate. Silicon wafer with an oxide layer (thickness ~1 μm) was spin-coated with photoresist (AZ4620) and pre-baked at 95 ℃ for 3 min. Then the wafer covered with a designed photomask was exposed to UV light for 50 s, followed by developing in a 0.7% NaOH aqueous solution. After post-baked at 95 ℃ for 20 min, the substrate was immersed into a BHF buffer (6% HF and 32% NH_4F) to remove part of the SiO_2 without photoresist protection. After washing off the remaining photoresist with acetone, a silicon wafer with a patterned SiO_2 layer was obtained. Then, the microchannel and silicon nanowires were prepared by wet etching and a two-step metal assistant chemical etching (MACE) with a patterned SiO_2 layer as an etching mask. Briefly, the wet etching was performed in a 30% KOH solution at 90 ℃ for 20 min. The depth of the channel reached about 40 μm. After that, the microchannel silicon wafer was immersed into 0.02 M $AgNO_3$ solution for 20 s to deposit silver particles on its surface. Then the microchannel silicon wafer with sliver particles was soaked in an etching solution containing 4.8 M HF and 0.4 M H_2O_2 for 15 min at room temperature to produce SiNWs. After etching, the McSiNWs substrate was immersed into diluted nitric acid (1:1, v/v) to remove the silver particles. At last, rinsed the McSiNWs with deionized water and dried with nitrogen.

Con A modification. Before modification, the McSiNWs substrates were oxidized in a tube furnace at 500 ℃ for 1 hour to form a dense oxide layer in case the degradation during bacteria capture. First, the substrates were immersed into a 4% (v/v) 3- (3-Aminopropyl) triethoxysilane solution in ethanol for 1 hour. Then, rinsed the substrates with ethanol and dried with nitrogen. Next, the substrates were incubated with 10 mM bis(N-succinimidyl) carbonate for 15 min and rinsed with acetonitrile. After drying with nitrogen, the substrates were immersed in a 30 μg/mL Con A solution in PBS buffer for 1h. Last, rinsed with PBS to remove the non-specific adsorption. The whole produce was carried out at room temperature.

Vancomycin modification. McSiNWs substrates were oxidized in a tube furnace at 500 ℃ for 1 hour. Then, the substrates were immersed in a 4% (v/v) (3-Aminopropyl) triethoxysilane solution in ethanol for 1 hour. After that, the substrates were rinsed with ethanol and dried with nitrogen. Vancomycin (2 mg/mL) and EDC (1 mg/mL) are dissolved in PBS buffer (pH=7.2) and activation for 15 min. Then, the McSiNWs substrates were immersed in the activated vancomycin dissolved in PBS buffer for 6 hours. finally, the

McSiNWs substrates attached with vancomycin were rinsed with PBS to remove the non-specific adsorbed molecules. The whole produce was carried out at room temperature.

Culture of bacteria. *Escherichia coli* (*E. coli*), and *Staphylococcus aureus* (*S. aureus*) were used as model bacteria in our experiments. The bacteria were grown overnight in LB medium at 37 ℃ with consecutive shaking at 200 rpm. Then, the bacteria in the LB medium were separated by centrifugation. The supernatant was discarded and the cell pellet was resuspended in PBS or urine samples. The concentration was monitored by the optical density (OD) at the wavelength of 570 nm using a microplate reader (Infinite F50, Tecan Co.). Prior to bacteria capture experiments, the OD_{570} values of bacteria stock solutions were adjusted to 0.2 for *E. coli* and 0.1 for *S. aureus*, corresponding to 4×10^8 CFU/mL measured by colony counting. Culture of bacteria in urine samples was conducted by spiking *E. coli* (4×10^6, 4×10^5, $4\times10_4$, 4×10^3 CFU/mL) in urine collected from volunteers. To improve the bacterial growth rate, LB liquid medium was added to provide nitrogen and carbon sources. 3 mL spiked urine sample was mixed with 3 mL LB medium, then cultured at 37 ℃ with consecutive shaking at 200 rpm. After culture, urine samples directly flowed through the microfluidic device for bacterial capture.

Bacteria capture. A series of bacteria suspension in PBS and urine (4×10^8, 4×10^7, 4×10^6, 4×10^5, 4×10^4 CFU/mL) were prepared, and 0.5 mL of the bacterial suspension was flowed through the microfluidic device at 15 μL/min for bacteria capture. Then, 0.03 mL of sterilized water was using to elution non-specific adsorption. Bacteria that enriched by the McSiNWs substrate were identified by MALDI-TOF MS.

MALDI-TOF MS detection and data analysis. After bacterial culture, the microfluidic device could be disassembled by unscrewing the screws on the PMMA plates. Since the PDMS film and the McSiNWs substrate were compressed by PMMA plates, the combination is reversible. The PDMS film could be directly peeled off from the McSiNWs substrate. The McSiNWs substrate with captured bacteria was overlaid with 1 μL matrix solution (saturated CHCA in 50% acetonitrile and 2.5% trifluoroacetic acid) and dried at room temperature. Then the McSiNWs chip was fixed onto a costume-made plate designed to match with the UltrafleXtreme MALDI TOF MS instrument (Bruker Daltonics Corp.) for bacterial identification.

The raw spectra were processed using FlexAnalysis 3.4 software (Bruker Daltonics Corp.) for baseline subtraction and smoothing. Peak picking was completed by ClinproTools software (Bruker Daltonics Corp.) with S/N ≥5. Autof Analyzer (Autobio Diagnostics Co., Ltd) software was used for database searching and bacterial identification. A proprietary algorithm was used for spectral pattern matching and a score of ≥9 indicates species identification, a score between 6 and 9 indicates genus identification, and a score of < 6 indicates no identification.

2. Supplementary Figures

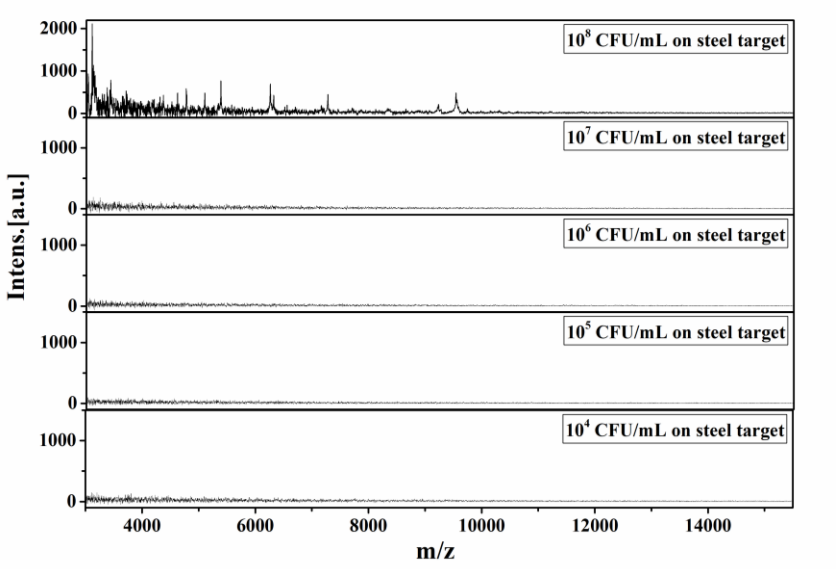

Fig. S1 MALDI-TOF MS spectra of *E. coli* by dropping bacterial suspension with different concentrations on the steel target.

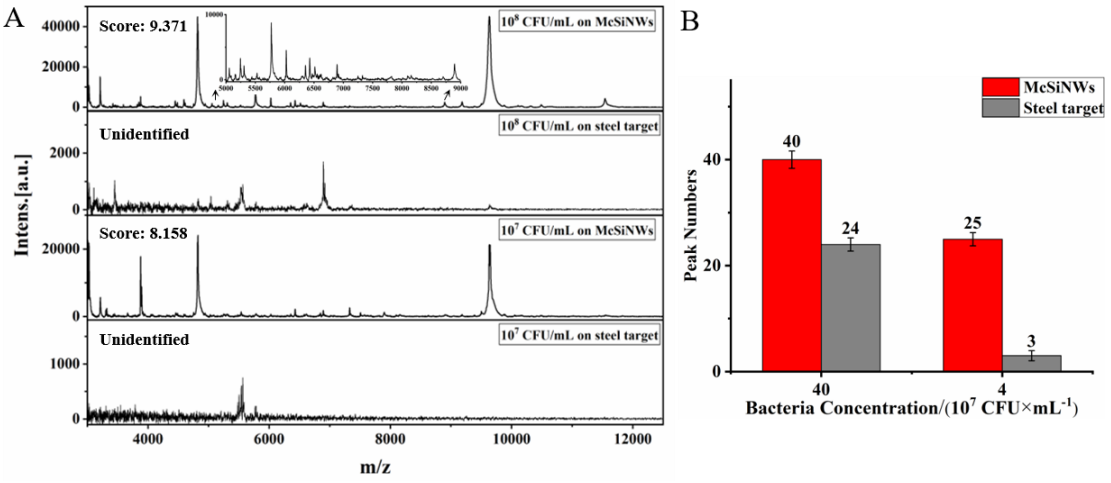

Fig. S2 (A) MALDI-TOF MS spectra of *S. aureus* enriched by McSiNWs-vancomycin substrate (0.5 mL) and MS spectra of 1 μL *S. aureus* suspension droplet on a steel target at a concentration of 4×10^8 and 4×10^7 CFU/mL. (B) Average peak numbers of three corresponding MS spectra ranging from 3-15 kDa with S/N ≥3.

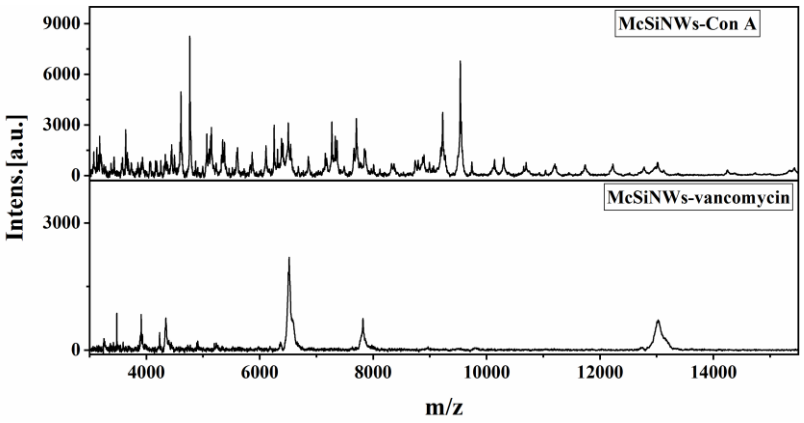

Fig. S3 MALDI-TOF MS spectra of *E. coli* enriched by McSiNWs-Con A and McSiNWs-vancomycin substrates from suspensions in PBS buffer (0.5 mL, 4×10^8 CFU/mL).

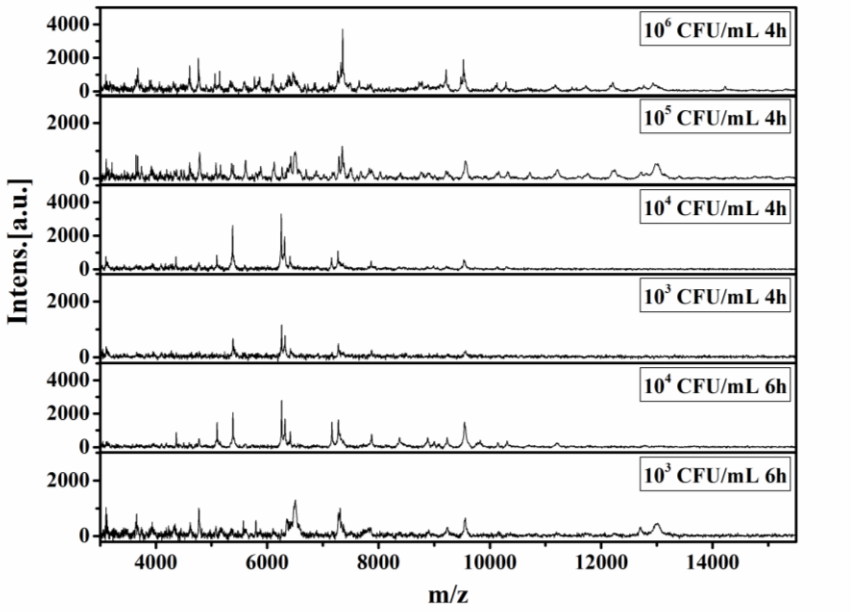

Fig. S4 MALDI-TOF MS spectra of *E. coli* enriched from spiked urine samples with various initial concentrations after culture.

3. Supplementary Tables

Table S1. Average optical intensity of the fluorescent images of four substrates with different structures.

Chip structure	McSiNWs-Con A	McSiNWs	SiNWs-Con A	McSi-Con A
Average optical intensity/pixel	0.5971	0.0698	0.0727	0.0564

Table S2. MALDI-TOF MS identification scores for *E. coli* at different counts in PBS and urine sample.

Bacterial concentration /(CFU·mL^{-1})	4×10^8	4×10^7	4×10^6	4×10^5	4×10^4
Scores in PBS buffer	9.871	9.581	9.012	8.918	<6
Score in spiked urine sample	9.547	9.450	7.479	<6	<6